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Spatial distribution of soil mechanical strength in a controlled traffic farming system as determined by cone index and geostatistical techniques

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Abstract. Controlled traffic farming (CTF) is a mechanisation system in which all load-bearing wheels are confined to the least possible area of permanent traffic lanes and where crops are grown in permanent, non-trafficked beds. In well-designed systems, the area affected by traffic represents less than 15% of the total field cropped area. The extent and distribution of soil compaction at locations laterally outboard of the permanent traffic lanes may explain the performance of the crop on the rows located either side of the wheeling. This compaction is due to lateral displacement of soil caused by repetitive wheeling, the effect of soil-tyre interaction and the soil conditions (strength) at the time of traffic. The impact of compaction on crop rows adjacent to permanent traffic lanes is also dependent on the seasonal effect of weather, because of changes in soil water availability. This work was conducted to model the spatial distribution of soil mechanical strength under increasing number of tractor passes to simulate the soil conditions that may be encountered in CTF systems at locations near-permanent traffic lanes. The study was conducted on a *Typic Argiudoll* (26% clay, 72% silt, 2% sand) with four traffic intensities (0, 6, 12 and 18 passes) using a 120 HP tractor (overall mass: 6.3 Mg). Traffic treatments were applied to experimental plots using a completely randomized block design with three replications per treatment. The spatial distribution of soil strength within wheeled and non-wheeled zones was determined using a cone penetrometer (depth range: 0–300 mm) and geostatistical techniques. In all treatments, cone index showed a quadratic response with depth, which explained between 67% and 88% of the variation in soil strength. The number of tractor passes had no effect on the range of spatial dependence of residuals. No differences were observed in the proportion of grid cells where penetration resistance was greater than 2 MPa (considered to be the soil strength limit for root growth of most arable crops) between-traffic treatments, or wheeled and non-wheeled zones, respectively. The overall mean proportion (± 95% confidence interval) of grid cells (4.9 ± 4.5%) suggested that this measure has a relatively high variability and therefore may not be a reliable parameter to be used in the design of future experimental work.

Key words: field traffic, soil compaction, soil mechanical properties, soil penetration resistance.

INTRODUCTION

The development and adoption of higher capacity, and therefore heavier agricultural vehicles, raises concerns over the long-term sustainability of intensively-managed arable cropping systems, because of increased potential for deterioration of soil structure due to traffic compaction (Chamen et al., 2003; Spoor et al., 2003; Keller et al., 2019). In permanent (e.g., > 15 years) zero-tillage (ZT) systems without controlled traffic, compaction may aggravate other soil degradation processes (e.g., erosion, runoff, loss of soil organic carbon), which may be conducive to reduced crop productivity and increased the risk of greenhouse gas emissions (Soane & van Ouwerkerk, 1995; Li et al., 2007; Antille et al., 2015a). Studies conducted in Argentina have shown that long-term ZT systems often exhibit widespread soil compaction (e.g., Díaz-Zorita et al., 2002; Antille et al., 2015b; Masola et al., 2020). This is explained by relatively high traffic intensities ($\geq$ 40 Mg km^{-1} ha^{-1}) commonly observed in those systems (e.g., Botta et al., 2007; Mašek et al., 2014). The effects of traffic-induced compaction are often persistent (e.g., > 5 years), particularly in the subsoil (Spoor, 2006; Radford et al., 2007). In intensively-managed soils (e.g., double-cropping) under ZT, these effects are exacerbated by the frequency of traffic, which therefore restricts the opportunities for soil repair through natural processes (Dexter, 1991). Soil mechanical resistance influences root penetration into the soil, and exploitation of soil water and nutrients by the plant, thereby affecting crop growth and yield (Goss, 1977; Letey, 1985; Lipiec & Stępniewski, 1995). Soil penetration resistance is widely used to determine soil strength both under field and laboratory conditions (e.g., Ayers & Bowen, 1985; Nawaz et al., 2013; Rooney & Lowery, 2000). Increased soil strength above a critical value of 2 MPa (suggested limit value for most arable crops) at soil water contents near-drain upper limit severely restricts root elongation (Taylor & Gardner, 1963; Carter & Tavernetti, 1968). Controlled traffic farming (CTF) systems are regarded as a practical and cost-effective technology to minimise the impact of field traffic-induced soil compaction (Kingwell & Fuchsbichler, 2011; Chamen et al., 2015). The underlying principle of this technology is the establishment of two distinctive zones within a field; namely: permanent crop beds (non-wheeled soil) and permanent traffic lanes (wheeled soil), respectively (Taylor, 1983; Tullberg et al., 2007). Both anecdotal and reported evidence indicates CTF systems to have positive impacts on crop productivity, and importantly on resource use-efficiency, particularly water (rainfall and irrigation), fertiliser and on-farm energy use (Hussein et al., 2018; Bluett et al., 2019; Tullberg, 2000). Acknowledgement of these benefits has been the main driver for increased adoption of this technology (Tullberg et al., 2007; Chamen, 2015), although at a much slower rate in some cropping systems (e.g., Braunack & McGarry, 2006; McPhee & Aird, 2013; Antille et al., 2016).

Only few studies available in the scientific literature have investigated the spatial variability of soil penetration resistance as affected by vehicular traffic at the field-scale; these studies include 2D and 3D approaches (e.g., Castrignano et al., 2002; Ferrero et al., 2005; Carrara et al., 2007; Alesso et al., 2012). There appears to be a paucity of reported information about the spatial distribution of soil compaction within CTF systems. This knowledge is required to advance the understanding of crop performance and root behaviour at locations near-permanent traffic lanes, and to quantify impacts on crop yield and quality at the field-scale. In well-designed, fully matched CTF systems with 3-m centres and 12.2-m (40-ft) modules or greater, the traffic footprint is fairly small, and may represent 15% or less of the field cropped area (Antille et al., 2019). Therefore, compaction at locations laterally outboard of the permanent traffic lanes affects a relatively small number of crop rows. For other CTF systems, in which track gauge widths are not fully matched, the traffic footprint is greater (typically, ≥ 15% of the field cropped area). This occurs in CTF systems with unmodified machinery and also when narrower modules are used (e.g., 6-m or 8-m wide, Galambošová et al., 2017). Consequently, the number of crop rows affected by compaction in these latter systems is higher and will depend upon the specific design of the CTF system. This can increase the spatial variability in crop yield and quality, as shown by Jensen et al. (2000).

The objective of this study was to apply geostatistical techniques to analyse cone index data collected from transects perpendicular to the direction of tramlines, which were established to capture the spatial variability in soil mechanical strength in wheeled and non-wheeled soil. Tramlines were created to represent recently established and older CTF systems by controlling the number of passes with a medium-sized tractor. It was hypothesised that the spatial structure of soil penetration resistance and the soil profile area affected by compaction (cone index greater than 2 MPa) would be significantly affected the number of tractor passes.

MATERIALS AND METHODS

The study was conducted in a commercial farm located in Aurelia, Argentina (31°29'8.50" S, 61°27'25.88" W). The area is characterised by a flat relief with deep and moderately well-drained, silty-clay loam soils originated from loess sediments. These soils have relatively high susceptibility to soil structural damage due compaction when moist (Imhoff et al., 2016). The soil at the experimental site was a *Typic Argiudoll* Rafaela Series with the following granulometric composition in the top 0–20 cm: 20 g kg^{-1} (sand), 720 g kg^{-1} (silt), and 260 g kg^{-1} (clay), respectively (INTA, 1991). The site selected for the study had 30 ha and had been under conventional tillage for more than 50 years, but it has been managed under zero-tillage (ZT) in the five years prior to the experiment using a continuous wheat-soybean-wheat crop sequence. In spring 2014, an area of 40×80-m was tilled to remove historical compaction, as shown by Godwin et al. (2015), using a chisel plough fitted with 11-curved shanks, which were mounted on the frame of the implement at regular intervals of 35-cm. The implement was operated at 20 cm deep and at a forward speed of 7 km h^{-1}. Thirty days after this operation was performed, controlled traffic conditions were imposed by applying soil compaction treatments to experimental plots (dimensions: 40×6-m) using a completely randomised block design with three replications (n = 3). The experimental treatments represented permanent crop bed (zero-traffic) and permanent traffic lanes of a CTF system, which had 6, 12, and 18 passes of a tractor, respectively. Traffic treatments were completed in two stages, which were spaced seven days apart, and comprised 4+2, 8+4, and 12+6 passes, respectively. The tractor used at the site was a Pauny 120 HP with an overall mass of 6.3 Mg equipped with single, radial tyres (front axle: 14.9×26, rear axle: 23.1×30) inflated to the manufacturer's recommended inflation pressure for load and speed. Average soil water content at the time of traffic was 0.28 g g^{-1}, which was slightly higher than that (0.25 g g^{-1}) required for the Proctor density (1.42 g cm^{-3}).

Soil penetration resistance was measured within each plot to determine the effect of traffic intensity (number of passes) on soil compaction. For this, penetration resistance readings were taken from 110-cm transects perpendicular to the tramline's direction, and at random locations along the tramline (Fig. 1).

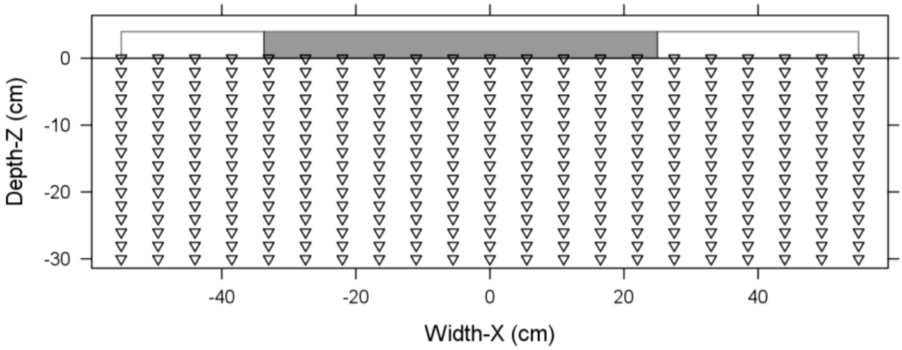

Figure 1. Grid indicating the soil sampling scheme. Inverted triangles denote points where soil penetration resistance measurements were taken. Soil penetration resistance was recorded on transects perpendicular to tramlines with points spaced at 5.5 cm in the *X*-direction, and to a depth of 30 cm at 2 cm depth intervals. The measured area across the tramline is represented by the rectangles on the top of the graph, which show wheeled (darker gray) and non-wheeled zones (lighter gray), respectively.

Measurements were taken on either side of the rut's centreline at regular intervals of 5.5 cm (horizontal direction, *X*-coordinate) and at 2 cm depth increments to a depth of 30 cm (vertical direction, *Z*-coordinate), and the force was digitally recorded using a soil compaction meter (PNT-2000®). Since the centreline of the rut matched the centre of the 110-cm transect ($X = 0$), readings included a wheeled (W) zone of ≈60 cm and two non-wheeled (NW) zones of ≈20 cm and 30 cm either side of the centreline, respectively. The cone had 125 mm^2 base area and 30° apex, and measurements were taken based on ASABE (2019). The 2D grid at each sampling location consisted of 336 observations. Soil water content was determined gravimetrically by taking soil cores at 10 cm depth increments in the 0–30 cm depth range. Cores were taken from both trafficked and non-trafficked soil, respectively. Exploratory spatial data analysis of soil penetration resistance readings was conducted to identify outliers and check for assumptions of geostatistical techniques. Due to skewness (lack of symmetry), data were transformed based on the approach of Webster & Oliver (2007). The non-stationarity of the process was modelled by polynomial functions using spatial coordinates (*X* and *Z*, respectively) as explanatory variables. Estimation of trend coefficients was performed by applying the generalised least squares (GLS) method. The spatial structure of penetration resistance's residuals was examined by computing 2D omnidirectional sample variograms. Box plot diagrams were used to identify outliers (Figure 2). Given outliers were present in the dataset, the Cressie's robust semi-variance estimator (Cressie, 1993) was used to compute the variograms, as shown in Eq. (1).

$$\gamma(h) = \frac{\left[\frac{1}{N(h)}\sum Z(S_i)Z(S_i+h)^{1/2}\right]^4}{0.914 + 0.988[N(h)]^{-1}} \qquad (1)$$

where $\gamma(h)$ is the semivariance estimator, $N(h)$ is the number of pairs of observations separated by the vector $h, Z(S_i)$ and $Z(S_i + h)$ are the observations at S_i and $S_i + h$ locations, respectively.

Isotropic exponential, spherical and Gaussian models were fitted to sample variograms and cross-validated by 30-fold cross-validation procedure. The models were selected based on the sum square error and the following diagnostic criteria: correlation coefficient (r) between observed and predicted values, the root mean squared error (RMSE), and the mean squared deviation ratio (MSDR) (Webster & Oliver, 2007). The selected model was used to obtain surfaces of penetration resistance on the *X*-*Z* plane by block-kriging on a 1×2.5-cm of the grid. Finally, to synthesise the effect of controlled traffic on soil compaction, the proportion of grid cells with cone index > 2 MPa on each *X*-*Z* plane was accounted for and used as a response variable for ANOVA. The data was modeled using a split-plot design with number of passes applied to the main plot following a completely randomized block design, and zones as sub-plots. The blocks were regarded as the random term. Data management and geostatistical analyses were undertaken using the statistical programming language *R* (R Development Core Team, 2014), and the gstat (Pebesma, 2004) and nlme (Pinheiro et al., 2013) packages.

RESULTS AND DISCUSSION

Median values of soil penetration resistance profiles (depth range: 0–30 cm) corresponding to the traffic treatments are shown in Fig. 2. Untrafficked soil between-traffic lanes exhibited higher soil penetration resistance compared to control soil (0 passes or crop bed). Median values of soil penetration resistance at this location were below the suggested threshold of 2 MPa, but with some exceptions at depths greater than 10 cm. Table 1 shows soil water content recorded in the plots in both wheeled (W) and non-wheeled (NW) zones. Corrections of soil penetration resistance readings for soil water content (Ayers & Perumpral, 1982; ASABE, 2019) were not required given that soil water values were within about 5% difference in all three depths. There were no differences in soil water contents between W and NW zones ($P = 0.15$), which was observed in all measured depths (*P*-values between 0.25 and 0.96).

Geostatistical analyses of soil penetration resistance data for all 12 plots consistently showed non-stationarity in the mean. With the exception of the 12 passes treatment in Block 1, quadratic functions on both coordinates accounted for the trend and explained between 67% and 88% of the variation in soil penetration resistance (Table 2). The trend on *Z* could be better explained by the natural increase in soil penetration resistance with increasing soil depth, possibly due to densification of the soil profile and increased clay content (Bt horizon). The trend on *X* is explained by the effect of tractor passes, which shows significant differences between wheeled and non-wheeled soil, respectively. Readings recorded at the centreline of the rut were consistently higher compared with those at locations laterally outboard of the wheeling or in non-wheeled soil, which was therefore consistent with previous studies (e.g., Way et al., 2009; Antille et al., 2013). Such response is explained by the soil stress distribution at depth and the soil-tyre contact pressure (Misiewicz et al., 2015).

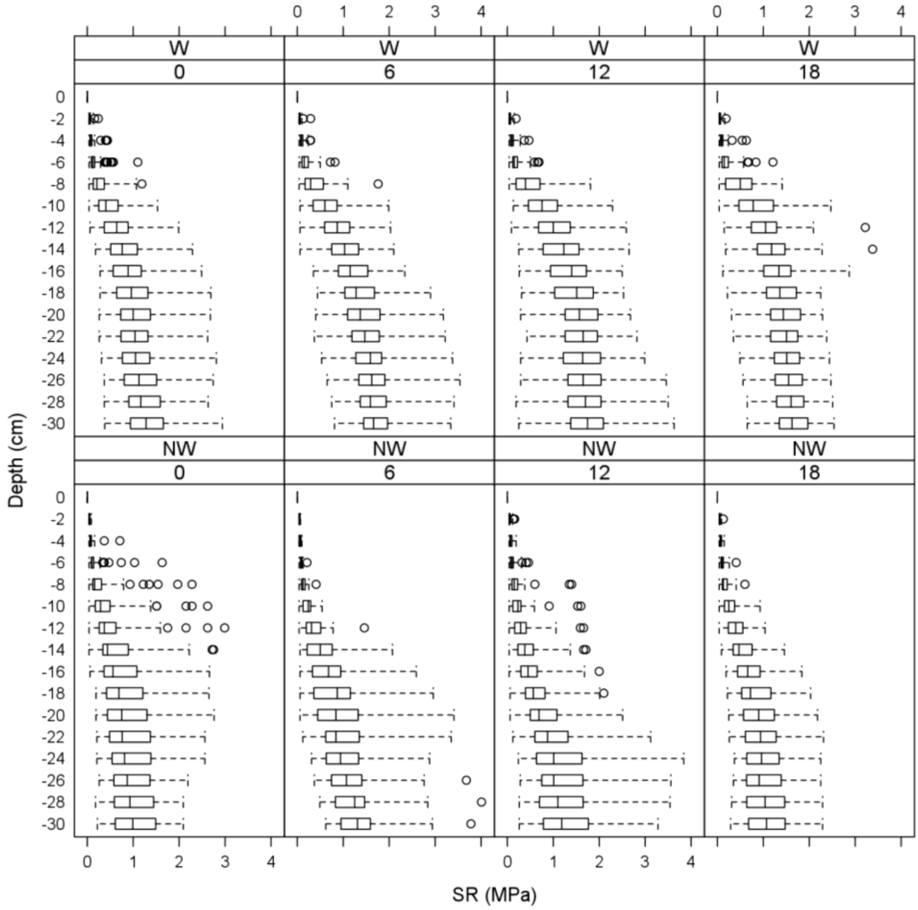

Figure 2. Box plots (Min, Q_1, Med, Q_3, Max, Outliers) showing the distribution of soil penetration resistance (SR) readings for each traffic treatment (0, 6, 12, 18 passes of a tractor) in both wheeled (W) and non-wheeled (NW) zones, respectively. Sample medians of penetration resistance from each depth show the trend of this measurement as a function of depth.

Table 1. Mean soil moisture content (±SD) at the three measured depths in wheeled (W) and non-wheeled (NW) zones in each of the traffic treatments, respectively

Treatment		Soil depth		
No. of passes	Zone	0–10 cm	10–20 cm	20–30 cm
0	Wheeled	0.28 ± 0.005	0.27 ± 0.010	0.27 ± 0.014
0	Non-wheeled	0.29 ± 0.033	0.28 ± 0.006	0.27 ± 0.012
6	Wheeled	0.27 ± 0.003	0.27 ± 0.016	0.27 ± 0.017
6	Non-wheeled	0.28 ± 0.034	0.27 ± 0.011	0.28 ± 0.011
12	Wheeled	0.26 ± 0.024	0.28 ± 0.017	0.27 ± 0.016
12	Non-wheeled	0.27 ± 0.032	0.28 ± 0.013	0.27 ± 0.017
18	Wheeled	0.26 ± 0.002	0.28 ± 0.007	0.27 ± 0.005
18	Non-wheeled	0.26 ± 0.010	0.27 ± 0.020	0.28 ± 0.025

Residuals obtained after applying the GLS procedure exhibited a structure depicted by the spherical isotropic models presented in Table 2. Fitted ranges varied between 10 and 18 cm, and no differences were detected between traffic treatments ($P = 0.17$). Due to the configuration of the sampling grid (5.5×2-cm), the analysis of directional variograms revealed that the spatial continuity was mainly attributed to the spatial structure in the vertical direction, whereas in the horizontal direction, the spatial structure could be under the minimum lag distance. Despite this, the models shown in Table 2 were fitted assuming isotropy for soil penetration resistance data. Cross-validation of results shows that, even if the process was regarded as isotropic, reasonable good predictions of penetration resistance could be obtained from non-sampled locations.

Table 2. Parameters and 30-fold cross-validation results for the spatial models of soil resistance fitted for each traffic treatment and block

Treatment	Block	Transf.	Trend model			Variogram				N-fold cross-validation		
			Z	*X*	R^2_{adj}	Model	Nugget	Sill	Range (cm)	r	RMSE	MSDR
0 passes	1	none	Q	Q	0.68	Sph	0	0.169	15.1	0.98	0.143	0.57
	2	sqrt	Q	Q	0.80	Sph	0	0.032	12.8	0.98	0.113	1.48
	3	sqrt	Q	none	0.70	Sph	0	0.067	17.9	0.98	0.138	1.62
6 passes	1	none	Q	Q	0.78	Sph	0	0.084	13.6	0.98	0.096	0.44
	2	none	Q	Q	0.79	Sph	0	0.226	13.3	0.99	0.149	0.40
	3	sqrt	Q	Q	0.89	Sph	0	0.021	11.3	0.99	0.077	1.00
12 passes	1	sqrt	Q	C	0.78	Sph	0	0.071	13.9	0.98	0.176	1.95
	2	none	Q	Q	0.77	Gau	0.015	0.164	11.7*	0.97	0.146	0.85
	3	sqrt	Q	Q	0.86	Sph	0	0.025	10.7	0.98	0.093	1.15
18 passes	1	none	Q	Q	0.79	Sph	0	0.107	13.7	0.99	0.106	0.45
	2	sqrt	Q	Q	0.78	Sph	0	0.060	18.4	0.99	0.108	1.11
	3	none	Q	Q	0.81	Sph	0	0.105	15.3	0.99	0.094	0.40

*****Effective range; Trend model: Q = quadratic; C = cubic; Model: Sph = Spherical; Gau = Gaussian; sqrt = squared root-transformed, respectively. RMSE is root mean squared error, and MSDR is mean squared deviation ratio.

The spatial distribution of soil penetration resistance observed in each plot is shown in Fig. 3. The proportion of grid cells with cone index > 2 MPa showed non-significant interaction ($P = 0.28$) and non-significant main effect of treatments ($P = 0.57$) or zones ($P = 0.36$), which was due to high variability of this measure (CV ≥ 0.65), also shown by Alesso et al. (2017, 2018). Over a total of 1395 grid cells, the estimated overall mean proportion of grid cells (± 95% confidence interval) with cone index > 2 MPa was 4.9 ± 4.5%. Several studies (e.g., Botta et al., 2019; Horn et al., 2003) have shown that soil compaction increases with increased traffic intensity because of progressively greater soil displacement beneath the tyres (Ansorge & Godwin, 2007). However, in our study, a relatively low compaction was observed within tramlines, even with 18 passes, suggesting that true replication of CTF conditions were not achieved, possibly due to the moderately light-mass tractor used to establish the tramlines.

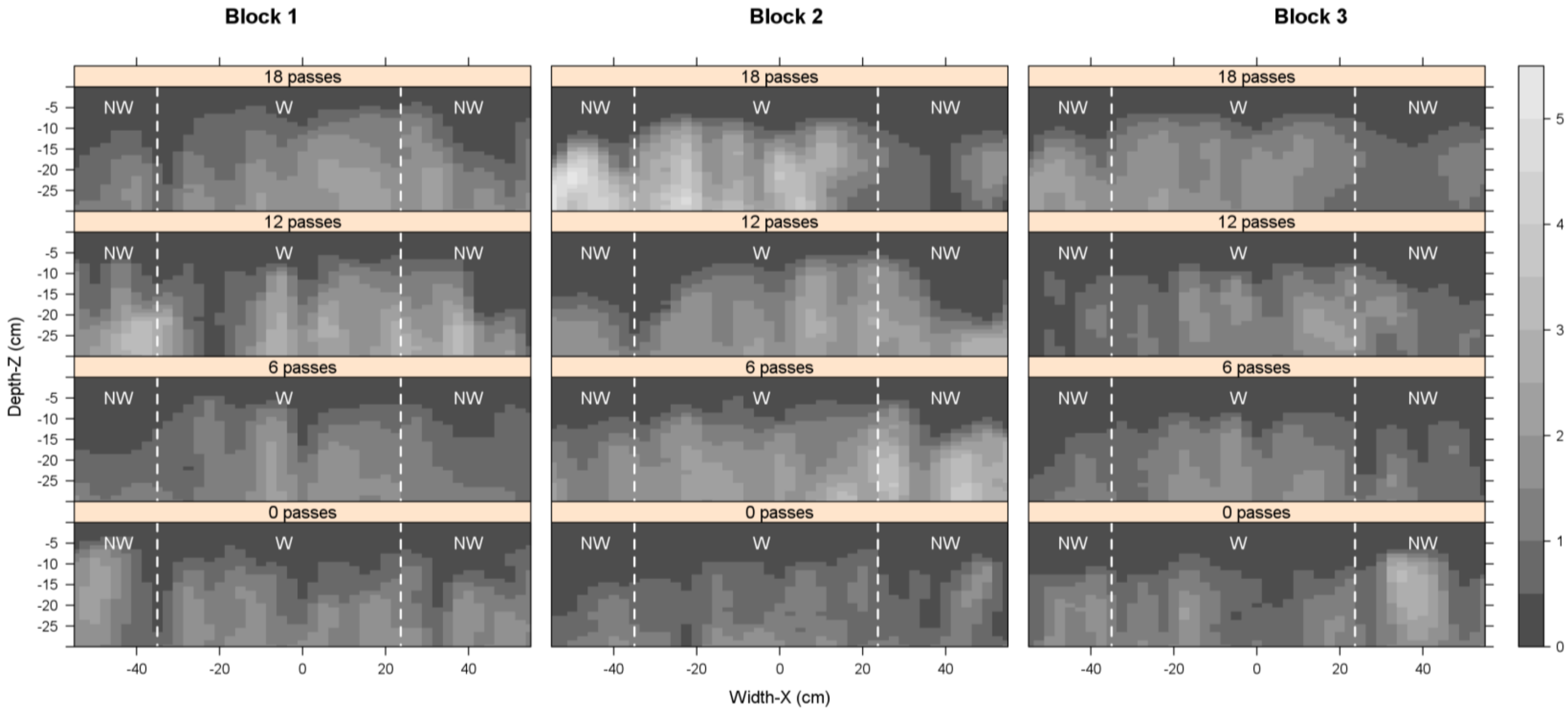

Figure 3. Spatial distribution of soil penetration resistance in vertical planes obtained by block-kriging over sample grids of 110 cm (width-*X*) by 30 cm (depth-*Z*) perpendicular to the direction of established tramlines in each traffic treatment (0, 6, 12 and 18) and block (1–3) in wheeled (W) and non-wheeled (NW) zones, respectively. The grey colour-scale represents levels of soil penetration resistance (MPa).

CONCLUSIONS

The main results derived from this work are summarised below:

– Modelled data showed a significant trend and spatial structure of soil penetration resistance in the vertical plane. Variation in soil penetration resistance with depth explained between 67% and 88% of the variation,
– Residuals were autocorrelated with ranges between 10 and 18 cm. However, the hypotheses formulated prior to this study, could not be verified because the number of tractor passes showed no significant effects (P >0.05) either on the range of spatial dependence of generalised least squares (GLS) residuals or the proportion of grid cells with cone index > 2 MPa,
– The overall mean proportion of grid cells (±95% confidence interval) with values of cone index > 2 MPa (4.9 ± 4.5%) reflects relatively high variability of this measure, and therefore does not appear to be a reliable parameter to inform future experimental designs.

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REFERENCES

Alesso, C.A., Masola, M.J., Carrizo, M.E. & Imhoff, S.D.C. 2017. Estimating sample size of soil cone index profiles by bootstrapping. *Revista Brasileira de Ciencia do Solo* **41**, Article number: e0160464.

Alesso, C.A., Masola, M.J., Carrizo, M.E., Cipriotti, P.A. & Imhoff, S.C. 2018. Spatial variability of short-term effect of tillage on soil penetration resistance. *Archives of Agronomy and Soil Science* **65**, 822–832.

Alesso, C.A., Pilatti, M.A., Imhoff, S.C. & Grilli, M. 2012. Spatial variability of soil chemical and physical attributes in a flat pampa Santa Fe field. *Ciencia del Suelo* **30**, 85–93.

Ansorge, D. & Godwin, R.J. 2007. The effect of tyres and a rubber track at high axle loads on soil compaction, Part 1: Single axle-studies. *Biosystems Engineering* **98**, 115–126.

Antille, D.L., Ansorge, D., Dresser, M.L. & Godwin, R.J. 2013. Soil displacement and soil bulk density changes as affected by tire size. *Transactions of the ASABE* **56**, 1683–1693.

Antille, D.L., Bennett, J.McL. & Jensen, T.A. 2016. Soil compaction and controlled traffic considerations in Australian cotton-farming systems. *Crop and Pasture Science* **67**, 1–28.

Antille, D.L., Chamen, W.C.T., Tullberg, J.N. & Lal, R. 2015a. The potential of controlled traffic farming to mitigate greenhouse gas emissions and enhance carbon sequestration in arable land: A critical review. *Transactions of the ASABE* **58**, 707–731.

Antille, D.L., Imhoff, S.C., Alesso, C.A., Chamen, W.C.T. & Tullberg, J. N. 2015b. Potential to increase productivity and sustainability in Argentinean agriculture with controlled traffic farming: a short discussion. *Acta Technologica Agriculturae* **18**, 83–87.

Antille, D.L., Peets, S., Galambošová, J., Botta, G.F., Rataj, V., Macák, M., Tullberg, J.N., Chamen, W.C.T., White, D.R., Misiewicz, P.A., Hargreaves, P.R., Bienvenido, J.F. & Godwin, R.J. 2019. Review: Soil compaction and controlled traffic farming in arable and grass cropping systems. *Agronomy Research* **17**, 653–682.

ASABE. 2019. ASAE Standard EP542.1-NOV2019: Procedures for using and reporting data obtained with the soil cone penetrometer. St. Joseph, Mich.: American Society of Agricultural and Biological Engineers.

Ayers, P.D. & Bowen, H.D. 1987. Predicting soil density using cone penetration resistance and moisture profiles. *Transactions of the ASAE* **30**, 1331–1336.

Ayers, P.D. & Perumpral, J.V. 1982. Moisture and density effect on cone index. *Transactions of the ASAE* **25**, 1169–1172.

Bluett, C., Tullberg, J.N., McPhee, J.E. & Antille, D.L. 2019. Soil and Tillage Research: Why still focus on soil compaction? *Soil and Tillage Research* **194**, Article number: 104282.

Botta, G.F., Bienvenido, J.F., Antille, D.L., Rivero, E.R.D., Contessotto, E.E., Ghelfi, D.G. & Nistal, A.I. 2019. Effect of traffic with a light-weight tractor on physical properties of an Aridisol soil in Almeria, Spain. *Revista de la Facultad de Ciencias Agrarias* **51**, 270–279.

Botta, G.F., Pozzolo, O., Bomben, M., Rosatto, H., Rivero, D., Ressia, M., Tourn, M., Soza, E. & Vazquez, J. 2007. Traffic alternatives for harvesting soybean (*Glycine max* L.): Effect on yields and soil under a direct sowing system. *Soil and Tillage Research* **96**, 145–154.

Braunack, M.V. & McGarry, D. 2006. Traffic control and tillage strategies for harvesting and planting of sugarcane (*Saccharum officinarum*) in Australia. *Soil and Tillage Research* **89**, 86–102.

Carrara, M., Castrignano, A., Comparetti, A., Febo, P. & Orlando, S. 2007. Mapping of penetrometer resistance in relation to tractor traffic using multivariate geostatistics. *Geoderma* **142**, 294–307.

Carter, L.M. & Tavernetti, J.R. 1968. Influence of precision tillage and soil compaction on cotton yields. *Transactions of the ASAE* **11**, 65–67.

Castrignano, A., Maiorana, M., Fornaro, F. & Lopez, N. 2002. 3D spatial variability of soil strength and its change over time in a durum wheat field in Southern Italy. *Soil and Tillage Research* **65**, 95–108.

Chamen, T. 2015. Controlled traffic farming-from worldwide research to adoption in Europe and its future prospects. *Acta Technologica Agriculturae* **18**, 64–73.

Chamen, T., Alakukku, L., Pires, S., Sommer, C., Spoor, G., Tijink, F., & Weisskopf, P. 2003. Prevention strategies for field traffic-induced subsoil compaction: a review. Part 2. Equipment and field practices. *Soil and Tillage Research* **73**, 161–174.

Chamen, W.C.T., Moxey, A.P., Towers, W., Balana, B. & Hallett, P.D. 2015. Mitigating arable soil compaction: a review and analysis of available cost and benefit data. *Soil and Tillage Research* **146**, 10–25.

Cressie, N. 1993. *Statistics for spatial data*. Revised Edition. New York, USA: Wiley-InterScience. doi: 10.1002/9781119115151.

Dexter, A.R. 1991. Amelioration of soil by natural processes. *Soil and Tillage Research* **20**, 87–100.

Díaz-Zorita, M., Duarte, G.A. & Grove, J.H. 2002. A review of no-till systems and soil management for sustainable crop production in the subhumid and semiarid Pampas of Argentina. *Soil and Tillage Research* **65**, 1–18.

Ferrero, A., Usowicz, B. & Lipiec, J. 2005. Effects of tractor traffic on spatial variability of soil strength and water content in grass covered and cultivated sloping vineyard. *Soil and Tillage Research* **84**, 127–138.

Galambošová, J., Macák, M., Rataj, V., Antille, D.L., Godwin, R.J., Chamen, W.C.T., Žitňák, M., Vitázková, B., Ďuďák, J. & Chlpík, J. 2017. Field evaluation of controlled traffic farming in Central Europe using commercially available machinery. *Transactions of the ASABE* **60**, 657–669.

Godwin, R., Misiewicz, P., White, D., Smith, E., Chamen, T., Galambošová, J. & Stobart, R. 2015. Results from recent traffic systems research and the implications for future work. *Acta Technologica Agriculturae* **18**, 57–63.

Goss, M.J. 1977. Effects of mechanical impedance on root growth in barley (*Hordeum vulgare* L.) I. Effects on the elongation and branching of seminal root axes. *Journal of Experimental Botany* **28**, 96–111.

Horn, R., Way, T. & Rostek, J. 2003. Effect of repeated tractor wheeling on stress/strain properties and consequences on physical properties in structured arable soils. *Soil and Tillage Research* **73**, 101–106.

Hussein, M.A., Antille, D.L., Chen, G., Kodur, S. & Tullberg, J.N. 2018. Agronomic performance of sorghum (*Sorghum bicolor* (L.) Moench) and fertilizer use efficiency as affected by controlled and non-controlled traffic of farm machinery. ASABE Paper No.: 1800250. St. Joseph, MI.: 2018 *ASABE Annual International Meeting, American Society of Agricultural and Biological Engineers*. https://doi.org/10.13031/aim.201800250

Imhoff, S., Da Silva, A.P., Ghiberto, P.J., Tormena, C.A., Pilatti, M.A. & Libardi, P.L. 2016. Physical quality indicators and mechanical behavior of agricultural soils of Argentina. *PLoSONE* **11**, Article number: e0153827.

INTA. 1991. Carta de suelos de la República Argentina: Hojas 3160-25-25 Esperanza-Pilar. Rafaela, Argentina: Estación Experimental Agropecuaria Rafaela, Instituto Nacional del Tecnología Agropecuaria. Available at: http://rafaela.inta.gov.ar/mapas/suelos/__series/pil/Pilar_datos_analiticos.htm.

Jensen, T., Powell, G. & Neale, T. 2000. Yield and protein variation within a planter width. In: Proceedings of the Society for Engineering in Agriculture. Barton, ACT, Australia: Engineers Australia. (www.engineersaustralia.org.au/resources-and-library).

Keller, T., Sandin, M., Colombi, T., Horn, R. & Or, D. 2019. Historical increase in agricultural machinery weights enhanced soil stress levels and adversely affected soil functioning. *Soil and Tillage Research* **194**, Article number: 104293.

Kingwell, R. & Fuchsbichler, A. 2011. The whole-farm benefits of controlled traffic farming: An Australian appraisal. *Agricultural Systems* **104**, 513–521.

Letey, J. 1985. Relationship between soil physical properties and crop production. *Advances in Soil Science* **1**, 277–294.

Li, Y.X., Tullberg, J.N. & Freebairn, D.M. 2007. Wheel traffic and tillage effects on runoff and crop yield. *Soil and Tillage Research* **97**, 282–292.

Lipiec, J. & Stępniewski, W. 1995. Effects of soil compaction and tillage systems on uptake and losses of nutrients. *Soil and Tillage Research* **35**, 37–52.

Mašek, J., Kroulik, M., Chyba, J., Novak, P. & Kumhala, F. 2014. Traffic intensity in fields and technical possibilities for reduction of machinery passes. *Engineering for Rural Development* **13**, 216–220.

Masola, M.J., Alesso, C.A., Carrizo, M.E., Berhongaray, G., Botta, G.F., Horn, R. & Imhoff, S. 2020. Advantages of the one-wheeled tramline for multiple machinery widths method on sunflower (*Helianthus annus* L.) and maize (*Zea mays* L.) responses in the Argentinean Flat Pampas. *Soil and Tillage Research* **196**, Article number: 104462.

McPhee, J.E. & Aird, P.L. 2013. Controlled traffic for vegetable production: Part 1. Machinery challenges and options in a diversified vegetable industry. *Biosystems Engineering* **116**, 144–154.

Misiewicz, P.A., Blackburn, K., Richards, T.E., Brighton, J.L. & Godwin, R.J. 2015. The evaluation and calibration of pressure mapping system for the measurement of the pressure distribution of agricultural tyres. *Biosystems Engineering* **130**, 81–91.

Nawaz, M.F., Bourrié, G. & Trolard, F. 2013. Soil compaction impact and modelling. A review. *Agronomy for Sustainable Development* **33**, 291–309.

Pebesma, E.J. 2004. Multivariable geostatistics in S: The GSTAT package. *Computers and Geosciences* **30**, 683–691.
Pinheiro, J.C., Bates, D.M., Debroy, S. & Sarkar, D. 2013. Linear and nonlinear mixed effects models: The NLME Package. Vienna, Austria: R Foundation for Statistical Computing.
R Development Core Team. 2014. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.
Radford, B.J., Yule, D.F., McGarry, D. & Playford, C. 2007. Amelioration of soil compaction can take 5 years on a Vertisol under no till in the semi-arid subtropics. *Soil and Tillage Research* **97**, 249–255.
Rooney, D.J. & Lowery, B. 2000. A profile cone penetrometer for mapping soil horizons. *Soil Science Society of America Journal* **64**, 2136–2139.
Soane, B.D. & van Ouwerkerk, C. 1995. Implications of soil compaction in crop production for the quality of the environment. *Soil and Tillage Research* **35**, 5–22.
Spoor, G. 2006. Alleviation of soil compaction: requirements, equipment and techniques. *Soil Use and Management* **22**, 113–122.
Spoor, G., Tijink, F.G.J. & Weisskopf, P. 2003. Subsoil compaction: risk, avoidance, identification and alleviation. *Soil and Tillage Research* **73**, 175–182.
Taylor, H.M. & Gardner, H.R. 1963. Penetration of cotton seedling taproot as influenced by bulk density, moisture content, and strength of soil. *Soil Science* **96**, 153–156.
Taylor, J.H. 1983. Benefits of permanent traffic lanes in a controlled traffic crop production system. *Soil and Tillage Research* **3**, 385–395.
Tullberg, J.N. 2000. Wheel traffic effects on tillage draught. *Journal of Agricultural Engineering Research* **75**, 375–382.
Tullberg, J.N., Yule, D.F. & McGarry, D. 2007. Controlled traffic farming – From research to adoption in Australia. *Soil and Tillage Research* **97**, 272–281.
Way, T.R., Kishimoto, T., Torbert, H.A., Burt, E.C. & Bailey, A.C. 2009. Tractor tire aspect ratio effects on soil bulk density and cone index. *Journal of Terramechanics* **46**, 27–34.
Webster, R. & Oliver, M.A. 2007. Geostatistics for Environmental Scientists, 2nd Edition. John Wiley & Sons, Ltd. ISBN: 978-0-470-02858-2

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Growth performance and carcass characteristics of finishing Boer goats

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Abstract. In developing countries, goat kids are usually reared naturally under extensive conditions, but kids that are fed high concentrate diets generally have higher daily gains, dressing percentage and carcass quality then those produced in extensive system. Feeding of goat kids is the main factor affecting growth performance and carcass characteristics of finishing Boer goats. A study was carried out within the framework of the project 'Zootechnical and economic efficiency of feeding of fodder pulses to ruminant's meat production'. Eight kids of Boer breed were individually fattened for 60 days under an intensive management system, of which 4 kids received *ad libitum* concentrated feed of melted grain mix (GG) that was produced in farm and consisted of 25% oats, 25% barley and 50% fodder beans, but 4 kids received pelleted concentrated feed (CG) produced in feed company. Fattening performance, slaughter traits and carcass characteristics were evaluated. The carcasses were analyzed by weight and proportion of tissue: muscle, bone, fat. Daily live weight gain during 60 days of the fattening period was 235 g per day for CG kids, which is by 42.4% greater than for GG group kids (165 g per day ($P < 0.05$)). Feeding of GG kids by grain mix showed a lower growth rate of kids than feeding by pelleted concentrated feed (31.1 kg and 35.5 kg pre-slaughter, respectively), but had a higher dressing percentage (49.5% and 43.5%, respectively). Consequently, there was no significant difference in carcass weight (14.36 kg and 14.50 kg, respectively). GG carcasses had a higher fat content - 11.8% vs. 9.4%. In terms of the muscle-to-fat ratio, the carcasses of the kids of the two groups were significantly different ($P < 0.05$), where in the GG group there was 5.0 kg of muscle tissue per kg of fat and in the CG group there was 6.8 kg of muscle tissue. Total feed costs per day per animal and per kg live weight gain were higher in the CG group, as the feed price from a feed company was significantly higher than for farm-based grain meal. The results suggest that diet has an impact on the goat kid growth performance and carcass quality, and in intensive fattening systems it is more profitable to use a self-produced grain mix.

Key words: kids, fattening performance, carcass characteristics, meat quality.

INTRODUCTION

Today, goats are spread throughout the world, with the exception of extremely cold areas. Goats are widely present in the countries with an extensive agricultural production, although their population is rising in richer countries, mainly due to intolerance of certain groups of people to cow's milk (Ivanovic, 2016). The world population of goats (1.05 billion in 2017) increased by 49% in the last 20 years, whereas that of sheep (1.20 billion in 2017) and cattle (1.49 billion in 2017) increased more slowly (FAOSTAT, 2019). According to FAO (FAO, 2017) the number of meat goats worldwide totals 464 million heads and the estimated goat meat production is 5.8 million tons. In spite of the numerical relevance of the stock, goat meat consumption is low, when compared to beef consumption. In Latvia the number of goats for meat production is 4 thousand heads, but there is no production of goat meat in the market (FAO, 2017). Demand for low-fat meats and meat products have increased in recent years to avoid the health risks associated with excessive fat intake. Goat is known to produce relatively lean meat. Information on the characteristics of goat meat and meat products in Latvia and the Baltic's remains limited and needs to be further investigated. The breeding of goat meat in Latvia started in 2005, when the German Cattle breed was purchased from a German farm. The most popular meat goat breed in the world is the Boer goat. Currently, the Latvian goat-rearing industry is at an early stage of development. Although there is a demand for goat meat in Latvia, the number of breed goats is decreasing. In order to successfully develop goat meat production, it is necessary to increase the number of animals and to continue to popularize goat meat production in Latvia (Report on Agriculture ...). With increasing of living standards and increasing of economy, requirements for the qualitative livestock products have increased (Ni et al., 2017).

According to Teixeira et al. (2017) the most popular goat meat in the world is chivo meat from goats aged 6 to 9 months with a carcass weight of more than 11 kg. Nutritionally, goat meat is a source of high quality protein and fat, low in calories, intramuscular fat, saturated fatty acids and sodium (Horcada et al., 2012). The goat carcass fat content is low and mostly all parts of carcass are consumed (Webb, 2014).

Major goat rearing and feeding systems for kid meat production are extensive grazing and intensive rearing systems (Joy et al., 2008). Therefore, it is necessary to optimize productivity of goats with naturally available feed resources, and goat producers have to adopt new technologies and better feeding practices to improve the performance and yield of goats (Webb, 2014). The feeding system affects the animal's growth rate, carcass weight, dressing percentage, fat content, muscle / fat ratio, organoleptic properties of meat (Carrasco et al., 2009; 2010; Toplu et al., 2013). Feed rations with concentrates can affect various carcass characteristics as well as internal and carcass fat levels (Goetsch et al., 2011). The level of concentrate has few effects on meat quality, but the total level of fat in meat tissues is greater for the highest vs. intermediate dietary concentrate level. The level of subcutaneous fat is greater among diets with higher level of concentrated feed (Ryan et al., 2007). Including of protein crops into the goat compound feed results in a greater live weight gain, but also a greater accumulation of adipose tissue in the carcass (Aplocina et al., 2019). Limited nutrient intake increases lean meat yield and reduces fat deposition, regardless of animal sex.

All goat breeds, ages and genders have similar requirements for the main nutrients - protein, energy, minerals, vitamins and water. The diet should contain sufficient protein, as no other nutrient can replace it. Energy is provided by dietary carbohydrates (starch and crude fiber), fat and protein surplus. If the forage is low in protein, additional protein supplements should be provided. It has been found that the cheapest source of protein is high quality forage. If necessary, the feed for fattening may be supplemented with additional protein sources - soya beans, peas or beans, gluten feed, urea or other feedstuffs, but the effectiveness and economy of these feedstuffs must be evaluated (Feeding programs ...).

The objective of our study was to assess the efficiency of feeding different concentrated feed to the growth performance and carcass quality of Boer goat kids.

MATERIALS AND METHODS

The groups of kids were formed by selecting for each group kids that were born as twins (Table 1) with similar live weight after weaning and transferring them to the study site. Goat kids were given *ad libitum* access to water, salt block, hay and concentrated feed. Every day were accounted consumed feedstuffs. Four animals were housed together in pens equipped with elevated slatted floors, automatic waterers, grain mix and hay feeders.

Table 1. The research scheme

Group	Concentrated feed	Fattening period, days	Number of kids per pen
1st trial	Melted fodder beans (50%), barley (25%), oats (25%)	60	4
2nd trial	Pelleted commercial feed	60	4

The goat kids were weighed regularly with electronic scales and the depth of the dorsal muscle and adipose tissue was measured with the Mindray ultrasonograph at 13 ribs.

The following feed nutrient chemical parameters were established before the start of the trial according to generally accepted methods of analysis: dry matter (DM) according to ISO 6496:1999 method; neutral detergent fibre (NDF) according to LVS EN ISO 16472:2006; acid detergent fibre (ADF) according to LVS EN ISO 13906:2008; crude protein (CP) according to LVS EN ISO 5983-2:2009; fat (EE) according to ISO 6492:1999; starch according to LVS EN ISO 10520:2001, calcium (Ca) according to LVS EN ISO 6869:2002; phosphorus (P) according to ISO 6491:1998; but the net energy for lactation (NEL) was calculated based on the results of the analysis performed (Moe et al., 1976). The quality indicators for nutrients were determined by the accredited scientific laboratory of Biotechnologies of the Latvia University of Life Sciences and Technologies.

In the trial period goat kids were weighed at birth and at the age of 50 days and also at the beginning (91–92 days of age) and at the end (152–153 days of age) of fattening with electronic scales (accuracy of 0.01 kg). The absolute live weight gain per day of the period for the kids subject to the analysis was calculated by using the following formula (1):

$$a = \frac{W_t - W_0}{t} \quad (1)$$

where a – live weight gain per day, g; W_t – live weight at the end of the period, g; W_0 – live weight at the beginning of the period, g; t – the period, days.

At the end of the feeding period kids were held overnight without feed before slaughtering. Each goat kid was weighed before slaughtering to determine the slaughter live weight. Hot carcass weights were taken immediately after slaughtering and removal of non-carcass components. Carcasses were chilled for 24 h at 4°C. In 24 h *post mortem*, the carcasses were reweighed. Dressing percentage was computed as a proportion of the cold carcass weight to the slaughter live weight by using the following formula (2).

$$K = \frac{K_m}{W_k} \times 100 \qquad (2)$$

where K – dressing, %; W_k – live weight before slaughter, kg; K_m – carcass weight, kg

Each carcass was evaluated for conformation and fatness according to the SEUROP carcass classification system for lambs (Commission Regulation (EC) No. 1249/2008). Carcass was classified for conformation (scale from S = Superior (0) to P = poor (5)) and fatness (scale from 1 = traces of or no fat visible to 5 = very thick fat cover) according to the visual scores in the SEUROP system. After the evaluation of conformation and fatness, the length of the carcass and the circumference of hips were measured using a tape measure. The neck was then separated and the carcass was split lengthwise by a saw. The left side of the carcass was also separated into five prime cuts: shoulder blade with shoulder, hip with leg, ribs, flank, sirloin.

The cuts were weighed and expressed as a percentage of the total weight of the carcass. The *M. longissimus dorsi* (MLD) were carefully dissected from the left side sampled, weighted and the ribeye area (REA) was measured on a transparent plastic grid. The guidance for grid assessment of REA was based on a mathematical theorem for determining the area of a polygonal object located on a grid of equally spaced points, Pick's Theorem (Pick, 1899).

For the calculations of REA we used Pick (1899) theorem (3).

$$A = ID + \frac{BD}{2} - 1 \qquad (3)$$

where A – area, cm^2; ID – interior dots; BD – boundary dots.

Each cut was dissected into the components of meat, bone and fat. The dissection technique used for measuring muscle, bone, and fat composition was described by Colomer-Rocher et al. (1987). First, the muscle was individually removed from their attachment and then the external fat was removed. Then, muscle, fat, and bone were separated and weighed individually. After boning the carcasses, the tissue ratios were calculated: meat (muscle tissue + adipose tissue) and bone tissue, muscle tissue and bone tissue, muscle tissue and adipose tissue.

Using the data on the consumed feed and the obtained live weight gain, the economic efficiency of the use of concentrated feed was calculated.

Fattening performance and carcass parameters were analyzed with mathematical data processing methods using Microsoft Excel software. In all analyses, statistical significance was declared at $P < 0.05$. The physical data were subjected to analysis of variance (ANOVA). The results are reported as least square means (LSM) and the standard error of the mean (SEM).

RESULTS AND DISCUSSION

The evaluation of the results of the research was started with the analysis of the composition of the concentrated feed.

During the study, the 1st trial group kids were fed a cereal mix containing 50% beans, 25% oats and 25% barley as an energy source, whereas 2nd group kids received concentrated feed of pelleted commercial feed. The grain mix and pelleted feed were fed without restriction from loose troughs and hay was freely accessible throughout the fattening period.

The cereal mix has a higher content of net energy for lactation (NEL), protein, starch and Ca compared to commercially produced concentrated feed, while lower total crude fiber, neutral detergent fibre (NDF), acid detergent fibre (ADF) and total fats, but the difference isn't significant. The quality of hay is low, which is evidenced by the low protein (10.71%) and energy (5.55 MJ kg^{-1} DM) content and the very high NDF content (62.64%), which signifies that the grass forage consists mainly of perrenial grasses that have already been overgrown. In Table 2 we can see the feed nutrient intake per goat kid per day.

Table 2. Average feed nutrient intake per head per day

Indices	1st trial group			2nd trial group		
	Hay	Concentr. feed	Total	Hay	Concentr. feed	Total
The amount, kg	0.605	1.289	1.894	0.580	1.359	1.939
Dry matter (DM), kg	0.501	1.084	1.585	0.481	1.177	1.658
Crude protein (CP), g	53.7	206.3	260.0	51.5	232.1	283.6
NDF, g	314.1	185.9	500.0	301.3	331.6	632.9
ADF, g	195.5	104.8	300.3	184.2	226.6	401.8
NEL, MJ	2.8	8.5	11.3	2.7	8.3	11.0
Fats, g	-	23.5	23.5	-	29.6	29.6
Starch, g	-	485.7	485.7	-	311.6	311.6
Ca, g	2.7	24.9	27.6	2.5	13.5	16.0
P, g	1.2	5.0	6.2	1.15	5.7	6.85

Because of the unrestricted access to food, we can see that, during the entire fattening period, the animals in the 1st trial group ate slightly less concentrated feed than in group 2. Kids in the 2nd trial group consumed more protein (by 9.08%), fat (by 25.9%) and P (10.5%), while they consumed less starch (by 35.9%) and Ca (by 42.0%).

The analysis of the results of the study continued with growth rates, i.e. changes in the kids' live weight during the fattening.

The 1st trial group kids started fattening at the mean age of 92.7 days and mean live weight of 21.3 kg, while the 2nd group kids were on average by 1.2 days younger and 0.1 kg heavier, indicating that groups were very similar in terms of live weight and the age of the kid (Table 3).

Table 3. The age and live weight of kids at the beginning of fattening

Group	Age at beginning of fattening, days				Live weihgt at beginning of fattening, kg			
	$\bar{x} \pm S_{\bar{x}}$	min.	max.	V,%	$\bar{x} \pm S_{\bar{x}}$	min.	max.	V, %
1st trial	92.7 ± 0.75	92	95	1.6	21.3 ± 1.33	18.0	24.5	12.5
2nd trial	91.5 ± 1.65	87	95	3.6	21.4 ± 0.98	18.5	23.0	9.2

Where V, % – coefficient of variation; s_x – standard error of the mean.

The results in Table 3 show that the kids in both groups were age-adjusted and weight-adjusted at the start of the study - the coefficient of variation is 1.6–12.5%. At the start of the trial, the adaptation period of 10 days was provided to allow the kids to get used to the new housing and feeding conditions.

Kid's age and live weight before slaughter are summarized in Table 4.

Table 4. The age and live weight of kids before slaughter

Group	Age before slaughter, days				Live weihgt before slaughter, kg			
	$\bar{x} \pm S_{\bar{x}}$	min.	max.	V,%	$\bar{x} \pm S_{\bar{x}}$	min.	max.	V, %
1st trial	152.8 ± 0.75	152	155	1.0	31.1 ± 2.07	25	34	13.3
2nd trial	151.5 ± 1.66	147	155	2.2	35.5 ± 1.54	31	37	8.7

Where V, % – coefficient of variation; s_x – standard error of the mean.

The first group kids were by 1 day older and 4.38 kg lighter than kids in the 2nd group. At the begginning of the trial, goat kids in both groups had nearly the same live weight, but after fattening with a supplement of commercial concentrated feed, the body weight in group 2 was by 14.1% higher than in cases when fattening was achieved with grain mix, but there was no significant difference ($P > 0.05$).

The fattening period of goat kids in both groups was 60 days, during which the daily live weight gain was 165 and 235 g, respectively (Table 4). Daily live weight gain for 60 days of the fattening period was by 42.4% greater in the 2nd group kids than in the 1st group kids, and this difference was significant ($P < 0.05$).

Table 5. Average kid's live weight and live weight gain (± s_x)

Group	Birth live weight, kg	Live weight at 50th day, kg	Live weight gain from birth till 50th day., g per day	Live weight gain until the end of fattening, g per day	Live weight gain during fattening, g per day
1st group	3.7 ± 0.25	13.0 ± 0.72	186 ± 15.3	179 ± 14.3	165^a ± 21.3
2nd group	3.9 ± 0.36	13.3 ± 0.78	186 ± 10.6	208 ± 9.5	235^b ± 13.3

Where s_x – standard error of the mean; a, b – signifficant differences ($P < 0.05$).

According to Table 5 the first group kids had a 0.22 kg lower birth weight than the kids of the 2nd group. In the 1st group, one kid was born alone, but all other kids in both groups were born as twins. Goat kids for the trial were purchased from a farm that is engaged in breeding of meat goats, but as this is a relatively new livestock breeding sector in Latvia, the farm has Boer goats with different crossing level. In Latvia, only 10 male goats and 34 female goats are pure-bred animals (https://www.ldc.gov.lv/upload/doc/BK_15112019_K.PDF).

For both goups kids crossing level is 66.02–90.6%. The daily increase in live weight up to the age of 50 days is 186 g per day. Boer goat fertility should be 180–200%, and live weight gain till 50 days of age should be 160–220 g per day (https://www.ldc.gov.lv/upload/doc/BK_15112019_K.PDF). As can be seen from the data in Table 5, the farm should focus on feeding mother goats in order to achieve a better fasting of kids during the first 50 days. During the fattening period the weight gain reached 165 to 235 g per day ($P < 0.05$). These results are higher compared to other reported research where average daily gain for Boer goats during *ad libitum* feeding reached only 147 g per day per animal (Solaiman et al., 2011).

After slaughtering, the resulting carcass weight and dressing was calculated (Table 6).

Table 6. Means (± S.E.) of carcass measurements

Group	Dressing, %	Carcass length, cm	Hip circumference cm	Body fat thickness, mm	Thickness of dorsal muscle, mm	Ribeye area, cm^2
1st group	49.5 ± 6.61	68.0 ± 2.33	54.0 ± 1.92	1.9 ± 0.21	24.3 ± 1.19	12.3 ± 1.09
2nd group	43.1 ± 4.20	70.0 ± 1.52	55.0 ± 0.41	2.4 ± 0.16	25.5 ± 0.71	13.1 ± 0.97

Dressing percentage can be calculated as the ratio between hot carcass weight and live weight (McGregor, 2012). For the 1st group kids dressing percentage ranged from 32.0 to 64.0%, while for the second study group it was from 38.5 to 55.7%. The results showed lower dressing percentage by 13.0% for the 2nd group kids, but the differences were not significant. Although there were differences in the thickness of the adipose tissue, in the thickness of the muscle tissue and in the size of the ribeye muscle area, no significant differences were found ($P > 0.05$). McMillin et al. (2013) found that the average dressing percentage of goats is 48%, but other researchers found it in the range of 44.2–57.0% (Gurung et al., 2009; Kadim et al., 2003). Dressing percentage can have a large variation due to many factors including muscling, fat thickness, mud or debris on the hide, gut fill, amount of bone and horns (Schweihofer, 2011). According to Ruvuna et al. (1992) dressing percentage can increase with age. Intensive feeding can also increase the dressing percentage (Ryan et al., 2007). In our research carcasses weighing on average 14.36 kg (1st group) to 14.50 kg (2nd group) were obtained, and this difference isn’t significant.

Overall, three carcasses in each group were graded O class and one carcass in each group was graded P class. The fat cover is a very important indicator for kid carcasses, because the quality of meat during storage depends on how much subcutaneous fat is present. If the fat in carcasses is low, storing and freezing may be difficult as the carcasses are damaged by dehydration. In the present study values of fat thickness and *Longissimus* muscle area are higher than in the studies of other researchers (Owen & Norman, 1977; Solamain et al., 2011). The carcass fat content is quite varying and depends on goat breed, age, sex, nutrition, live weight, physiological condition (Kirton, 1988). Visually the whole carcasses did not show differences to fat cover, but it should be noted that the carcass fat in the 1st group were notably yellow, and any tint could influence the consumers' choice when buying meat.

In view of the interests of consumers, in the market most carcasses are cut into cuts and then sold at a different price. The most valuable carcass cuts are hips, sirloin and ribs, which are classified by meat consumers in the ‘Extra’ category, while less valuable parts are either in category 1 – shoulder blade and shoulder or category 2 – flank and neck (Colomer-Rocher et al., 1987, Table 7).

According to previous findings (Aplocina et al., 2019), half carcass weight is quite similar despite the fact that kids in the present trial were by 57–62 days younger. The proportion of the most valuable and expensive parts is basically the same for the carcasses of kids in both groups, representing 70.9% to 71.4% of the weight of a half carcass. The least valuable part - flank - formed a bigger part in the carcass in the 1st group.

The results and ratios of the parts of the carcass tissue are shown in Figure 1. In respect of muscle tissue that is highly demanded by customers the highest carcass yield was obtained from the 2nd group carcasses, 55.8% on average, indicating an increased tendency for muscle formation in kids that are fed with commercially produced feed. This concentrated feed has a high fat content that gives a kid more nutritional energy during fattening, which is beneficial for the kid's growth and fattening performance. The largest proportion of low-value bone tissue - 32.4% - was obtained from the 1st group carcasses. Values reported for the proportion of muscles in the carcass in the present study are slightly lower (Aplocina et al., 2019) or consistent with the findings of other researchers, but the proportion of fats is significantly lower than in the studies of other researchers (Solamain et al., 2011; Aplocina et al., 2019).

Table 7. Carcass parts

Carcass parts	1st group		2nd group	
	kg	%	kg	%
Half carcass	7.12	100	6.85	100
Hip + leg	2.14	30.2	2.25	32.9
Flank	0.57	7.8	0.45	6.5
Sirloin	0.42	5.9	0.41	6.0
Ribs + chest vertebrae + back vertebrae	2.48	34.8	2.23	32.5
Shoulder blade + shoulder	1.51	21.3	1.51	22.1

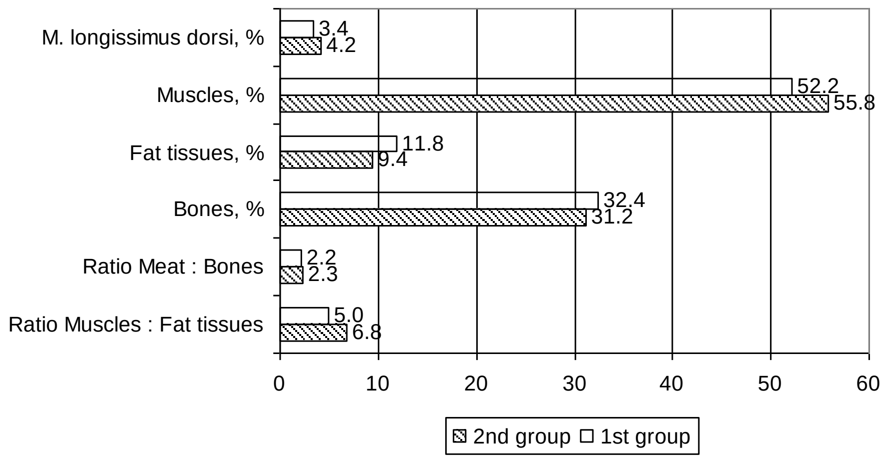

Figure 1. Carcass tissue parts and the ratio.

The meat-to-bone ratio was the same for both groups of kids, with 2.2 to 2.3 kg of meat per kg of bone. However, in terms of the muscle-to-fat ratio, the carcasses of both groups were significantly different ($P < 0.05$). Compared to our previous research, where goat kids received restricted amount of concentrated feed (Aplocina et al., 2019), the meat-to-fat ratio in the present study is significantly higher, which means that carcasses had less fat and much more lean meat. It is the result of *ad libitum* feeding of concentrated feed at the fattening period.

During the fattening of kids, the consumed concentrated feed and hay were recorded. The amount and economical effect of feeding during the fattening is summarized in Table 8.

Table 8. Feedstuffs consumed during the study

Indices	1st group		2nd group	
	Amount, kg	Cost, EUR	Amount, kg	Cost, EUR
Concentrated feed per day per animal	1.289	0.31	1.359	0.49
Concentrated feed per 1kg of ADG	7.83	1.88	5.77	2.08
Hay per day per animal	0.605	0.04	0.580	0.03
Hay per 1kg of ADG	3.67	0.22	2.46	0.15
Total daily feed costs per animal, EUR	0.35		0.52	
Total feed costs per 1 kg ADG, EUR	2.10		2.23	

Where ADG – Average daily gain.

The results show that the kids in the 2nd group used more concentrated feed per day per kid than the kids in the 1st group, and the cost of consumed concentrated feed per day by one kid was 58.1% higher than in the 1st group. Conversely, the amount of concentrated feed consumed per kg of live weight gain was lower in the 2nd group kids, but also in this case the cost of feed per kg of live weight gain was by 0.20 euros or 10.6% higher than in the 1st group group animals. The amount of hay consumed per animal per day did not differ between groups. The total feed costs per animal per day and per kg of live weight gain were higher in the 2nd group, as it is known that commercially produced feed has higher price than that of grain mix.

CONCLUSIONS

Feed rations with *ad libitum* concentrates affected various carcass characteristics as well as carcass fat level. During the whole fattening period, the feed intake was slightly less in the 1st group. The 2nd group kids consumed more protein (9.08%), fat (25.9%) and P (10.5%), while they consumed less starch (by 35.9%) and Ca (by 42.0%). Daily live weight gain of the 60 days fattening period was by 42.4% higher for the kids of the 2nd group that consumed commercially produced concentrated feed ($P < 0.05$). Feeding of grain mix resulted in a lower growth rate in the 1st group kids (31.1 kg and 35.5 kg pre-slaughter live weight, respectively), but kids of the 1st group have a higher dressing rate (49.5% and 43.5%, respectively). Consequently, there is no significant difference in carcass weight (14.36 kg and 14.50 kg, respectively). Carcasses in the 1st group had a higher fat content. In terms of muscle-to-fat ratio, the carcasses of kids in both groups were significantly different ($P < 0.05$), with 5.0 kg of muscle tissue per 1 kg of fats in the 1st group and 6.8 kg muscles in the 2nd group. Total feed costs per animal per day and per 1 kg of live weight gain were higher in the 2nd group, as commercially produced concentrated feed has a significantly higher price than grain mix feed. As there is no difference in obtained carcass weight and consumers ultimately pay for the amount of meat, not quality of meat, farmers can decide which feedstuffs are more suitable and profitable for fattening of goat kids.

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REFERENCES

Aplocina, E. & Degola, L. 2019. Effect of concentrate supplementation on fattening performance and carcass composition of finished meat–goat kids. *Agronomy Research* **17** (S2), 1273–1286.

Carrasco, S., Ripoll, G., Sanz, A., Alvarez-Rodriquez, J., Panea, B., Revilla, R. & Joy, M. 2009. Effect of feeding system on growth and carcass characteristics of Churra Tensina light lambs. *Livestock Science*. **121**, 56–63.

Colomer-Rocher, E., Morand-Fehr, P. & Kirton, A.H. 1987. Standard methods and procedures for goat carcass evaluation, jointing and tissue separation. *Livestock Production Science* **17**, 149–159.

Commission Regulation (EC) No 1249/2008. Available https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:337:0003:0030:EN:PDF

FAOSTAT. 2019. Available http://www.fao.org/faostat/en/#home

Feeding programs for meat goats. By Pinkerton F. and Pinkerton B. http://www.goatworld.com/articles/nutrition/feedingprograms.shtml.

Food and Agriculture Organization of the United Nations (FAO), 2017. Available: http://www.fao.org/faostat/en/#data/QL

Goetsch, A.L., Merkel, R.C. & Gipson, T.A. 2011. Factors affecting goat meat production and quality. *Small Ruminant Research* **101**, 173–181.

Gurung, N.K., Solaiman, S.G., Rankins, D.L. & McElhenney, W.H. 2009. Effects of distillers dried grains with solubles on feed intake, growth performance, gain efficiency and carcass quality of growing Kiko x Spanish male goats. *J. Anim. Vet. Adv.* **8**(10), 2087–2093.

Horcada, A., Ripoll, G., Alcalde, M.J., Sanudo, C., Teixeira, A. & Panea, B. 2012. Fatty acid profile of three adipose depots in seven Spanish breeds of suckling kids. *Meat Science* **92**, 89–96.

ISO 10520:2001. Native starch. Determination of starch content. ISO/TC93, 8 pp.

ISO 13906:2008. Animal feeding stuffs. Determination of acid detergent fibre (ADF) and acid detergent lignin (ADL) contents. ISO/TC34/SC10, 17 pp.

ISO 16472:2006. Animal feeding stuffs. Determination of amylase-treated neutral detergent fibre content. ISO/TC34/SC10, 15 pp.

ISO 5983-2:2009. Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content. ISO/TC34/SC10, ICS: 65.120, ed. 2. part 2, 15 pp.

ISO 6491:1998. Animal feeding stuffs. Determination of phosphorus content. ISO/TC34/SC10, 7 pp.

ISO 6492:1999. Animal feeding stuffs. Determination of fat content. ISO/TC34/SC10, 9 pp.

ISO 6496:1999. Animal feeding stuffs. Determination of moisture and other volatile matter content. ISO/TC34/SC10, 7 pp.

ISO 6869:2002. Animal feeding stuffs. Determination of calcium, magnesium and sodium. ISO/TC34/SC10, ed.1,15 pp.

Ivanovic, S., Pavlovic, I. & Pisinov, B. 2016. The quality of goat meat and it's impact on human health. *Biotechnology in Animal Husbandry* **32**(2), 111–122.

Joy, M., Ripoll, G. & Delfa, R. 2008. Effects of feeding system on carcass and non-carcass composition of Churra Tensina light lambs. *Small Ruminant Research* **78**, 123–133.

Kadim, I.T., Mahgoub, O., Al-Ajmi, D.S., Al-Maqbaly, R.S., Al-Saqri, N.M. & Ritchie, A. 2003. An evaluation of the growth, carcass, and meat quality characteristics of Omani goat breeds. *Meat Sci.* **66**, 203–210.

Kirton, H. 1988. Characteristics of goat meat, including carcass quality and methods of slaughter. In: *Goat Meat Production in Asia. Proc. of a Meat Workshop.* Tando Jam, Pakistan, pp. 13–18.

McGregor, B.A. 2012. The role of objective and subjective evaluation in the production and marketing of goats for meat. In: Mahgoub, O.I.T. & Kadim, E.C. Webb (eds): *Goat Meat Production and Quality*. CAP international, Cambridge, MA. p 212.

McMillin, K., Gregorie, J., Braden, K., Cope, R. & Pinkerton, F. 2013. Live animal and carcass measurements of meat goats: A USDA National Institute of Food and Agriculture project. *Proc 28th Ann. Goat Field Day*. Langston University. Langton, OK. pp. 14–16.

Moe, P.W. & Turrell, H.F. 1976. Estimating metabolizable and net energy of feeds. In: *Proc. 1st Intern. Symp. on Feed Composition, Animal Nutrient Requirement and Computerization of Diets.* Utah State University, pp. 232–237.

Ni, K.K., Wang, F.F., Zhu, B.G., Yang, J.X., Zhou, G.A., Pan, Y. & Zhong, J. 2017. Effects of lactic acid bacteria and molasses additives on the microbial community and fermentation quality of soybean silage. *Bio resource Technology* **238**, 706–715.

Owen, J.E. & Norman, G.A. 1977. Studies on the meat production characteristics of Botswana goats and sheep – Part II: General body composition, carcass measurement sand joint composition. *Meat Sci.* **1**, 283–306.

Report on Agriculture 2014. https://www.zm.gov.lv/public/files/CMS_Static_Page_Doc /00/00/00/63/66/LS_gadazinojums_2015.pdf (in Latvian).

Ruvuna, F., Taylor, J.F., Okeyo, M., Wanyoike, M. & Ahuya, C. 1992. Effects of breed and castration on slaughter weight and carcass composition of goats. *Small Rumin. Res.* **7**, 175–183.

Ryan, S.M., Unruh, J.A., Corrigan, M.E., Drouillard, J.S. & Seyfert, M. 2007. Effects of concentrate level on carcas traits o fBoer crossbred goats. *Small Ruminant Research* **73**, 67–76.

Solaiman, S., Min, B.R., Gurung, N., Behrends, J. & Taha, E. 2011. Assessing feed intake, growth performance, organ growth, and carcass characteristics of purebred Boer and Kiko male kids fed high concentrate diet. *Small Ruminant research* **98**, 98–101.

Schweihofer, J.P. 2011. Carcass dressing percentage and cooler shrink. Native Plants and Ecosystem Services. https://www.canr.msu.edu/news/carcass_dressing_percentage_and_cooler_shrink.

Teixeira, A., Fernandes, A., Pereira, E., Manuel, A. & Rodrigues, S. 2017. Effect of salting and ripening on the physicochemical and sensory quality of goat and sheep cured legs. *Meat Science* **134**, 163–169.

Toplu, H.D.O., Goksov, E.O., Nazligul, A. & Kahraman, T. 2013. Meat quality characteristics of Turkish indigenous Hair goat kids reared under traditional extensive production system: effects of slaughter age and gender. *Tropical Animal Health and Production* **45**(6), 1297–1304.

Webb, E.C. 2014. Goat meat production, composition and quality. *Animal Frontiers* **4**(4), 33–37

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Experimental Analysis of IoT Based Camera SI-NDVI Values for Tomato Plant Health Monitoring Application

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Abstract. This paper reveals an IoT based camera design to capture SI-NDVI parameters and describes first obtained data analysis regarding luminary spectrum impact on readings in real greenhouse application. For experimental comparison, measurements of Encore, Strabena, Audiance, Bolzano, Forticia and Chocomate tomato plants, both for the 'best' and the 'weakest' plant sample, using IoT based camera solution and portable leaf spectrometer. First experimental results show that this approach can be applied for tomato plant monitoring, and reveals some ideas about possible precision improvements.

Key words: SI-NDVI, tomato plant, industrial greenhouse, precision agriculture, IoT.

INTRODUCTION

Industrial greenhouses are large production buildings, having a lot of crops inside, where vegetation process is determined by natural and artificial light parameters, nutrition quality, indoor and outdoor climate parameters and many other factors. Normalized Difference Vegetation Index (NDVI) can be an important remote measurement in agriculture as it has a high correlation with plant growth and yield result. Currently, measurement methods, like chlorophyll Fluorescence and Multispectral imaging, are used to obtain many photobiological parameters done by experienced personnel. Greenhouse automation asks for more automated quality parameter measurements for feedback, and nowadays systems with Internet of Things (IoT) become more and more popular for such applications. Also non-destructive or non-contact sensing techniques of plant stress or 'health' status would enable continuous monitoring of plants and enable automated feedback for greenhouse control systems. A good overview of crop reflectance monitoring as a tool for water stress detection in greenhouses is given by (Katsoulas et al., 2016), it also reveals that most common approach is to use photochemical reflectance index (PRI) and the normalised difference vegetation index (NDVI) parameters, however it deals with precision measurement equipment. Spectral reflectance by vegetation (Botha, 2001) in ranges of 0.4 μm to 2.5 μm can indicate healthy, stressed or severely stressed vegetation, by using leaf spectrometer data. Also (Kharat et al., 2016) analyses the normalized difference vegetation index (NDVI), using the normalized ratio of red reflectance and near-infrared reflectance, as a vegetation index for tomato plant monitoring, indirectly also relating to nitrogen status. There are various literature sources on NDVI measurements as it is widely used metric for plants health monitoring, where healthy plants absorb light in the photosynthetically active and reflect at nearinfrared region. According to (Beisel et al., 2018) a single image NDVI (SI-NDVI) gives a new way to derive spectral character from a single RGB image, thus it can be applied to indoor greenhouses using artificial lighting such as LED. This is new technique, therefore this paper deals with practical application of developed IoT based Camera SI-NDVI data for tomato plant health monitoring and comparison to leaf spectrometer results in real tomato crop greenhouse.

MATERIALS AND METHODS

Description of experimental place

Experiments were conducted on 18.11.2019. in industrial greenhouse of Mezvidi, that grows several sorts of tomatoes (Encore, Strabena, Audience, Bolzano, Forticia, Chocomate) and therefore can be treated as a complex environment, as it has a relatively large growing area (5,062 sq. meters), height of 6 meters, 40 sections of tomato growing rows top-lighted by 1,760 pieces of Hellight Helturn 400 W high-pressure sodium vapour lamps, and uses GreenPower LED Interlighting module DR/B (115W) for tomato crop interlighting. More info about growing details can be seen in article (Rakutko et al., 2019), but due to industrial nature of the Mezvidi greenhouse, more detailed info is business sensitive. During the measurements only daylight and light from LED interlight modules were used. The tomato plants were grown already in production stage, thus this can be treated as a normal production process in greenhouse in autumn and winter start season. The proposed system and experiments is addition to the uMOL IoT sensor system described in previous articles (Avotins et. al., 2018), (Bicans et. al., 2019). Spectral measurements are used to determine nutritional quality of greenhouse vegetables (Olle & Alsina, 2019), but can be done only periodically and not every day.

Figure 1. Sample of measurements in Mezvidi industrial tomato greenhouse.

Equipment used for measurements

Leaf spectrophotometer CID BioScience Cl-710 - wavelengths 350–1,000 nm and spectroradiometer RS3500 (Spectral evolution) wavelengths 350–2,500 nm were used for non-destructive determination of physiological and biochemical status of plants (Alsina et al., 2019). The measurement process can be seen in Fig. 1, c, and the measurements were performed for the same leaf both with spectrometer (see Fig. 1, a) and focusing on the same spot also with the SI-NDVI camera (see Fig. 1, b). For these experiments SI-NDVI camera was mobile and powered from battery pack.

In our experimental setup we used proposed SI-NDVI calculation method and deployed 5 NDVI sensors for long term plant monitoring which consists of Raspberry Pi Foundation produced single-board computer Raspberry Pi 3 Model B with PiNoIR Camera V2 sensor (see Fig. 2).

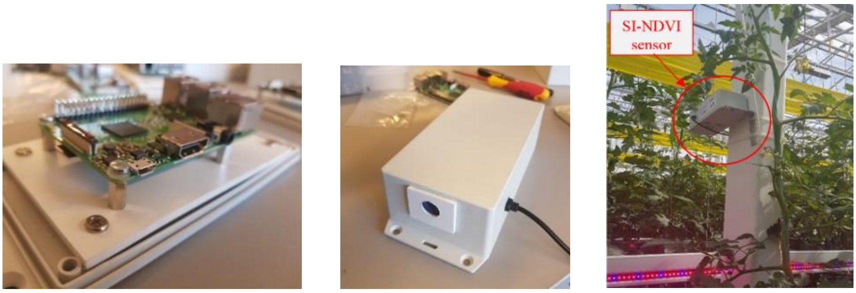


Figure 2. SI-NDVI sensor prototype and placement in the greenhouse.

Blue filter to the camera was added in order to meet SI-NDVI method requirements. SI-NDVI calculation method requires camera sensor with additional blue light filter which allow transmission in the 400–575 nm and 675–775 nm ranges. The SI-NDVI values were calculated according to common formula (1).

$$SI - NDVI = \frac{NIR - Blue}{NIR + Blue} \quad (1)$$

Developed experimental NDVI sensor principal circuit diagram (see Fig. 3) consists the following elements: RPi – Raspberry Pi 3 model B; NoIR cam – Raspberry Pi NoIR Camera v2 with blue filter; Plant – tomato plant; Internet – Wi-Fi Router that is connected to the internet; API – IoT Cloud endpoint; AC/DC – power supply; EN – Electrical Network. RPi is powered by an AC / DC network voltage adapter with an output voltage of 5V and a maximum current of 1A. SI-NDVI calculation program is written in Python 3.5.

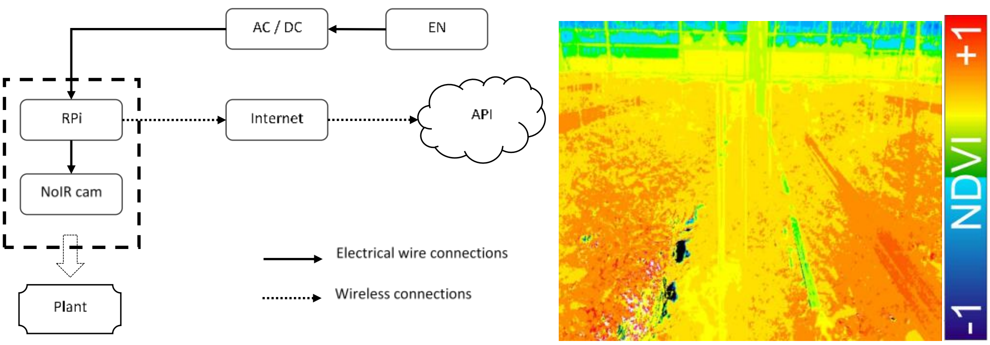


Figure 3. Architecture of the SI-NDVI sensor and IoT system and obtained picture.

Process of SI-NDVI value data obtaining in five steps is given in Fig. 4. In first step *.jpg format picture (640×480) is obtained remotely and saved on Raspbery device for further calculations and analysis. In step 2, the picture is split in three multispectral layers of R (red), G (green) and B (blue). As we have blue filter, NIR layer replaces the R layer. Thus three data matrix can be created, where we need only NIR and B matrix for further calculations by formula (1). Further we obtain a new matrix of SI-NDVI values (see Fig. 5) for each pixel of 640×480 resoltuion, that are further divided in 40 histogram intervals from -1 to +1 by step of 0.05.

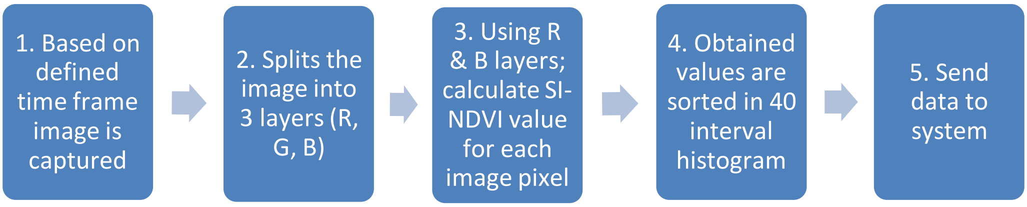
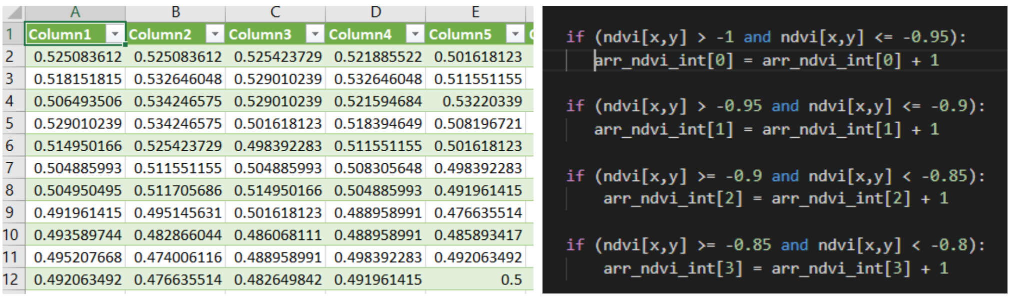


Figure 4. SI-NDVI value derivation process and initial calculation algorithm.



	A	B	C	D	E
1	Column1	Column2	Column3	Column4	Column5
2	0.525083612	0.525083612	0.525423729	0.521885522	0.501618123
3	0.518151815	0.532646048	0.529010239	0.532646048	0.511551155
4	0.506493506	0.534246575	0.529010239	0.521594684	0.53220339
5	0.529010239	0.534246575	0.501618123	0.518394649	0.508196721
6	0.514950166	0.525423729	0.498392283	0.511551155	0.501618123
7	0.504885993	0.511551155	0.504885993	0.508305648	0.498392283
8	0.504950495	0.511705686	0.514950166	0.504885993	0.491961415
9	0.491961415	0.495145631	0.501618123	0.488958991	0.476635514
10	0.493589744	0.482866044	0.486068111	0.488958991	0.485893417
11	0.495207668	0.474006116	0.488958991	0.498392283	0.492063492
12	0.492063492	0.476635514	0.482649842	0.491961415	0.5

```
if (ndvi[x,y] > -1 and ndvi[x,y] <= -0.95):
    arr_ndvi_int[0] = arr_ndvi_int[0] + 1

if (ndvi[x,y] > -0.95 and ndvi[x,y] <= -0.9):
    arr_ndvi_int[1] = arr_ndvi_int[1] + 1

if (ndvi[x,y] >= -0.9 and ndvi[x,y] < -0.85):
    arr_ndvi_int[2] = arr_ndvi_int[2] + 1

if (ndvi[x,y] >= -0.85 and ndvi[x,y] < -0.8):
    arr_ndvi_int[3] = arr_ndvi_int[3] + 1
```

Figure 5. SI-NDVI matrix (left) and histogram calculation algorithm (right).

Performed measurement and obtained results

Experimental measurements were performed on six sorts of tomato plants, where both leaf spectrometer measurements were done and also experimental prototype SI-NDVI readings with centred focus on same leaf. Measurements were taken for visually ‘weakest’ and ‘best’ (strongest) sample and in three levels of height: upper (close to flowers), middle and lower (close to fruits). Table 1 and Table 2 gives overview of samples taken and their allocation in the greenhouse.

Table 1. Samples taken from Encore, Starbena and Audiance

Total rows:	1–22		half of 23		half of 23	
Sort:	Encore		Strabena		Audiance	
Row:	16		Start of 23		End of 23	
Plant sample visual quality	Best	Weakest	Best	Weakest	Best	Weakest
Height level:	NDVI picture sample timestamp:					
Upper	11:40	11:48	11:58	12:07	12:17	12:27
Middle	11:37	11:50	12:01	12:09	12:19	12:30
Lower	11:45	11:53	12:03	12:12	12:22	12:32

Table 2. Samples taken from Bolzano, Forticia and Chocomate

Total rows:	24–26		37– half of 38		half of 38–40	
Sort:	Bolzano		Forticia		Chocomate	
Row:	25		Start of 38		39	
Plant sample visual quality	Best	Weakest	Best	Weakest	Best	Weakest
Height level:	NDVI picture sample timestamp:					
Upper	12:37	12:46	13:09	13:17	13:27	13:37
Middle	12:40	12:58	13:11	13:20	13:30	13:39
Lower	12:43	13:00	13:14	13:23	13:33	13:42

Figs 6–7 show reflectance readings of all sorts in TOP level sample array from weakest and strongest (best) sample. According to data, it seems that Bolzano sort is more stressed than the other sorts. For comparison, one not entirely whithered leaf with light green color was measured of unknown sort, and included in Figures. Fig. 8 shows Bolzano readings in all levels, both for the weakest and best sample. Logically it should have the worst readings in terms of stress, it can be seen from wavelengths centered about 450 nm and 670 nm, as chlorophyll doesnt absorbs energy so good as attached tomato leafs.

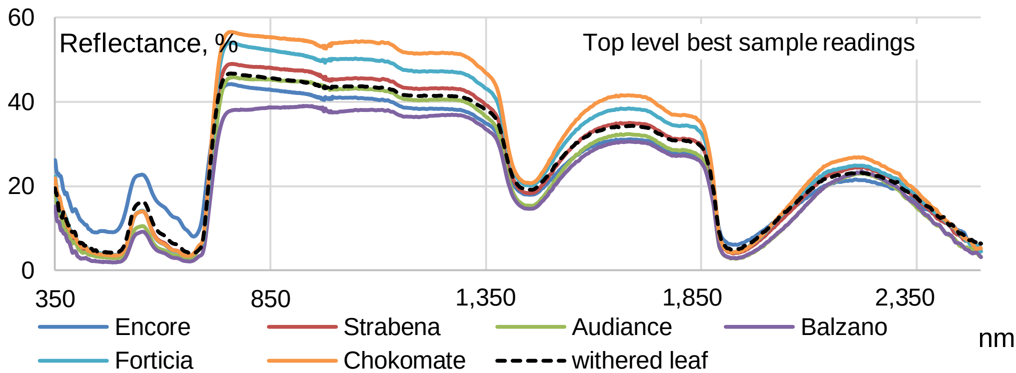


Figure 6. Reflectance values of Top level best sample of all tomato sorts.

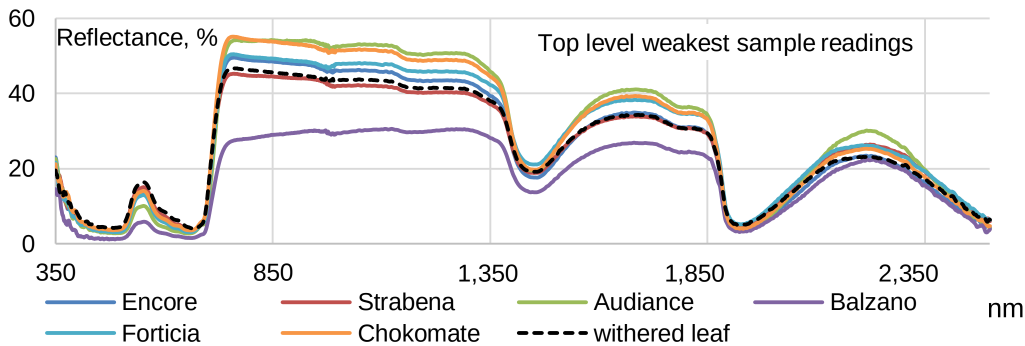


Figure 7. Reflectance values of Top level weakest samples of all tomato sorts.

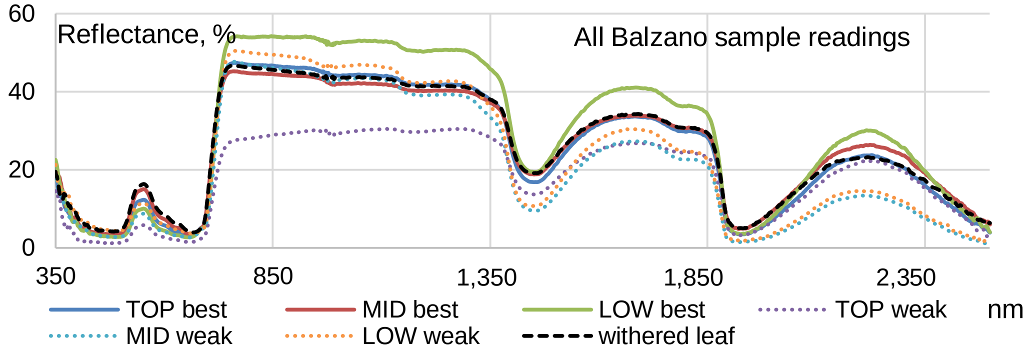


Figure 8. Reflectance values of all samples from Balzano tomato sort.

Table 3. NDVI values obtained from leaf spectrometer measurements for all tomato sorts and measurement levels

	NDVI	Leaf Chlorophyll Index		NDVI	Leaf Chlorophyll Index
Encore (best_TOP)	0.667	0.287	Encore (best_MID)	0.871	0.565
Encore (weak_TOP)	0.826	0.487	Encore (weak_MID)	0.838	0.568
Strabena (best_TOP)	0.826	0.469	Strabena (best_MID)	0.849	0.486
Strabena (weak_TOP)	0.818	0.427	Strabena (weak_MID)	0.841	0.462
Audiance (best_TOP)	0.821	0.539	Audiance (best_MID)	0.841	0.569
Audiance (weak_TOP)	0.852	0.590	Audiance (weak_MID)	0.841	0.586
Balzano (best_TOP)	0.821	0.539	Balzano (best_MID)	0.841	0.569
Balzano (weak_TOP)	0.852	0.590	Balzano (weak_MID)	0.841	0.586
Forticia (best_TOP)	0.823	0.490	Forticia (best_MID)	0.838	0.549
Forticia (weak_TOP)	0.827	0.471	Forticia (weak_MID)	0.854	0.511
Chokomate (best_TOP)	0.829	0.478	Chokomate (best_MID)	0.809	0.512
Chokomate (weak_TOP)	0.836	0.488	Chokomate (weak_MID)	0.807	0.470
Encore (best_LOW)	0.794	0.493	Balzano (best_LOW)	0.825	0.569
Encore (weak_LOW)	0.836	0.547	Balzano (weak_LOW)	0.816	0.559
Strabena (best_LOW)	0.848	0.514	Forticia (best_LOW)	0.812	0.475
Strabena (weak_LOW)	0.830	0.429	Forticia (weak_LOW)	0.835	0.577
Audiance (best_LOW)	0.825	0.569	Chokomate (best_LOW)	0.824	0.564
Audiance (weak_LOW)	0.816	0.559	Chokomate (weak_LOW)	0.803	0.491

NDVI values shown in Table 3 are calculated by formula (W800-W670)/(W800+W670), and Leaf Chlorophyll Index by formula (W850−W710)/(W850+W680). As it can be seen from the obtained data, NDVI values are ranging 0.79 to 0.85, with some exception of Encore (best sample at TOP level) with value of 0.667.

RESULTS AND DISCUSSION

Initial raw data calculations of proposed IoT camera based solution shows that pictures for the Encore, Audiance and Chokomate top (best sample) most pixels carry value of 0 to 0.25 (see Fig. 9. *'Encore Top Level', 'Audiance Top level' and 'Chokomate*

Top level'), which clearly is not the same as values obtained from leaf spectrometer measurements. As these calculations can be affected by picture quality, filter parameters, resolution and surrounding light spectrum (daylight and LED interlight), it can be assumed that correction coeficient must be applied to NIR value of calculation formula (1).

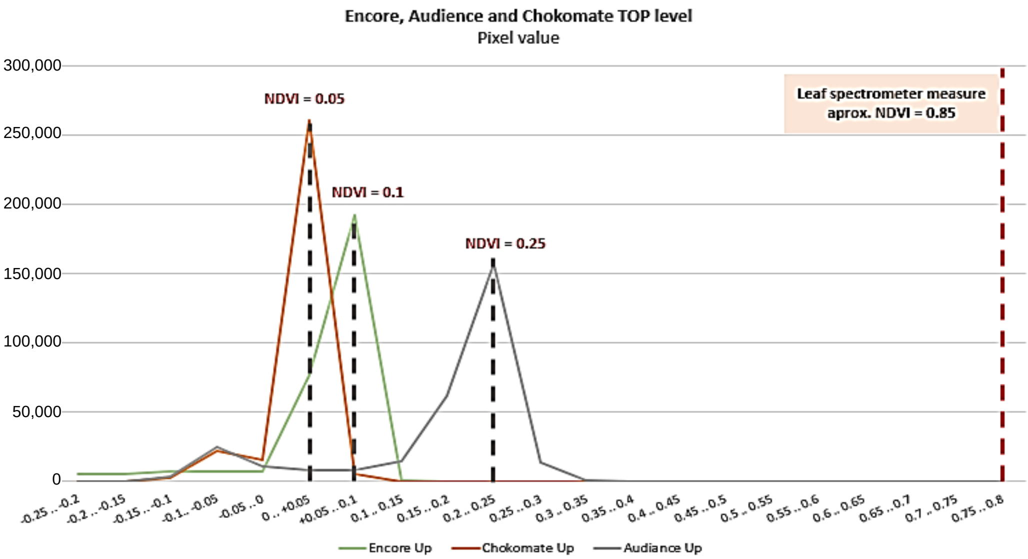


Figure 9. Obtained SI-NDVI histogram values without correction coefficient.

As we know that LED interlight module has one blue diode and four red diodes, we can assume that ratio could be 1:4, Based on this ratio, the settings are adjusted to take the necessary pictures, so the blue color gain is used 0.57 and the red chanel gain is used 2.26 (ratio 1:3.96).

Obtained new SI-NDVI histogram results (see Table 4) are shown in Fig. 10. where the blue areas are the raw measurements without the correction coeficient, whereas the red curve in each graph represents the readings after the coeficient adhibition. As can be seen in the following graphs, when applying the coefficient calculation method, the amount of values (peak values) deviates and is closer to the values which obtained by measuring with leaf spectrometer.

Table 4. NDVI values obtained from leaf spectrometer measurements for all tomato sorts and measurement levels

Level→	UP			MID			DOWN		
NDVI values	Spectr.	SI-NDVI	match, %	Spectr.	SI-NDVI	match, %	Spectr.	SI-NDVI	match, %
Encore	0.709	0.65	92%	0.896	0.65	73%	0.857	0.65	76%
Strabena	0.841	0.65	77%	0.854	0.65	76%	0.882	0.65	74%
Audiance	0.852	0.75	88%	0.875	0.65	74%	0.837	0.65	78%
Balzano	0.859	0.6	70%	0.858	0.65	76%	0.897	0.65	72%
Forticia	0.844	0.65	77%	0.832	0.65	78%	0.844	0.65	77%
Chokomate	0.855	0.65	76%	0.847	0.65	77%	0.859	0.65	76%

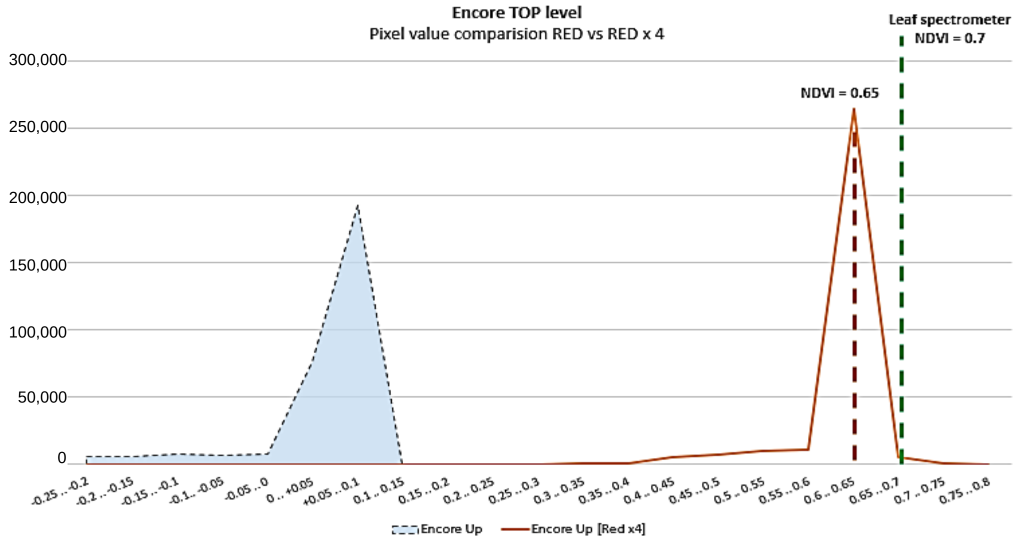


Figure 10. Obtained SI-NDVI value comparison with and without correction coefficient.

In our case we are not interested into photosynthesis activity in particular individual pixel of the image opposite to a satellite or an aerial imaging where each pixel represents area on the ground. We propose to use histogram data representation method. This method provides an accurate representation of the distribution of SI-NDVI numerical data, and seems to represent plants development tendency or processes.

If we look at the whole day measurments and to the solar raddation at the same time (see Fig. 11), then we obtain plants activity data when solar radiation is higher, at the period when lamps are swirched ON (around 2:00 UTCC time), dominant are values in the region +0.35 to + 0.6, showing that process is maintained, but not with high activity. This aproach must be studied further in more details, comparing data with plants behaviour.

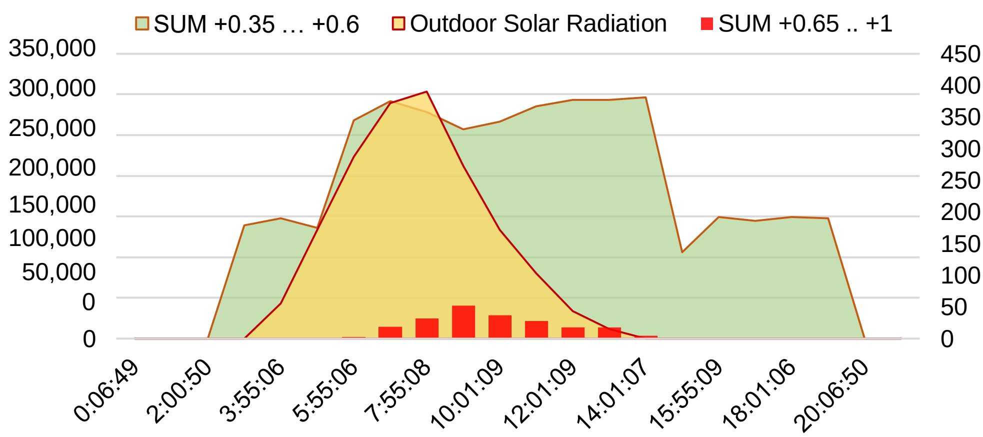


Figure 11. Obtained SI-NDVI value data from camera #1 with correction coefficient throughout whole day in the industrial Mezvidi tomato greenhouse.

CONCLUSIONS

It can be stated, that histogram approach of SI-NDVI sensor can be used as monitoring tool and indicate the daily NDVI value. From the single picture measurements, comparing them to the NDVI results measured by leaf-spectrometer, we obtained 73%–92% precision, if using the leaf-spectrometer result as reference value. After application of corrective coefficients, similar value pikes was obtained also for Encore, Strabena and others sorts.

Nevertheless the precision can be improved, by applying more precise correction coefficients. To determine its linear or non-linear nature is scope of the future research of the authors.

Furthermore obtained results shows potential application of this approach for controlling the greenhouse environment, same time monitoring the tomato plant growth quality parameters remotely and using long term hourly measurements. Data processing algorithm allows to get numerical values that are also comparable to those obtained by leaf spectrometer and calculated from certain wavelength data reflectance values.

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REFERENCES

Alsina, I., Dubova, L., Duma, M., Erdberga, I., Avotiņš, A. & Rakutko, S. 2019. Comparison of lycopene and β-carotene content in tomatoes determined with chemical and non-destructive methods. *Agronomy Research* **17**(2), 343–348.

Avotins, A., Apse-Apsitis, P., Bicans, J. & Gruduls, J. 2018. Development and Testing Results of IoT Based Air Temperature and Humidity Measurement System for Industrial Greenhouse. *Agronomy Research* **16**(A1), 943–951. https://doi.org/10.15159/AR.18.141

Beisel, N.S., Callaham, J.B., Sng, N.J., Taylor, D.J., Paul, A.-L. & Ferl., R.J. 2018. Utilization of single-image normalized difference vegetation index (SI–NDVI) for early plant stress detection. *Applications in Plant Sciences* **6**(10): e1186.

Bicans, J., Kviesis, K. & Avotins, A. 2019. IoT Camera-based Approach to Capture and Process SI-NDVI Sensor Data for Industrial Tomato Greenhouse, *2019 IEEE 7th IEEE Workshop on Advances in Information, Electronic and Electrical Engineering (AIEEE)*, Liepaja, Latvia, 2019, pp. 1–6.

Botha, E.J. 2001. Estimating nitrogen status on crops using non destructing remote sensing technique. Republic of South Africa. Academic Press. Inc. pp. 1–10.

Katsoulas, N., Elvanidi, A., Ferentinos, K.P., Kacira, M., Bartzanas, T. & Kittas, C. 2016. Crop reflectance monitoring as a tool for water stress detection in greenhouses: *A review, Biosystems Engineering* **151**, pp. 374–398, ISSN 1537–5110.

Kharat, R.B. & Deshmukh, R.R. 2016. Analysis of Effective Leaf Nitrogen Concentrations in Tomato Plant using Vegetation Indices, *International Journal of Engineering Science and Computing*, August 2016, **6**(8), pp. 2397–2400.

Olle, M. & Alsina, I. 2019. Influence of wavelength of light on growth, yield and nutritional quality of greenhouse vegetables. *Proceedings of the Latvian Academy of Sciences*, Section B: Natural, Exact, and Applied Sciences, **73**(1), 1–9.

Rakutko, S., Alsina, I., Avotins, A. & Berzina, K. 2018. Manifestation of effect of fluctuating asymmetry of bilateral traits of tomato growing in industrial greenhouses. *Engineering for Rural Development* **17**, 186–191.

Agronomy Research **18**(S2), 1147–1153, 2020
https://doi.org/10.15159/AR.20.072

Sorghum dry biomass yield for solid bio-fuel production affected by different N-fertilization rates

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Abstract. The objective of this study was to examine the effect on the dry biomass yield of two dfferent sorghum hybrids (H1 and H2) under five different N-fertilization levels (0, 70, 140, 210 and 280 kg ha^{-1}) in a soil which was formed by lacustrine deposits of Karla Lake and is characterized from the downward movement of calcium carbonate from the surface horizons due to leaching (*Fluventic Xerochrept*) during 2017. The results demonstrated a significant effect ($P < 0.05$) of fertilization only for one hybrid. Biomass yield ranged from 22.2 to 37.5 t ha^{-1}. For both hybrids, sorghum accumulated a high amount of biomass in stems. Dry stem/total biomass ratio was rather constant throughout the different fertilization treatments achieving 81.6 and 77.5% for the first (H1) and the second hybrid (H2), respectively. The second hybrid (H2) had a higher percentage of leaf biomass (20.1 vs. 13.8%) than the first (H1), but lagged behind in seed production (2.4 vs. 4.6%). Biomass dry matter partitioning and total dry weight are important selection criteria for energy crops, due to different gross calorific value and ash content but also because of the different economic importance they may have e.g. the seed is also used as animal feed. The above high biomass yields of sorghum, confirming the high potential of this crop, should be taken into serious consideration regarding land use planning, but further investigation for the gross calorific value and the ash content is needed as well as biomass characteristics that are quite important in case to improve the combustion process.

Key words: biomass, sorghum, dry weight, stems, leaves.

INTRODUCTION

Nowadays countries all over the world are facing a problem due to the depletion of the conventional fossil fuel sources and the prediction of doubling the energy demand within a decade, and thus have raised concerns about unsure energy supply in the coming future (Rehman et al., 2013; Nurmet et al., 2019). Therefore, the interest of new environmental friendly energy sources, has increased as well as the development of new technologies, which are the main reasons for using biofuels (Vitazek et al., 2018).

Bioenergy production from biomass has an increased interest during the last decades. Today the most known and cultivated energy crop due to its high specific biomass yield, the growing knowhow of almost all farmers worldwide and the investigation on its breeding, is maize (*Zea mays* L.; Graß et al., 2015). Bioenergy production results to green environmental friendly energy. Therefore, sustainable biomass and bioenergy production systems should adapt to climate and care for the environment (Lobell et al., 2013; Semenov et al., 2014). Biomass is one of the most important sources of producing energy and synthetic fuels. Therefore, carbon-trading laws are good motivation for greater usage of biomass (Urbancl et al., 2019).

One of the crops that attracted worldwide attention during the last fifteen years is sorghum, which produces non-food feedstock, enhancing energy production while helping to reduce greenhouse gas emissions (Liu et al., 2015). There are many reports where sorghum is reported for its short growth cycle, the high water and nitrogen use efficiency, the low-input requirements (Stone et al., 2001; Farré & Faci, 2006¸ Ananda et al., 2011), the high soil-climatic adaptability (Teetor et al., 2011) and finally due to its C4 photosynthesis efficient the high biomass yield (Wortmann et al., 2010; Zegada-Lizarazu et al., 2012; Xu et al., 2018). According to its use sorghum can be classified in groups and one of them is the energy sorghum (Shakoor et al., 2014), which can further be divided in two categories i) the sweet and ii) the biomass sorghum (Rooney et al., 2007).

Energy crops must produce high biomass quantities (Sanderson & Wolf, 1995). Sorghum biomass may be a reasonable alternative energy crop because it could easily fit into existing production systems and it has high biomass production (Rocateli et al., 2012).

In order to increase biomass yield, farmers are applying higher amounts of nitrogen (Sheriff, 2005; Le Noë et al., 2017), which is one of the most important nutrients and it could increase sorghum biomass yield (Zhao et al., 2005; Almodares et al., 2008; Good & Beatty, 2011; Han et al., 2011; Sowiński & Głąb, 2018).

Nitrogen plays a crucial role in plant growth (Stals & Inze, 2001; Zhao et al., 2005; Saraswathy et al., 2007) and a deficiency of N results in lower sorghum biomass production due to reductions in LAI (leaf area index) and photosynthetic rate (Zhao et al., 2005). The need to maximize biomass yield for biofuel production makes nitrogen management research a priority.

There are a few studies where minimal or statistically insignificant effectsof increased N-dressings on sorghum biomass yield have been reported (Barbanti et al., 2006; Wortmann et al., 2010; Tamang et al., 2011; Erickson et al., 2012; Adam et al., 2015). Furthermore, it is reported (Erickson et al., 2012) that the optimum requirement based on yield and nutrient recovery responses is about 90–110 kg N ha^{-1}, while rates lower than the 80 kg N ha^{-1} are not affecting sorghum biomass (Wortmann et al., 2010).

Although in previous studies it is reported that N-fertilizers had significant effects on sorghum growth (Ayoubet al., 2003; Almodares et al., 2008), only few are known for different nitrogen application rates on sorghum biomass yield, especially for higher rates over the 150 kg of nitrogen per hectare.

The aim of this study was to identify the efficient nitrogen fertilizer application rates for sustainable energy sorghum cultivation in a soil characterized from the downward movement of calcium carbonate from the surface horizons due to leaching with focuses on improvement of dry biomass production yield.

MATERIALS AND METHODS

Two field experiments were established for the study in the main agricultural plain of Greece (Thessaly; Velestino area) to evaluate the effect of different nitrogen fertilization levels on two new hybrids (H1: EJ7281 and H2: ES5200) of energy sorghum yield in 2017. The experimental site is located at 39°02´ N and 22°45´ E (Velestino area;

Magnesia). Velestino soil was classified as *Calcixerollic Xerochrept*, according to USDA (1975). Soil analysis of a depth 0–40 cm showed average organic matter of 2.4%, pH 8.1, total N 0.2 mg kg^{-1}, available P and K, 5 and 197 mg kg^{-1} respectively.

Sowing took place on the 20^{th} of June (due to the fact that there was pea cultivation in the field which was incorporated as green manure) with sowing distances, 75 cm between rows and 8 cm on the row (according to the instructions of the production company for the hybrids).

Five different nitrogen fertilization levels were applied under 4 replications (blocks) for each tested hybrid. Plot size was 20 m^2 (5 m width × 4 m length), while the total plots per crop were 20 (5 N-fertilization levels × 4 blocks). The type of fertilizer that was used wasurea 46-0-0, while all plots were irrigated using a drip irrigation system.

Final biomass yield measured on final samplings (end of October for both hybrids), where the whole aerial biomass were cut 8–10 cm above ground. From the center lines of each plot was selected for cutting 1 meter (0.75 m^2) so as to avoid any border effect. The samples were weighed in the field and then a sub-sample was taken for further laboratory measurements and air drying. Thereafter, the dry samples sub-samples were weighed.

Complete weather data were recorded on a daily basis by an automated meteorological station, which was installed in the experimental farm of the University of Thessaly.

Finally, the statistical package GenStat (7^{th} Edition) was used for the analysis of variance (ANOVA) within sample timings for all measured and derived data. The $LSD_{0.05}$ was used as the test criterion for assessing differences between means (Steel & Torrie, 1982) of the main and/or interaction effects.

RESULTS AND DISCUSSION

Weather conditions

The study area is characterized by a typical Mediterranean climate with cold humid winters and hot-dry summers.

In particular, the average air temperature ranged from 21 °C to 27.4 °C during the summer 2017. Precipitation in the same period was 146.4 mm, while the 108 mm were observed during the second ten days of July (Fig. 1).

The best temperature for sorghum growth is 20–30 °C, while its base temperature is 13 °C (*Ferraris* and *Charles-Edwards*, 1986). Therefore during this field

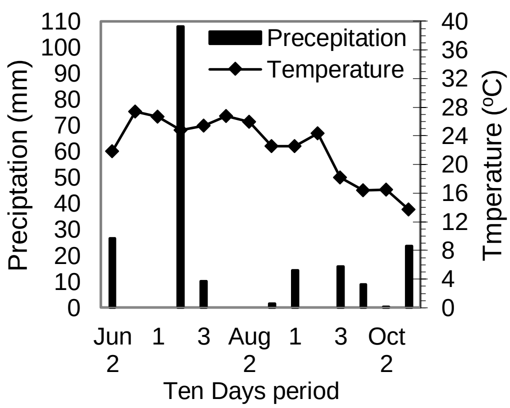

Figure 1. Temperature and precipitation (10-days mean values) occurring in studied site during the growing periods of sorghum in 2017.

study, mean temperature between 20 °C to 30 °C was consistently reached by June and maintained until almost end of September.

Biomass and seed yield

No statistical significant effects of nitrogen fertilization on total dry biomass yield for both tested sorghum hybrids were found (Tables 1, 2). Total dry yield was fluctuated between 22.2 to 37.5 t ha^{-1}, with the higher dry yield corresponding to the hybrid 2 under the higher N-fertilization level (280 kg N ha^{-1}), while the lower corresponded to the hybrid 1 without fertilization. Hybrid 1 had a negative effect of N-supply above the 210 kg N ha^{-1}, while hybrid 2 followed the principle the higher the nitrogen supply, the higher yield can be obtained indicating that hybrid 2 did not reach the potential biomass yield.

In both hybrids, sorghum accumulated a high amount of biomass in stems, while the stem/total biomass ratio was rather constant in each hybrid. Hybrid 1 produced higher stem yield than hybrid 2, which was affected from nitrogen fertilization (Table 1). This ratio achieved the 81.6 and 77.5%for the first (H1) and the second hybrid (H2), respectively. In the case of leaves the second hybrid (H2) had a higher percentage of leaf biomass (20.1 vs. 13.8%) than the first (H1). Petrova Chimonyo et al (2018) reported that the leaves yield at the harvest was the 30% of the total biomass, percentage higherthan found in the current study. Furthermore, it has been reported that in case of energy crops the gross calorific value of leaves is always lower comparing to the caloric value of the rest biomass (Gravalos et al., 2016) and thus the reduced biomass of leaves will lead to increased total calorific value for the studied hybrids. Hybrid 1 produced double the seed yield of hybrid 1 (1,400 vs. 720 kg ha^{-1}; Tables 1, 2), which can be used as animal feed.

Table 1. Effects of different N-fertilization levels (0, 70, 140, 210, 280 kg N ha^{-1}) biomass and seed yield of sorghum hybrid 1 (H1: EJ7281)

Fertilization	Total Dry Weight (t ha^{-1})	Dry Stem Weight (t ha^{-1})	Dry Leaves Weight (t ha^{-1})	Seed Weight (kg ha^{-1})
0	22.24	17.71	3.60	931
70	35.31	29.66	4.24	1,414
140	31.55	25.42	4.31	1,822
210	34.77	28.77	4.46	1,534
280	29.10	23.54	4.22	1,341
$LSD_{0.05}$	ns	9.072	ns	491.5
CV (%)	22.3	23.5	21.1	22.7

Table 2. Effects of different N-fertilization levels (0, 70, 140, 210, 280 kg N ha^{-1}) biomass and seed yield of sorghum hybrid 2 (H2: ES5200)

Fertilization (kg N ha^{-1})	Total Dry Weight (t ha^{-1})	Dry Stem Weight (t ha^{-1})	Dry Leaves Weight (t ha^{-1})	Seed Weight (kg ha^{-1})
0	25.98	19.88	5.27	830
70	24.17	18.51	5.08	580
140	25.28	19.41	5.25	620
210	29.26	23.01	5.67	580
280	37.44	29.28	7.17	990
$LSD_{0.05}$	ns	ns	ns	ns
CV (%)	22.3	23.5	21.1	22.7

The produced biomass yield is higher than the reported yield (Buxton et al., 1999; Regassa & Wortmann., 2014; Wannasek et al., 2017) and in agreement with previous reports for sweet sorghum (Zhao et al., 2009), biomass sorghum (Rooney et al., 2007) and forage sorghum hybrids (Venuto & Kindiger, 2008). The produced sorghum dry biomass of the unfertilized treatments in the current study agrees with the reported yield of 23 t ha^{-1} (Pannacci & Bartolini, 2018). In the case of hybrid 1, stems dry yield had been significant affected by nitrogen fertilization which has been reported in previous studies (Ayoub et al., 2003; Pholsen & Sornsungnoen, 2004; Pholsen & Suksri, 2007). Nitrogen fertilization (up to 140 kg ha^{-1}) had the same effect on sorghum dry biomass yield as with the reported effect to sunflower biomass (Skoufogianni et al., 2019).

CONCLUSIONS

The tested sorghum hybrids showed that high dry biomass yield can be produced even under low nitrogen fertilization or even without fertilization when pea cultivation is the previous one and has been used as green manure. Furthermore, a sufficient amount of seed yield could be produced and could boost animal feed production. A general conclusion could be that sorghum, should be taken seriously into consideration in land use planning, producing high dry biomass yields for solid biofuels, but further investigation of the gross calorific value and the ash content is needed.

REFERENCES

Adam, C.B., Erickson, J.E. & Singh, M.P. 2015. Investigation and synthesis of sweet sorghum crop responses to nitrogen and potassium fertilization. *Field Crops Res*. **178**, 1–7.

Almodares, A., Taheri, R., Chung, I.M. & Fathi, M. 2008. The effect of nitrogen and potassium fertilizers on growth parameters and carbohydrate contents of sweet sorghum cultivars. *J. Environ. Biol* .**29**, 849–852.

Ananda, N., Vadlani, P.V. & Prasad, P.V.V. 2011. Evaluation of drought and heatstressed grain sorghum (Sorghum bicolor) for ethanol production. *Ind. Crops Prod.* **33**, 779–782.

Ayoub, M., Nadeem, M.A., Tanveer, A. & Husnain, A. 2003. Effect of different levels of nitrogen and harvesting times on the growth: yield and quality of sorghum fodder. *Asian J. Plant Sci.* **1**, 304–307.

Barbanti, L., Grandi, S., Vecchi, A. & Venturi, G. 2006. Sweet and fiber sorghum (*Sorghum bicolor* (L.) Moench), energy crops in the frame of environmental protection from excessive nitrogen loads. *Eur. J. Agron.* **25**, 30–39.

Buxton, D.R., Anderson, I.C. & Hallam, A. 1999. Performance of sweet and forage sorghum grown continuously, double-cropped with winter rye, or in rotation with soybean and maize, *Agron. J.* **91**, 93–101.

Erickson, J., Woodard, K. & Sollenberger, L. 2012. Optimizing sweet sorghum production for biofuel in the Southeastern USA through nitrogen fertilization and top removal. *Bioenergy Res*. **5**, 86–94.

Farré, I. & Faci J.M., 2006. Comparative response of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench) to deficit irrigation in a Mediterranean environment. *Agric. Water Manage.* **83**, 135–143.

Ferraris, R. & Charles-Edwards, D.A. 1986. A comparative analysis of the growth of sweet and forage sorghum crops. I. Dry matter production, phenology and morphology. *Aust. J. Agric. Res.* **37**, 495–512.

Good, A.G. & Beatty, P.H. 2011. Fertilizing nature: a tragedy of excess in the commons. *PLoS Biol.* **9**, e1001124.

Graß, R., Thies, B., Kersebaum, K.C. & Wachendorf, M. 2015. Simulating dry matter yield of two cropping systems with the simulation model HERMES to evaluate impact of future climate change. *Eur. J. Agron.* **70**, 1–10.

Gravalos, I., Xyradakis, P., Kateris, D., Gialamas, T., Bartzialis, D. & Giannoulis, K. 2016. An Experimental Determination of Gross Calorific Value of Different Agroforestry Species and Bio-Based Industry Residues. *Natural Resources* **7**, 57–68.

Han, L.P., Steinberger, Y., Zhao, Y.L. & Xie, G.H. 2011. Accumulation and partitioning of nitrogen: phosphorus and potassium in different varieties of sweet sorghum. *Field Crops Res.* **120**, 230–240.

Le Noë, J., Billen, G. & Garnier, J. 2017. How the structure of agro-food systems shapes nitrogen, phosphorus, and carbon fluxes: the generalized representation of agro-food system applied at the regional scale in France. *Sci. Total Environ.* **586**, 42–55.

Liu, H., Ren, L., Spiertz, H., Zhu, Y. & Xie, G.H. 2015. An economic analysis of sweet sorghum cultivation for ethanol production in North China. *GCB Bioenergy* **7**, 1176–1184.

Lobell, D.B., Hammer, G.L., McLean, G., Messina, C., Roberts, M.J. & Schlenker, W. 2013. The critical role of extreme heat for maize production in the United States. *Nat. Clim. Chang.* **3**, 497.

Nurmet, M., Mõtte, M., Lemsalu, K. & Lehtsaar, J., 2019. Bioenergy in agricultural companies: financial performance assessment. *Agronomy Research* **17** (3), 771–782

Pannacci, E. & Bartolini, S. 2018. Effect of nitrogen fertilization on sorghum for biomass production. *Agronomy Research* **16**(5), 2146–2155.

Petrova Chimonyo, V.G., Modi, A.T. & Mabhaudhi, T. 2018. Sorghum radiation use efficiency and biomass partitioning in intercrop systems. *South African Journal of Botany* **118**, 76–84.

Pholsen, S. & Sornsungnoen, N. 2004. Effects of nitrogen and potassium rates and planting distances on growth, yield and fodder quality of a forage sorghum (*Sorghum bicolor* L. Moench). *Pak. J. Biol. Sci.* **7**, 1793–1800.

Pholsen, S. & Suksri, A. 2007. Effects of phosphorus and potassium on growth, yield, and fodder quality of IS 23585 forage sorghum cultivar (*Sorghum bicolor* L. Moench). *Pak. J. Biol. Sci.* **10**, 1604–1610.

Regassa, T.H. & Wortmann, C.S. 2014. Sweet sorghum as a bioenergy crop: literature review, *Biomass Bioenergy* **64**, 348–355.

Rehman, S.U.M., Rashid, N., Saif, A., Mahmood, T. & Han, J. 2013. Potential of bioenergy production from industrial hemp (*Cannabis sativa*): pakistan perspective. *Renew. Sust. Ener. Rev.* **18**, 154–164.

Rocateli, A.C., Raper, R.L., Balkcom, K.S., Arriaga, F.J. & Bransby, D.I. 2012. Biomass sorghum production and components under different irrigation/tillage systems for the southeastern U.S. *Ind. Crop. Prod.* **36**, 589–598.

Rooney, W.L., Blumenthal, J., Bean, B. & Mullet, J.E. 2007. Designing sorghum as a dedicated bioenergy feedstock. *Biofuel Bioprod. Biorefin.* **1**, 147–157.

Sanderson, M.A. & Wolf, D.D. 1995. Morphological development of switchgrass in diverse environments. *Agron. J.* **87**, 908–915.

Saraswathy, R., Suganya, S. & Singaram, P. 2007. Environmental impact of nitrogen fertilization in tea ecosystem. *J. Environ. Biol.* **28**, 779–788.

Semenov, M.A., Stratonovitch, P., Alghabari, F. & Gooding, M.J. 2014. Adapting wheat in Europe for climate change. *J. Cereal Sci.* **59**, 245–256.

Shakoor, N., Nair, R., Crasta, O., Morris, G., Feltus, A. & Kresovich, S. 2014. A Sorghum bicolor expression atlas reveals dynamic genotype-specific expression profiles for vegetative tissues of grain, sweet and bioenergy sorghums. *BMC Plant Biol.* **14**, 1–35.

Sheriff, G. 2005. Efficient waste? Why farmers over-apply nutrients and the implications for policy design. *Appl. Econ. Perspect. Policy* **27**, 542–557.

Skoufogianni, E., Giannoulis, K.D., Bartzialis, D. & Danalatos, N.G. 2019. Cost efficiency of different cropping systems encompassing the energy crop *Helianthus annuus*L. *Agronomy Research* **17**(6), 2417–2427.

Sowiński, J. & Głąb, L. 2018. The effect of nitrogen fertilization management on yield and nitrate contents in sorghum biomass and bagasse. *Field Crops Research***227**, 132–143.

Stals, H. & Inze, D. 2001. When plant cells decide to divide. *Trends Plant Sci.* **8**, 359–364.

Stone, L.R., Goodrum, D.E., Jaafar, M.N. & Khan, A.H. 2001. Rooting front and water depletion depths in grain sorghum and sunflower. *Agron. J.* **93**, 1105–1110.

Tamang, P.L., Bronson, K.F., Malapati, A., Schwartz, R., Johnson, J. & Moore-Kucera, J. 2011. Nitrogen requirements for ethanol production from sweet and photoperiod sensitive sorghums in the Southern High Plains. *Agron. J.* **103**, 431–440.

Teetor, V.H., Duclos, D.V., Wittenberg, E.T., Young, K.M., Chawhuaymak, J., Riley, M.R. & Ray, D.T. 2011. Effects of planting date on sugar and ethanol yield of sweet sorghum grown in Arizona. *Ind. Crop. Prod.* **34**, 1293–1300.

Urbancl, D., Krope, J. & Goričanec, D. 2019. Torrefaction – the process for biofuels production by using different biomasses. *Agronomy Research* **17**(4), 1800–1807.

Venuto, B. & Kindiger, B. 2008. Forage and biomass feedstock production from hybrid forage sorghum and sorghum-sudan grass. *Grassl. Sci.* **54**, 189–196.

Vitazek, I., Tulik, J. & Klucik, J., 2018. Combustible in selected biofuels. *Agronomy Research* **16** (2), 593–603.

Wannasek, L., Ortner, M., Amon, B. & Amon, T. 2017. Sorghum, a sustainable feedstock for biogas production? Impact of climate, variety and harvesting time on maturity and biomass yield. *Biomass Bioenergy* **106**, 137–145.

Wortmann, C.S., Liska, A.A., Ferguson, R.B., Lyon, D.J., Klein, R.N. & Dweikat, I.M. 2010. Dryland performance of sweet sorghum and grain crops for biofuel in Nebraska. *Agron. J.* **102**, 319–326.

Wortmann, C.S., Liska, A.J., Ferguson, R.B., Lyon, D.J., Klein, R.N. & Dweikat, I. 2010. Dryland performance of sweet sorghum and grain crops for biofuel in Nebraska. *Agron J.* **102**, 319–326.

Xu, Y.J., Li, J., Moore, C., Xin, Z.G. & Wang, D.H. 2018. Physico-chemical characterization of pedigreed sorghum mutant stalks for biofuel production. *Ind. Crops Prod.* **124**, 806–811.

Zegada-Lizarazu, W., Zatta, A. & Monti, A. 2012. Water uptake efficiency and above-and belowground biomass development of sweet sorghum and maize underdifferent water regimes. *Plant Soil.* **351**, 56–60.

Zhao, D., Reddy, K.R., Kakani, V.G. & Reddy, V.R. 2005. Nitrogen deficiency effects on plant growth, leaf photosynthesis and hyperspectral reflectance properties of sorghum. *Eur. J. Agron.* **22**, 391–403.

Zhao, Y.L., Dolat, A., Steinberger, Y., Wang, X., Osman, A. & Xie, G.H. 2009. Biomass yield and changes in chemical composition of sweet sorghum cultivars grown for biofuel. *Field Crops Res.***111**, 55–64.

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Impact of different fertilisers on elemental content in young hybrid aspen stem wood

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Abstract. The biomass production using fast-growing tree species such as hybrid aspen (*Populus tremuloides* Michx. *x Populus tremula* L.) has been recognized as an environmentally friendly and cost-effective approach. Growing these species can reduce the negative impact of earlier land mismanagement and at the same time provide additional biomass growth. The application of fertilisers may introduce not only the necessary macro elements (N, P, K) but also significant amounts of toxic heavy metals. Therefore, the knowledge about elemental flows from fertilised soil to the different parts of hybrid aspen trees is essential and especially meaningful for the evaluation of element content in specific environmental ecosystems. The impact of different fertilisers (sewage sludge, digestate and wood ash) on the concentrations of micro- and macro elements in the wood of six-year-old hybrid aspen stands grown on former agricultural land was studied. The determination of element concentrations in different tree rings of hybrid aspen trees was accomplished by inductively coupled plasma mass spectrometry (ICP–MS). Isotope ratio mass spectrometry (IRMS) was used to determine the nitrogen and carbon content and isotope ratios in different parts of hybrid aspen trees. Stem disc samples from hybrid aspen trees were obtained from agricultural land in the central part of Latvia. Samples were taken from six-year-old hybrid aspen trees that at the moment of planting were fertilised with sewage sludge, a residue of biogas production (digestate) and wood ash. The obtained results indicated that the chemical element accumulation in hybrid aspen was affected by the applied fertiliser type. In this study, the use of wood ash, as well as digestate, affected the elemental content in hybrid aspen to a greater extent than the use of sewage sludge, relative to unfertilised (control) subplot. The analysed elements varied in the analysed stem plane (across the tree rings). The most significant changes between the rings were observed for the content of K and Ca.

Key words: hybrid aspen, heavy metals, macro elements, ICP-MS, IRMS.

INTRODUCTION

Aspens are fast-growing trees with wide distribution. European (or Eurasian) aspen (*Populus tremula* L.) and North American quaking aspen (*P. tremuloides* Michx.) are among the most common tree species in Eurasia and North America (Tullus et al., 2020). Despite the wide distribution and high biodiversity of aspen trees, their economic value was low a few decades ago. However, the use of aspen and aspen hybrids has now significantly increased. The fast-growing aspen trees and their hybrids such as hybrid aspen (*Populus tremuloides* Michx. × *P. tremula* L.) are used for the production of pulp and energy wood (Zeps et al., 2015; Tullus et al., 2020). Previous studies indicate that the wood of hybrid aspen is suitable for high-quality paper production as well as for the production of wood chips, especially from the logging residues of such plantations (Smilga et al., 2015).

Moreover, the plantations of hybrid aspen are environmentally friendly because there is no need for annual tillage nor the use of mineral fertilisers, herbicides and pesticides. Growing of these species can reduce the negative impact on lands that have suffered from intensive management (agricultural practices, quarries etc.), while at the same time enhancing additional biomass growth in comparison to set-aside lands. Biomass is an essential renewable resource that provides a basis for the climate-neutral economy. For example, plantations have significant potential to compensate for CO_2 emissions in order to meet the European Union goals for carbon sequestration (Bardule et al., 2016; Fahlvik et al., 2019).

Plants can accumulate and store biologically essential elements as well as contaminants from the soil, thus acting as passive samplers. The approach of using plants for the remediation of polluted areas is considered environmentally friendly and inexpensive. Studies have shown that many plant species have great potential for accumulating heavy metals. This property of plants can be used for the stabilisation, phytoremediation and treatment of problematic soils (Mala et al., 2007; Mandre, 2014). Literature studies indicate that some clones of hybrid aspen can grow well in reclaimed surface mines and under the impact of elevated concentrations of industrially emitted gases and fly ash (Mandre, 2014). Nikula et al., (2011) investigated the growth and leaf traits of hybrid aspen growing close to a motorway in comparison to a control location and suggested that the species would be suitable for plantations in moderately polluted areas alongside roads carrying heavy traffic. Since hybrid aspen is one of the species characterized by fast growth even under bad climatic conditions, as well as capable of growing in poor soils heavily polluted with industrial waste, it has significant potential for phytoremediation (Mala et al., 2007; Mandre, 2014).

The growth of poplars and aspens especially at a young age is fast compared to conifers. Their rotation cycle often comprises only the maturation phase, and their tree ring archive contains hardly any non–juvenile rings (Meyer et al., 2018). Furthermore, the relatively wide aspen tree rings enable the analysing of the annual element concentrations in aspen wood. The tree ring samples are suitable for the analysis by advanced techniques like LA-ICP-MS, ICP-MS, and isotope ratio mass spectrometry (IRMS).

The aim of this study was to investigate the micro- and macro element content in hybrid aspen stem wood, and to evaluate the accumulation and distribution of different elements within aspen stem plane (across the tree rings).

MATERIALS AND METHODS

Stem disc samples of six-year-old hybrid aspen trees were obtained from an experimental plantation area that was part of a large-scale multifunctional plantation with a total area of about 16 ha in the central part of Latvia (56.6919 N, 25.1370 E). The hybrid aspen seedlings (clone No. 4) were produced in a nursery of the Joint Stock Company ‘Latvian State Forests’ were planted in the spring of 2011. The plantation density was 2.0×2.0 m between the trees. Short rotation energy crops and other deciduous trees were also grown in the plantation. According to the Food and Agriculture Organization of the United Nations classification (2006), the type of soil was *Luvic Stagnic Phaeozem* (*Hypoalbic*) or *Mollic Stagnosol* (*Ruptic, Calcaric, Endosiltic*) with predominantly loam and sandy loam soil texture at 0–20 cm depth and sandy loam soil texture at 20–80 cm depths. Four different types of fertilisation subplots were established in the plantation, each with four replications. The subplots were fertilised with sewage sludge, residue of biogas production (digestate), wood ash, and in one control subplot no fertiliser was applied. The subplots were established in the spring of 2011 and the size of each plot was 24×30 m (720 m^2). Class I sewage sludge (according to the regulations No. 362 of the Cabinet of Ministers of the Republic of Latvia) was obtained from the municipal wastewater treatment plant of ‘Aizkraukles ūdens’ (Aizkraukle Water). Stabilised wood ash was obtained from a boiler house in Sigulda and digestate was obtained from the methane reactor in Vecauce. Sewage sludge and wood ash were spread mechanically before planting the hybrid aspen trees, dosed at 10 t_{DM} ha^{-1} and 6 t_{DM} ha^{-1}, respectively. The digestate was applied immediately after planting the hybrid aspen seedlings as a point source fertiliser, dosed at 30 t ha^{-1}. The major nutrient content in the applied fertilisers is shown in Table 1.

Table 1. The major nutrient content in the applied fertilisers

Fertiliser	Origin	Dose	Application form	The input of major nutrients through fertilisation, kg ha^{-1}		
				N_{tot}	P_{tot}	K_{tot}
Wood ash	Boiler house, Sigulda	6 t_{DM} ha^{-1}	Mechanically	2.6	65	190
Digestate	Methane reactor, Vecauce	30 t ha^{-1}	Point source	69	1.2	99
Sewage sludge	Municipal wastewater treatment plant, ‘Aizkraukles ūdens’	10 t_{DM} ha^{-1}	Mechanically	259	163	22

Three samples were prepared from each of the subplots (fertilised with sewage sludge, digestate, wood ash, and control), from a total of 12 trees (Table 2). The hybrid aspen stem discs were sawn at a height of 20 cm above ground for the representation of all six tree rings in each of the samples. The discs had an approximate thickness of 2 cm. These sample discs were air-dried, and a trace metal free sandpaper was used for polishing the sample surfaces.

The sample amount of about 0.2 g was cut out from each of the tree rings and each sample was analysed separately. Microwave-assisted acid digestion was applied for the mineralisation of the samples. Each sample was weighed into a Teflon microwave digestion vessel and sequentially treated with 6 mL of 65% HNO_3 (Trace metal grade, Fisher Scientific) and 2 mL of concentrated 30% H_2O_2 (Trace metal grade, Fisher

Scientific). The vessels were closed, and the samples were heated in a Milestone Start E microwave oven (program: heating to 150 °C over 15 min and holding at 150 °C for 30 min). After the digestion program was completed, the vessels were cooled to room temperature. The samples were then diluted to 25 mL with deionised water (prepared by using Millipore water deionisation equipment). The reliability of the measurements was verified by using a certified reference material (CRM) BCR-060 - Aquatic plant (*Lagarosiphon major*), obtained from the Institute for Reference Materials and Measurements (IRMM), Belgium. The results of analysis for the CRM showed a good agreement with the certificate values (the differences did not exceed 10%).

Table 2. The characteristics of hybrid aspens from different subplots

Subplot	The average height of trees, m	The average mass of stem (fresh biomass), kg	The average mass of branches (fresh biomass), kg	Average diameter at 1.3 m height, mm
Control	9.1 ± 0.5	15.6 ± 1.7	4.0 ± 0.6	69.7 ± 3.5
Sewage sludge	9.3 ± 0.3	19.9 ± 2.0	6.4 ± 1.3	83.7 ± 3.9
Digestate	10.6 ± 0.5	27.8 ± 3.5	9.6 ± 1.1	90.7 ± 3.8
Wood ash	7.6 ± 0.7	12.0 ± 3.5	5.0 ± 2.0	66.0 ± 8.4

For the determination of element concentrations in the samples, standard procedures were used - an Agilent 8900 ICP-QQQ Inductively Coupled Plasma Mass Spectrometer (ICP-MS) equipped with a MicroMist nebulizer was applied for the determination of the following elements – Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Rb, Sr, and Zn. The following instrumental parameters of ICP-MS were set: RF power – 1.550 W, sampling depth - 8 mm, auxiliary gas flow - 0.90 mL min^{-1}, plasma gas flow – 15 L min^{-1}, He cell gas flow - 5 mL min^{-1}. All of the analytical standard stock solutions were TraceCert (Sigma-Aldrich) for ICP (100 mg L^{-1}). For the calibration graph, five different standard solutions in the concentration range from 0.1 µg L^{-1} to 50.0 µg L^{-1} were prepared from stock standard solutions. A calibration graph with blank correction was used for calculating the concentrations of elements in the samples. An Internal Standard Mix solution from Agilent Technologies (10 mg mL^{-1}) was used as internal standard. Stability check of the ICP-MS system was performed by using two standard solutions after every ten samples. A MassHunter workstation program with its Instrument control and Offline data analysis subprograms was used.

For the determination of $\delta^{15}N$ and $\delta^{13}C$, the samples were weighed into tin capsules (the weight of each sample was ∼1 mg) and then analysed in duplicate on an EA3000 elemental analyzer (EuroVector) coupled to a Nu-horizon continuous flow isotope ratio mass spectrometer (Nu Instruments). An internal standard sample of glutamic acid was used to check the reproducibility of stable isotope ratio determination. Certified reference materials USGS-40 and USGS-41 (L-Glutamic acid) were used. The $\delta^{15}N$ values were expressed in ‰ relative to air N_2 and $\delta^{13}C$ values in ‰ relative to VPDB (Vienna Pee Dee Belemnite).

The analysis of variance (ANOVA) was used to identify significant differences in element content between wood samples from the differently fertilised subplots. The F value was used as a criterion for the significance of differences, where differences were considered significant if $F > F_{crit}$ at 95% confidence level ($p \leq 0.05$).

RESULTS AND DISCUSSION

The abundance of 17 elements in hybrid aspen wood depending on the applied fertiliser type is shown in Table 3. K, Ca and P were the main elements measured in hybrid aspen during this study, varying from 1,100 to 1,300 mg kg^{-1}, from 830 to 920 mg kg^{-1}, and from 235 to 270 mg kg^{-1}, respectively. Statistical analysis of the obtained results showed that the applied fertilisers did not affect the average content of K, Ca and P in the hybrid aspen stem wood. The obtained results for K, Ca, P, and Mg in hybrid aspen stem wood were similar to those found in previous studies (Rytter and Stener 2003). Statistical analysis also showed that there were no significant differences in the content of Cr and Ni which are considered to be heavy metals. There were also very negligible changes in the content of Na. The highest increase in element abundance occurred in the samples collected from those subplots where wood ash was applied as fertiliser. For example, the highest content of Fe, Mg, Mn, Pb, Rb, and Sr was found in these samples. The use of digestate as fertiliser produced somewhat less change in element content than observed in the case of wood ash. Fertilisation with sewage sludge gave the lowest increase in element content in hybrid aspen stem samples, with the content of some elements, for example Al, Mg, Na and Zn even lower than in the control samples.

Table 3. Element concentrations in hybrid aspen stem wood from different fertilisation subplots (mean ± SD, mg kg^{-1}) (F_{crit} = 3.24, p = 0.05, significant differences between groups denoted with letters a, b, c, d)

Element	Control			Digestate			Wood ash			Sewage sludge			*F*
Al	3.8a	±	1.3	5.8b	±	0.7	11.8c	±	3.6	2.2d	±	0.3	22.8
B	47a	±	14	7.6b	±	1.9	6.0b	±	0.7	45a	±	7	42.3
Ca	880a	±	115	840a	±	92	917a	±	68	831a	±	107	0.8
Cd	0.76a	±	0.15	0.29b	±	0.04	0.36b	±	0.07	0.58a	±	0.20	13.6
Cr	0.3a	±	0.1	0.6a	±	0.4	0.4a	±	0.1	0.3a	±	0.1	2.7
Cu	3.5a	±	0.5	1.3b	±	0.6	1.4b	±	0.7	2.5ab	±	1.0	10.9
Fe	16a	±	3	14a	±	3	30b	±	7	10c	±	2	19.7
K	1117a	±	110	1183a	±	444	1066a	±	235	1286a	±	375	0.4
Mg	307a	±	25	270b	±	36	340c	±	14	290a	±	24	6.6
Mn	6.8a	±	0.1	13.0b	±	1.4	11.6b	±	0.8	8.6a	±	1.5	32.8
Na	45a	±	21	24a	±	2	34b	±	6	23ab	±	13	3.3
Ni	0.11a	±	0.04	0.09a	±	0.03	0.13a	±	0.04	0.09a	±	0.03	1.6
P	235a	±	27	238a	±	47	270a	±	11	235a	±	40	1.3
Pb	0.15a	±	0.04	0.22b	±	0.03	0.26b	±	0.06	0.13a	±	0.03	10.0
Rb	0.41a	±	0.03	0.36a	±	0.09	0.61b	±	0.08	0.53b	±	0.13	8.2
Sr	7.1a	±	1.1	10.7b	±	1.1	12.3c	±	1.1	9.5b	±	1.0	21.1
Zn	17.4a	±	1.4	13.8b	±	2.0	11.3b	±	2.0	11.1b	±	3.9	6.9

According to the experimental results, the element content of hybrid aspen stem wood samples increased in this order: control → sewage sludge → digestate → wood ash. This can be explained with the fertiliser production process. Sewage sludge was obtained from the municipal wastewater treatment plant of Aizkraukle, a small town with no heavy industry, where mainly domestic wastewater with low abundance of trace elements is treated. Also, the produced sewage sludge was not subjected to further treatment, so it had the highest ratio of organic matter to element content. Digestate was obtained by anaerobic treatment of biological materials which degrades organic matter, thus decreasing the ratio of organic matter to element content. Wood ash is practically free of organic matter, giving the lowest ratio of organic matter to element content.

It is possible to use dendrochemistry as a tool for detecting environmental changes that influence a tree over all of its life. Conclusions about the soil properties and the available concentrations of elements during each year of tree growth can be reached by analysing the elemental composition of individual tree rings (Bardule et al., 2020).

The results obtained during this study indicated similar elemental composition of different tree rings. For example, it was shown (Fig. 1) that the concentration of Mg did not significantly change between different tree rings. The change in element content between different samples is shown as standard deviation. The same tendency was also observed for P and Ca. The obtained results can be explained by stable growth conditions – fertiliser was applied only at the time of planting, and the further growth conditions were similar.

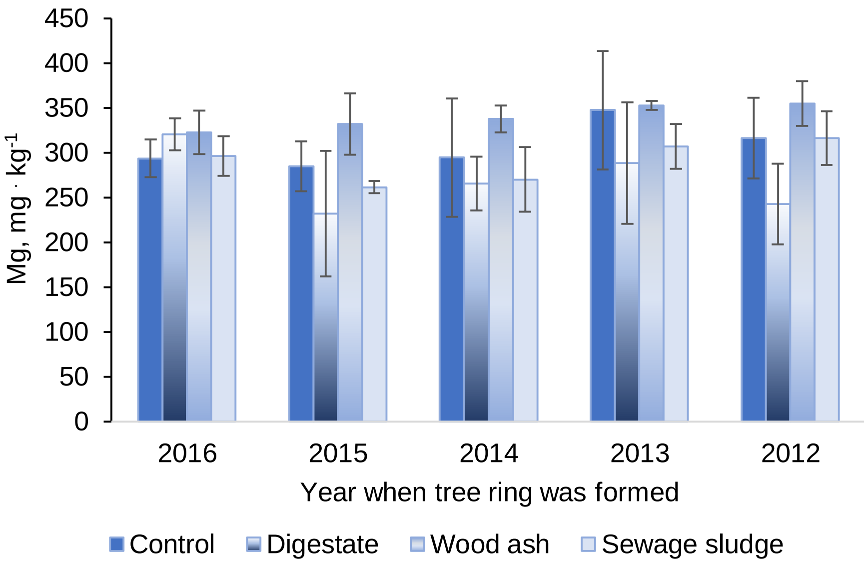

Figure 1. The magnesium content in hybrid aspen stem wood tree rings in a five year period.

The obtained results indicate that for all of the samples regardless of fertilisation type there was a higher amount of K in the more recently formed tree rings (Fig. 2). It can be explained by the fact that K is considered to be a highly mobile element and it is more effectively translocated to the growing parts of the plant (Ragel et al., 2019).

The concentration of Ca decreased in the more recently formed tree rings (Fig. 3). This trend can be explained with the low mobility of Ca, which is concentrated mostly inside cells, so it is largely immobilised in wood as the dying of old cells and formation of new cells takes place (Yang & Jie, 2005; Thor, 2019).

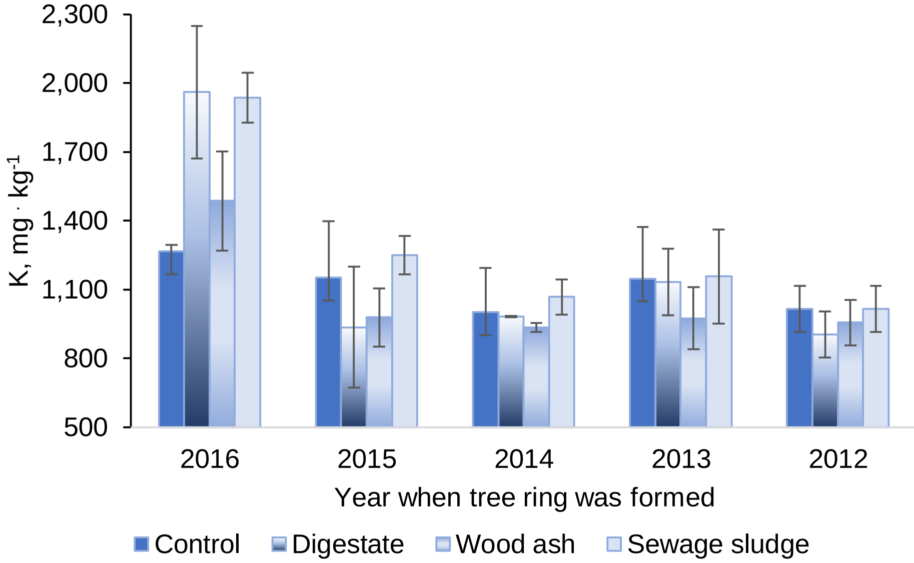

Figure 2. The potassium content in hybrid aspen stem wood tree rings in a five year period.

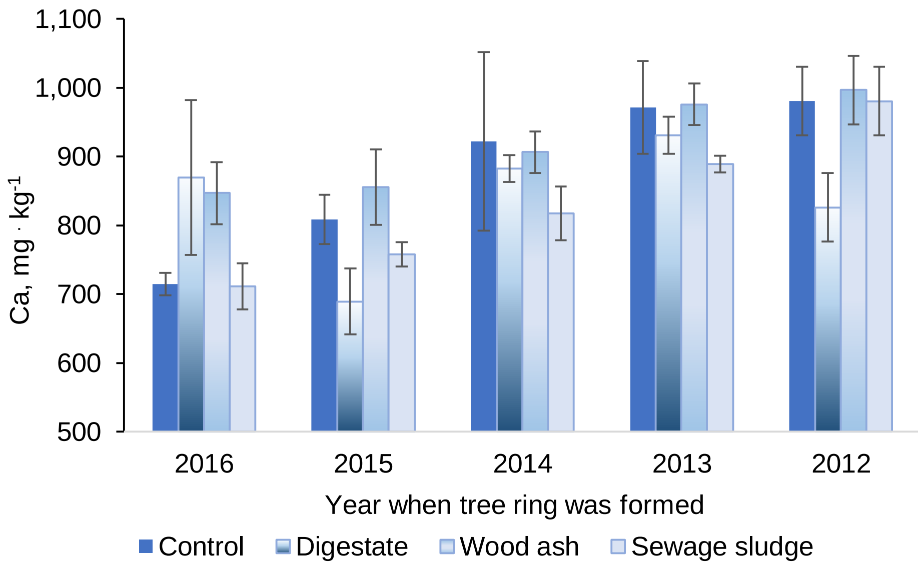

Figure 3. The calcium content in hybrid aspen stem wood tree rings in a five year period.

According to the measured N and C content (Table 4), there were no significant variations between different tree rings. The mass percentage for N was about 0.2%, and for C it was 45.5%. However, the determined isotope ratio values varied over a relatively wide range: $\delta^{13}C$ from -27.1 to -28.4 ‰ and $\delta^{15}N$ from -9.3 to -15.7 ‰.

Table 4. The $\delta^{15}N$, $\delta^{13}C$ values and mass percentages of N and C in hybrid aspen stem wood tree rings from different years (mean values ± SD)

	$\delta^{15}N$, ‰			wN, %			$\delta^{13}C$, ‰			wC, %		
Bark	-1.96	±	0.13	0.608	±	0.030	-30.54	±	0.23	46.92	±	0.08
2016	-15.70	±	0.19	0.170	±	0.003	-27.38	±	0.28	45.27	±	0.21
2015	-10.88	±	0.16	0.202	±	0.040	-26.96	±	0.08	45.76	±	0.80
2014	-11.50	±	0.26	0.171	±	0.009	-27.10	±	0.16	45.19	±	0.45
2013	-12.03	±	0.36	0.192	±	0.006	-27.91	±	0.26	45.45	±	0.21
2012	-10.20	±	0.14	0.208	±	0.019	-28.42	±	0.45	45.26	±	0.07
2011	-9.30	±	0.17	0.213	±	0.007	-28.24	±	0.06	45.56	±	0.11
Pit	-11.30	±	0.40	0.191	±	0.008	-28.27	±	0.02	44.84	±	0.71

The results of other studies show that the $\delta^{13}C$ value is mostly dependent on the type of plant and the photosynthesis mechanisms (Marshal et al., 2007). In addition, the variation in $\delta^{13}C$ values between different tree rings can be explained with the weather conditions during the particular years. However, the reasons for variations in $\delta^{15}N$ values are not as clear because that value may be affected both by the applied fertiliser and the natural nitrogen cycle (Pardo et al., 2013).

CONCLUSIONS

Although the applied fertilizers contained quite variable amounts of nutrients, the results showed that fertilisation treatments mostly did not affect concentrations of macro elements (e.g., P, K, and Ca) and some microelements (e.g., Cr, Ni) in stem wood of hybrid aspen. Thus, it seems that the soil had an adequate amount of these nutrients for the juvenile hybrid aspen trees or there is a large natural variation of nutrient and microelement content in the soil in the research object. Accumulation of the several microelements (e.g., Fe, Mg, Mn, Pb, Rb, and Sr) in hybrid aspen is affected by the type of fertiliser that has been applied. In this study, the use of wood ash or digestate affected the elemental content of hybrid aspen wood to a greater extent than the use of sewage sludge, compared to an unfertilised control plot.

When comparing the elemental concentrations between different tree rings, higher concentration of potassium was observed in the most recent tree ring, indicating the ability of plants to efficiently transport potassium to the growing parts of the plant.

The results obtained in this study indicated that the calcium content gradually increased from younger to older tree rings, thus pointing to the low mobility of Ca^{2+} ions.

The variability of elemental abundance between different tree rings was quite small for the majority of the studied elements, probably due to the stable growth conditions.

The $\delta^{15}N$ and $\delta^{13}C$ values showed greater variations that could provide data about growth conditions of processes that are not observable from the measurements of elemental abundance.

REFERENCES

Bardule, A., Lazdins, A., Sarkanabols, T. & Lazdina, D. 2016. Fertilized short rotation plantations of hybrid aspen (*Populus tremuloides* Michx. × *Populus tremula* L.) for energy wood or mitigation of GHG emissions. *Engineering for Rural Development,* 248–255.

Bardule, A., Bertins, M., Busa, L., Lazdina, D., Viksna, A., Tvrdonova, M., Kanicky, V. & Vaculovic, T. 2020. Variation of major elements and heavy metals occurrence in hybrid aspen (*Populus tremuloides* Michx. × *P. tremula* L.) tree rings in marginal land. *iForest* **13**, 24–32. doi: 10.3832/ifor2869-012

Fahlvik, N., Rytter, L. & Stener, L.-G. 2019. Production of hybrid aspen on agricultural land during one rotation in southern Sweden. *Journal of Forestry Research.* doi: 10.1007/s11676-019-01067-9

Mala, J., Machova, P., Cvrckova, H. & Vanek, T. 2007. Heavy metals uptake by the hybrid aspen and rowan-tree clone. *Journal of Forest Science* **53**(11), 491–497.

Mandre, M. 2014. Heavy metals uptake and assimilation by the hybrid aspen in alkalised soil. *Water, Air and Soil Pollution* **225**, 1808.

Marshal, J.D., Brooks, R. & Lajtha, K. 2007. Sources of variation in the stable isotopic composition of plants. Ed. Michener, R., Lajtha, K. *Stable isotopes in ecology and Environmental Science*, Second Edition, pp. 22–60.

Meyer, M., Krabel, D., Kniesel, B. & Helle, G. 2018. Inter-annual variation of tree-ring width, $\delta^{13}C$ and $\delta^{18}O$ in juvenile trees of five plantation poplar cultivars (*Populus* spp.). *Dendrochronologia* **51**, 32–39.

Nikula, S., Manninen, S., Vapaavouri, E. & Pulkkinen, P. 2011. Growth, leaf traits and litter decomposition of roadside hybrid aspen (*Populus tremula* L. × *P. Tremuloides* Michx.) clones. *Environmental Pollution* **159**, 1823–1830.

Pardo, L.H, Semaoune, P., Schaberg, P.G., Eagar, C. & Sebilo, M. 2013. Patterns in d15N in roots, stems, and leaves of sugar maple and American beech seedlings, saplings, and mature trees. *Biogeochemistry* **112**, 275–291.

Ragel, P., Raddatz, N., Leidi, E.O., Quintero, F.J. & Pardo, J. M. 2019. Regulation of K^+ nutrition in plants. *Frontiers in Plant Science* **10**, 281.

Rytter, L. & Stener, L-G. 2003. Clonal variation in nutrient content of woody biomass of hybrid aspen (*Populus tremula* L. x *P. tremuloides* Michx.). Silva Fennica **37**(3), 313–324. https://doi.org/10.14214/sf.491

Smilga, J., Zeps, M., Sisenis, L., Kalnins, J., Adamovics, A. & Donis, J. 2015. Profitability of hybrid aspen breeding in Latvia. *Agronomy Research* **13**(2), 430–435.

Thor, K. 2019. Calcium-Nutrient and messenger. *Frontiers in Plant Science* **10**, 440. doi: 10.3389/fpls.2019.00440

Tullus, A., Rosenvald, K., Lutter, R., Kaasik, A., Kupper, O. & Sellin, A. 2020. Copping improves the growth response of short-rotation hybrid aspen to elevated atmospheric humidity. *Forrest Ecology and Management* **459**, 117825.

Yang, H.Q. & Jie, Y.L. 2005. Uptake and transport of calcium in plants. *Journal of Plant Phyisiology and Molecular Biology* **31**(3), 227–234.

Zeps, M., Sisenis, L., Luguza, S., Purins, M., Dzerina, B. & Kalnins, J. 2015. Formation of height increment of hybrid aspen in Latvia. *Agronomy Research* **13**(2), 436–441.

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https://doi.org/10.15159/AR.20.063

Effect of cattle trampling and farm machinery traffic on soil compaction of an *Entic Haplustoll* in a semiarid region of Argentina

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Abstract. Soil compaction has detrimental effects on the physical, mechanical and hydraulic properties of soils, and affects important soil processes and function, and crop productivity. This work was conducted to investigate soil compaction impacts in integrated arable cropping-livestock systems managed under conventional tillage (CT) and no-tillage (NT). The work examined the combined effects of cattle trampling and farm machinery traffic on: soil strength, soil deformation, and water infiltration into soil. The following treatments were applied to soil (*Entic Haplustoll*, 60% sand) managed under CT and NT: three traffic intensities (1, 5, 7 passes) performed with light (2WD, 53 kN) and heavy (4WD, 100.4 kN) tractors, and two stocking densities (400 and 700 kg ha^{-1}), respectively. Controls were also used to represent the condition of the soil without any effect of livestock or field traffic. In both tillage systems, soil penetration resistance (strength) increased and water infiltration into soil decreased as traffic intensities or stocking rates applied increased. There was a significant traffic intensity × stocking rate interaction, which influenced the depth and extent of soil compaction at depth. Despite these results, stubble grazing during fallow should not be discouraged as this practice offers mixed farming systems several agronomic and financial benefits. If stubble was to be grazed, the system would need to be carefully managed: (1) avoid ‘random’ traffic using permanent or semi-permanent traffic paths to minimise the field wheeled area, (2) vacate livestock from the field, or confine it to a sacrificial area, when the soil water content exceeds a critical level above which soil damage is likely, and (3) maintain more than 60%–70% ground cover. Tillage repair treatments can be targeted to those sacrificial or 'hot-spots' areas so that localised, as supposed to widespread, compaction problems are rectified before the next crop is established.

Key words: axle load, cone index, ground cover, infiltration, soil deformation, traffic intensity.

INTRODUCTION

The western Pampas region of Argentina contributes a significant proportion of the country's agricultural production, including beef, dairy and arable cropping. The region comprises of approximately 8 M ha and is characterised by a semiarid climate. The mean annual rainfall is about 500 mm of which 75% falls between October and April. About 1.1 M ha of this land are used for arable cropping and is dominated by *Haplustoll* soils (INTA, 2009). Over the past 30 years, growers have progressively transitioned from conventional tillage (CT) to no-tillage (NT) systems, with the dual purpose of improving soil and water conservation and crop reliability, and as a result, be able to reduce financial risks and costs. The development of high-yielding GM crops, purposely-designed NT technology and sustained on-farm research effort have greatly enabled this transition (Peiretti & Dumanski, 2014). The net result has been a significant increase in crop productivity, facilitated by improved timeliness of field operations (planting, spraying, harvesting) and the use of farm equipment with increased capacity. However, a drawback of this process has been the progressive increase in machinery power, and consequently weight. Up to the early 1990's, the median size of a tractor in the region was 90 kW, which compares with 260 kW tractors commonly used at present. Such a shift in machinery size is also explained by the fact that key operations (e.g., spraying, harvest) are performed by contractors who rely on high-capacity equipment to remain profitable. Increased machinery weight has led to increased risk of soil damage due to compaction, particularly in the subsoil (e.g., ≥ 350 mm deep) (Chamen, 2015). Despite the seasonality of (summer) rainfall in this region, wet conditions at harvest, that is early autumn for summer-grown crops, are not uncommon, which means that the risk of compaction occurring is relatively high most years. The occurrence of soil compaction in this region is fairly well documented and has been attributed to a combination of tillage and traffic in some of the early work (e.g., Panigatti, 1964; Quiroga et al., 1999) and traffic in more recent studies (e.g., Botta, et al., 2019a; 2002).

Compaction adversely affects the physical and hydraulic properties of soils, and the ability of crops to efficiently use water (rainfall, irrigation) and applied nutrients; thus, reducing fertiliser recovery in grain and crop productivity (Soane & van Ouwerkerk, 1995; Hussein et al., 2018). Botta et al. (2004) showed that the yield of soybean (*Glycine max* L., Merr) crops established under NT decreases with increased soil compaction, and that yield penalties are proportional to the traffic intensity applied to the soil. Traffic intensity is determined by the combined effect of number of passes with a given farm vehicle over a given area and its overall load (Mg km^{-1} ha^{-1}). Botta et al. (2004) reported yield penalties of up to 38% compared with crops grown for three consecutive years on untrafficked soil. Deep compaction (≥ 350 mm) is often explained by the effect of traffic with high axle loads whereas shallower compaction is due to the pressure exerted at the soil-tyre contact area (e.g., Etana & Håkansson, 1994; Håkansson & Reeder, 1994; Botta et al., 2006a, 2006b). More recent studies (e.g., Ansorge & Godwin, 2007; Antille et al., 2013; Botta et al., 2019b) have also shown that while load is important, more so is how that load is spread over the contact area. Developments in low ground pressure systems, such as 'Hi-Flex' tyres and rubber belt technology, offer promise to mitigate compaction impacts associated with high axle loads (e.g., Ansorge & Godwin, 2008; Godwin et al., 2015; Desbiolles et al., 2019).

In CT systems, the load-carrying capacity of the soil is typically low; consequently, a single pass of a farm vehicle can cause up to about 80% of the maximum compaction and up to 90% of the maximum rut depth (Inns & Kilgour, 1978; Taylor et al., 1982). The initial soil strength is the main factor influencing the response of the soil to external stresses that are applied to that soil (Paz & Guérif, 2000). By contrast, soils under long-term NT generally exhibit greater load-carrying capacity than CT soils, because of natural consolidation in the absence of tillage as well as compaction caused by field traffic (Carter, 1990; Botta et al., 2010). In NT systems with stubble-grazing, relatively shallow compaction (e.g., ≤ 200 mm) has also been attributed to the effect of cattle trampling (e.g., Tollner et al., 1990; Fernández et al., 2011), but some studies have shown that the overall effect is negligible (e.g., Franzluebbers & Stuedemann, 2008). In rigid soils, such as those that occur in the region of interest to this study, subsoil compaction may be regarded as 'permanent' because of its long-term persistence (Arvidsson, 2001). The absence of natural processes such as swelling-shrinking or freezing-thawing cycles means that subsoil compaction cannot be easily reverted and amelioration through tillage is often required (Pollard & Webster, 1978; Dexter, 1991).

There appears to be a paucity of scientific information about the extent and degree of soil compaction in CT and NT systems of the western Pampas of Argentina; particularly, for systems that integrate arable cropping with stubble grazing during the fallow period. Potential soil impacts associated with stubble grazing during the winter fallow, which is commonly practiced in this region, are not well documented. Further, the few studies available appear to be outdated as they have been conducted with much lighter farm equipment, which are no longer used. The work reported in this article aims to inform the development of best soil and stubble management practices relevant to integrated arable cropping-livestock systems. This requires that potential impacts on soil associated with field traffic and stubble grazing be quantified so that appropriate intervention measures, such as controlled traffic and occasional tillage (e.g., Melland et al., 2017; Dang et al., 2018), can be undertaken, and that timing of stubble grazing and stocking rates (e.g., Swan et al., 2018) can be optimised. The dataset provided by this study can be used to inform the selection of appropriate farm machinery (size, capacity) with a view to minimising impacts on soil. As such, this study was undertaken to address this apparent lack of information by conducting a set of field observations that involved measurements of key soil physical/mechanical and hydraulic properties. Measurements were undertaken before and after compaction treatments were applied using a factorial combination of traffic intensities and stocking rates. Treatments were selected to represent common management practices in the region. It was hypothesised that the impact of traffic and stubble grazing on soil compaction would occur to lesser extent in soils managed under long-term (e.g., > 10 years) NT compared with soils managed under CT, but the overall effect would be significant in both soils.

MATERIALS AND METHODS

Site description

The study was conducted in a commercial farm located in Trenque Lauquen, Argentina (36°04'33" S, 62°29'14" W, 300-m above-sea level). The soil is classified as *Entic Haplustoll* (USDA Soil Taxonomy). Surface runoff at the site is negligible as the soil has a gentle slope (<1%) and is well-drained with moderately high permeability.

Soil textural analyses (Bouyoucos, 1962) for the 0–150 mm and 150–450 mm depth intervals reported 565 and 583 g kg^{-1} sand (50–500 μm), 274 and 239 g kg^{-1} silt (2–50 μm), and 161 and 178 g kg^{-1} clay (< 2 μm), respectively. Analysis of soil organic carbon (Walkley & Black, 1934) showed 12.3 g kg^{-1} and 5.85 g kg^{-1} SOC for the 0–150 mm and 150–450 mm depth intervals, respectively. A full description of the soil profile (0–1200 mm) is given by Botta el al. (2019a).

Experimental

A description of the two tractors used in the study is shown in Table 1. The mean pressure at the soil-tyre contact area was measured using a Tekscan® sensor (Tekscan, Inc., www.tekscan.com) and resulted from fifteen data points (n = 15) taken for each of the tractor tyres. The tyre inflation pressure was adjusted based on the manufacturer's recommendation for load and speed, and the tractors were operated at 8 km h^{-1}.

Table 1. Specification of tractors used in the experiments

Tractor, Unit	John Deere 4730
Drivetrain	2WD
Engine power (IRAM8005), HP	102
Tractor weight, kN	53
Weight (rear axle), kN	36
Weight (front axle), kN	17
Front tyres	10.00×16
Inflation pressure (front tyres), kPa	170
Rear tyres	23.1R30
Inflation pressure (rear tyres), kPa	130
Tractor, Unit	Zanello 540
Drivetrain	4WD
Engine power (IRAM8005), HP	210
Tractor weight, kN	100.4
Weight (rear axle), kN	34.28
Weight (front axle), kN	65.72
Front tyres	18.4R34
Inflation pressure (front tyres), kPa	140
Rear tyres	18.4R34
Inflation pressure (rear tyres), kPa	140

The following soil properties were measured: (1) soil strength was determined with the use of an electronic cone penetrometer (FieldScout SC-900), which automatically recorded the force to a depth of 450-mm at regular increments of 25-mm, based on ASABE (2019). Measurements (n = 20 per plot) were conducted immediately after the traffic and stocking rates treatments were applied and followed a similar approach to that used by Smith and Dickson (1990), albeit with livestock as an added factor. Control plots were also used to allow the undisturbed soil condition to be determined, that is, without the traffic × livestock effect; (2) measurements of water infiltration into soil (n = 3 per treatment) were conducted using the double-ring infiltrometer (Parr & Bertrand, 1960), readings taken at 125 minutes after the infiltration test was initiated (t = 125 min) and reported; (3) rut depth was measured using a profile-meter; and (4) soil water content was also determined because of its effect on soil strength (Ayers & Perumpral, 1982; Aikins et al., 2019, 2020). For this, soil samples (n = 20 for each depth interval) were taken at regular depth intervals (0–150, 150–300, and 300–450 mm, respectively), and subsequently oven-dried at 105 ± 5 °C for 48–72 hours (MAFF, 1986).

Traffic treatments were applied to two plots in which the soil had been managed under continuous (> 10 years) conventional tillage (CT) and no-tillage (NT), respectively. The crop grown at the site had been maize (*Zea mays* L.), which has a dual purpose; after the grain is harvested in autumn, the stubble is grazed by cattle over the fallow time in winter. The traffic treatments consisted of three different traffic intensities that combined number of tractor passes (1, 5, 7, respectively) with vehicle weight (Table 1), and replicated controls. A complete factorial arrangement with three replications per treatment ($n = 3$) was established using 48, 50-m×50-m plots. Measurements of water infiltration into soil and penetration resistance were conducted before and after traffic and stocking rate treatments were applied. For both determinations, the 'after' measurements were conducted at the centreline of the tyre rut where changes in soil properties, relative to the 'before' condition, were expected to be greatest. Soil-tyre contact stresses tend to concentrate toward the centreline of the rut (Way et al., 1997; Antille et al., 2013). After the harvest operation was completed, and the traffic treatments applied, two stocking rates were imposed to both CT and NT plots, and these are referred to here as low (400 kg ha^{-1}) and high (700 kg ha^{-1}), respectively. To achieve these stocking rates, the number of animals per plot remained constant, but the area within each plot was adjusted by using an electric fence. The animals were allowed to consume the stubble until ground cover dropped to about 60%, consistent with standard agronomic practice. Thus, the amount of dry matter retained on the plots ensured a 'safe' level of ground cover that was required to reduce the risk of wind erosion (Mendez & Buschiazzo, 2015). Subsequently, the animals were removed from the plots. Each plot had an area that could not be entered by animals used as control.

Summary of experimental design: The final experimental design resulted from a factorial combination of the following treatments: two tillage management systems (CT: conventional tillage and NT: no-tillage), six traffic intensities given by light (2WD, 53 kN) and heavy (4DW, 100.4 kN) tractors and number of tractor passes (1, 5 and 7), and two stocking rates (low: 400 kg ha^{-1} and high: 700 kg ha^{-1}), respectively. Controls (zero-traffic, zero-stocking rate) were also used both in the conventional and no-tillage systems, respectively. All treatments were replicated three times ($n = 3$), which therefore resulted in 96 experimental plots.

Statistical analyses

Statistical analyses were undertaken with STATGRAPHICS 7.1® (www.statgraphics.com), and involved analysis of variance (ANOVA), which was applied to all measured variables. Mean treatment effects from the tillage system × traffic intensity × stocking rate combinations were determined by applying the Duncan test using a probability level of 1% ($P < 0.01$) (Little & Hills, 1978).

RESULTS AND DISCUSSION

Table 2 shows measurements of soil water content recorded at the time the treatments were applied to the CT and NT soils. Results showed that overall differences in soil water content were not significant ($P > 0.01$); therefore, values of penetration resistance were not corrected by soil water content (Unger, 1996; ASABE, 2019). Table 3 shows that the contact pressure measured in CT soil was less than that of NT soil ($P < 0.01$), consistent with measurements of rut depth (Table 6). Softer soil conditions in CT compared with NT resulted in greater soil deformation thus increasing the area and dimensions of the part of the tyre in contact with the ground ('contact patch') (Burt et al., 1987; Way et al., 2005). This reduces the (average) contact pressure under the tyre despite the fact that, at the same load and inflation pressure, tyre deflection may be concurrently reduced (Raper et al., 1995a; Misiewicz et al., 2015). Hence, under relatively soft soil conditions such as those of the CT soil, tyre inflation pressure may be reduced slightly compared with the recommended pressure for the same load on a firmer soil condition (Raper et al., 1994; 1995b). This will also help reduce rolling resistance and improve traction (Wood & Burt, 1987; Ejsmont et al., 2016; Luhaib et al., 2017).

Table 2. Gravimetric water contents (θ_g) recorded at the site at the time the experiments were conducted. Mean values ± standard deviation ($n = 20$). Based on the Duncan test, letters show that mean values are not statistically different at a 1% probability level

Tillage system	Conventional tillage	No-tillage
Depth interval (mm)	$\theta_g (\%, w/w)$	$\theta_g (\%, w/w)$
0–150	13.2 ± 1.71*a*	7.2 ± 1.60*a*
150–300	10.3 ± 1.67*a*	9.1 ± 1.54*a*
300–450	9.5 ± 1.52*a*	8.3 ± 1.47*a*

Table 3. Mean contact pressure recorded at the soil-tyre interface ($n = 15$) for the two tractors used in the experiments. Values are given in kPa

Tyre, tillage system	John Deere 4730	Zanello 540
Front tyre, conventional tillage	74.7	49.0
Front tyre, no-tillage	100.3	57.8
Rear tyre, conventional tillage	38.9	30
Rear tyre, no-tillage	43.2	33.4

For the control soils, differences in water infiltration rates between CT and NT were significant ($P < 0.01$). At $t = 125$ min, mean infiltration rates were 90 and 55 mm h^{-1} for CT and NT, respectively. Overall, and before the treatments were applied, NT soil showed significantly higher values of penetration resistance up to a depth of 375 mm ($P < 0.01$), which reflected increased soil carrying capacity compared with CT soil (Fig. 1). At greater depths, differences between the two soil conditions were non-significant ($P > 0.01$).

Penetration resistance in CT soil peaked at 250 mm deep where it exceeded the suggested 2,000 kPa threshold (Taylor & Ratliff, 1969a, 1969b) above which root elongation may be significantly affected. Increased soil strength at this depth also suggested a plough pan was present, likely as a result of tillage operations conducted consistently at the same depth, and over a relatively long period of time (Cresswell et al., 1992). Below that depth, values of penetration resistance declined a little, but they remained around the 2,000 kPa range throughout the profile. In NT soil, values of penetration resistance peaked at a 200 mm deep (≈ 4,250 kPa) and declined to about

2,500 kPa or less below 250 mm deep suggesting that this soil may benefit from occasional or 'strategic' deep tillage (Spoor & Voorhees, 1986; Dang et al., 2018).

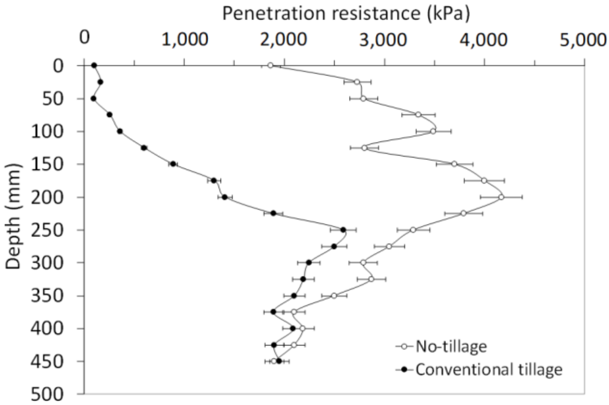

Figure 1. Soil penetration resistance recorded at the experimental site before the traffic and stocking rate treatments were applied. Error bars on mean data points (n = 20) denote the standard deviation.

A summary of soil penetration resistance, water infiltration rates, and rut depth measurements for all combinations of traffic intensities and stocking rates is shown in Tables 4–6. In both tillage systems, penetration resistance data showed that the depth and extent of soil compaction increases with the number of passes and axle load. A single pass of the heavier tractor used in this study was sufficient to cause compaction to the full measured depth. The lighter tractor required five or more passes. At shallow depths (≤ 150 mm), compaction was always significant regardless of the number of passes or the weight of the tractor. Pulido-Moncada et al. (2019) showed that the degree of soil compactness can be higher than 95% if multiple passes (e.g., 4–5) with 3–5 Mg wheel loads are performed. Measured pressures at the soil-tyre contact area in this study were slightly higher than the maximum value recommended by Alakukku (1996) for prevention of soil compaction. Maximum wheel loads have been suggested (e.g., Håkansson & Petelkau, 1994; van den Akker, 1998) in attempts to limit compaction to relatively shallow depths, but such limits can be easily exceeded with current editions of farm machinery. Technical solutions are available (e.g., Soane et al., 1982) including low (ground) pressure tyre technology and controlled traffic farming (e.g., Antille et al., 2015, 2019; Bluett et al., 2019), which have been shown to be cost-effective (e.g., Chamen et al., 2015; Godwin et al., 2015; Galambošová et al., 2017).

Water infiltration into soil was significantly affected by compaction in all treatments (Table 5), and the effect was proportional to the traffic intensity and stocking rate applied, which was consistent with other studies on light soils (e.g., Usman, 1994; Chyba et al., 2014; Ngo-Cong et al., 2020). This is an important practical consideration for water conservation in semiarid regions, such as the western Pampas, that rely on rainfall and soil water storage for successful crop establishment.

Table 4. Mean values (n = 20) of soil penetration resistance (kPa) recorded after traffic and stocking rate treatments were applied to soil managed under conventional tillage and no-tillage. Different letters (horizontally and within the same stocking rate) indicate that mean values are significantly different at a 1% probability level based on the Duncan test

Tillage system	Conventional tillage							
Tractor	John Deere 4730							
Stocking rate	700 kg ha^{-1}				400 kg ha^{-1}			
Depth (mm), Traffic	1 Pass	5 Passes	7 Passes	Control	1 Pass	5 Passes	7 Passes	Control
0–150	1,590*b*	1,801*c*	1,953*d*	889*a*	1,330*b*	1,421*b*	1,677*c*	695*a*
150–300	2,443*b*	2,410*b*	2,627*c*	2,190*a*	17,91*b*	1,811*c*	2,114*d*	1,460*a*
300–450	2,527*a*	2,595*a*	2,748*b*	2,500*a*	2,024*a*	2,110*b*	2,423*c*	1,992*a*
Tractor	Zanello 540C							
Stocking rate	700 kg ha^{-1}				400 kg ha^{-1}			
Depth (mm), Traffic	1 Pass	5 Passes	7 Passes	Control	1 Pass	5 Passes	7 Passes	Control
0–150	1,910*b*	2,357*c*	2,500*d*	889*a*	1,710*b*	1,829*c*	2,201*d*	695*a*
150–300	2,500*b*	2,660*c*	2,810*d*	2,190*a*	2,145*b*	2,368*c*	2,619*d*	1,267*a*
300–450	2,801*b*	2,929*c*	2,921*c*	2,500*a*	2,300*b*	2,502*c*	2,834*d*	1,992*a*
Tillage system	No-tillage							
Tractor	John Deere 4730							
Stocking rate	700 kg ha^{-1}				400 kg ha^{-1}			
Depth (mm), Traffic	1 Pass	5 Passes	7 Passes	Control	1 Pass	5 Passes	7 Passes	Control
0–150	3,288*b*	3,803*c*	3,970*d*	3,110*a*	2,940*b*	3,123*b*	3,478*c*	2,812*a*
150–300	3,735*a*	3,821*a*	4,127*b*	3,791*a*	3,145*a*	3,200*a*	3,600*b*	3,791*a*
300–450	2,830*a*	2,888*a*	2,902*a*	2,821*a*	2,778*a*	2,791*a*	2,900*a*	2,710*a*
Tractor	Zanello 540C							
Stocking rate	700 kg ha^{-1}				400 kg ha^{-1}			
Depth (mm), Traffic	1 Pass	5 Passes	7 Passes	Control	1 Pass	5 Passes	7 Passes	Control
0–150	3,299*b*	3,890*c*	4,110*d*	3,110*a*	3,156*b*	3,300*c*	3,432*d*	2,812*a*
150–300	4,002*b*	4,133*b*	4,318*c*	3,791*a*	3,897*b*	3,933*b*	3,990*c*	3,791*a*
300–450	3,133*b*	3,254*b*	3,634*c*	2,821*a*	3,102*b*	3,119*c*	3,401*d*	2,821*a*

Table 5. Mean values (n = 3) of water infiltration into soil (mm h^{-1}) measured at t = 125 min after traffic and stocking rate treatments were applied to soil managed under conventional tillage and no-tillage. Different letters (vertically) indicate that mean values are significantly different at a 1% probability level based on the Duncan test

Tillage system	Conventional tillage				No-tillage			
Stocking rate	400 kg ha^{-1}				400 kg ha^{-1}			
Tractor, Traffic	Control	1 Pass	5 Passes	7 Passes	Control	1 Pass	5 Passes	7 Passes
Zanello 540	90*a*	54*a*	31*a*	17*a*	55*a*	32*a*	24*a*	13*a*
John Deere 4730	90*a*	71*b*	38*b*	32*b*	55*a*	43*b*	37*b*	31*b*
Stocking rate	700 kg ha^{-1}				700 kg ha^{-1}			
Tractor, Traffic	Control	1 Pass	5 Passes	7 Passes	Control	1 Pass	5 Passes	7 Passes
Zanello 540	90*a*	40*a*	28*a*	15*a*	55*a*	29*a*	22*a*	8*a*
John Deere 4730	90*a*	61*b*	33*b*	26*b*	55*a*	45*b*	31*b*	24*b*

Table 6. Maximum rut depth (mm) measured at the centreline of the tyre rut after traffic treatments were applied to soil managed under conventional tillage and no-tillage. Mean values ± standard deviation (n = 3). Different letters (vertically) indicate that mean values are significantly different at a 1% probability level based on the Duncan test

Tillage system	Conventional tillage			No-tillage		
Tractor, Traffic	1 Pass	5 Passes	7 Passes	1 Pass	5 Passes	7 Passes
Zanello 540	44.4 ± 4.2*a*	39.2 ± 3.2*a*	45.3 ± 3.0*a*	20.0 ± 1.2*a*	23.1 ± 2.1*a*	24.0 ± 1.5*a*
John Deere 4730	40.2 ± 3.7*a*	38.7 ± 2.9*a*	42.3 ± 3.1*b*	8.5 ± 8.5*b*	10.1 ± 1.7*b*	7.30 ± 1.7*b*

Overall, soil mechanical and hydraulic responses to livestock grazing stubble showed detrimental effects in both tillage systems. The impact was greater on CT because of significantly lower soil strength and soil carrying capacity compared with NT. However, stubble grazing may not be discouraged as it offers mixed farms a 'low cost' cattle feed at a time of the year when other sources of forage are more expensive (e.g., winter cover crops) or not readily available. Winter cover or dual-purpose crops will tend to reduce plant available water for the subsequent summer crop (e.g., maize) and will likely have an impact on yield. By contrast, stubble grazing can have no significant impact on soil water availability for the following crop in the rotation, provided livestock was carefully managed. This requires that a minimum of 60% to 70% ground cover, or about 2–3 Mg ha^{-1} of stubble, is retained to ensure the soil is protected from erosion and evaporative losses are minimised (Swan et al., 2018). To minimise the risk of soil damage due to compaction, livestock should be removed from the field when the soil water deficit (SWD) declines below a critical level. This critical SWD should ensure livestock compaction is negligible or only confined to very shallow depths (e.g., ≤ 75 mm) so that it can be removed by the sowing operation without causing any impact on crop establishment. Similar approaches have been satisfactorily used to establish SWD thresholds for limits to trafficability with farm equipment (e.g., Earl, 1997; Vero et al., 2014), and these could be adopted for integrated arable cropping-livestock systems.

CONCLUSIONS

This article has presented and discussed results derived from field investigations that were conducted to quantify soil compaction impacts in integrated arable cropping-livestock systems. Results can be used to inform the development of best soil and stubble management practices in those systems.

Soil compaction occurred to lesser extent in soil managed under long-term (10 years) no-tillage (NT) compared with soil managed under conventional tillage (CT), but the overall effect of compaction was significant in both soils. This observation confirmed the hypothesis formulated prior to this study. This work also showed that soil compaction was higher with increased traffic intensities or stocking rates. There was a significant traffic intensity × stocking rate interaction, which influenced the depth and extent of compaction at depth. Despite these results, stubble grazing during winter fallow should not be discouraged as this practice offers mixed farming systems several tangible benefits, including agronomic and financial. If stubble was to be grazed, the system would need to be carefully managed: (1) avoid 'random' traffic using permanent or semi-permanent (seasonal) traffic paths that minimise the wheeled area within the field, (2) vacate livestock from the field, or confine it to a sacrificial area within the field. This may be performed when the soil water deficit reaches a critical level below which the risk of soil damage due to compaction (reduced bearing capacity) is significant. This should also ensure that compaction is limited to very shallow depths and that it can be removed by the sowing operation without affecting crop establishment, and (3) maintain no less than 60%–70% ground cover (stubble retention) to reduce the risk of erosion and evaporative losses.

Tillage repair treatments can be targeted to those sacrificial or 'hot-spots' areas so that localised, as supposed to widespread, compaction problems are rectified before the next crop is established.

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REFERENCES

Aikins, K.A., Barr, J.B., Jensen, T.A. & Antille, D.L. 2019. Measuring soil surface and furrow profiles using a portable and affordable 3D scanner. ASABE Paper No.: 1900038. St. Joseph, MI.: 2019 ASABE Annual International Meeting. *American Society of Agricultural and Biological Engineers.* https://doi.org/10.13031/aim.201900038

Aikins, K.A., Jensen, T.A. & Antille, D.L. 2020. Three-dimensional scanning of soil surface and furrow profiles using a portable and affordable unit. *Biosystems Engineering* **193**, 279–289. https://doi.org/10.1016/j.biosystemseng.2020.03.002

Alakukku, L. 1996. Persistence of soil compaction due to high axle load traffic. Short term effects on the properties of clay and organic soils. *Soil and Tillage Research* **37**, 211–222.

Ansorge, D. & Godwin, R.J. 2007. The effect of tyres and a rubber track at high axle loads on soil compaction, Part 1: Single axle-studies. *Biosystems Engineering* **98**, 115–126.

Ansorge, D. & Godwin, R.J. 2008. The effect of tyres and a rubber track at high axle loads on soil compaction-Part 2: Multi-axle machine studies. *Biosystems Engineering* **99**, 338–347.

Antille, D.L., Ansorge, D., Dresser, M.L. & Godwin, R.J. 2013. Soil displacement and soil bulk density changes as affected by tire size. *Transactions of the ASABE* **56**, 1683–1693.

Antille, D.L., Imhoff, S.C., Alesso, C.A., Chamen, W.C.T. & Tullberg, J.N. 2015. Potential to increase productivity and sustainability in Argentinean agriculture with controlled traffic farming: a short discussion. *Acta Technologica Agriculturae* **18**, 83–87.

Antille, D.L., Peets, S., Galambošová, J., Botta, G.F., Rataj, V., Macák, M., Tullberg, J.N., Chamen, W.C.T., White, D.R., Misiewicz, P.A., Hargreaves, P.R., Bienvenido, J.F. & Godwin, R.J. 2019. Review: Soil compaction and controlled traffic farming in arable and grass cropping systems. *Agronomy Research* **17**, 653–682.

Arvidsson, J. 2001. Subsoil compaction caused by heavy sugarbeet harvesters in southern Sweden: I. Soil physical properties and crop yield in six field experiments. *Soil and Tillage Research* **60**, 67–78.

ASABE. 2019. ASAE Standard EP542.1-NOV2019: Procedures for using and reporting data obtained with the soil cone penetrometer. St. Joseph, Mich.: American Society of Agricultural and Biological Engineers.

Ayers, P.D. & Perumpral, J.V. 1982. Moisture and density effect on cone index. *Transactions of the ASABE* **25**, 1169–1172.

Bluett, C., Tullberg, J.N., McPhee, J.E. & Antille, D.L. 2019. Soil and Tillage Research: Why still focus on soil compaction? *Soil and Tillage Research* **194**, Article number: 104282.

Botta, G.F., Jorajuria, D., Balbuena, R. & Rosatto, H. 2004. Mechanical behavior of an agricultural soil under different traffic intensities: effect on soybean (*Glycine max* L.) yields. *Soil and Tillage Research* **78**, 53–58.

Botta, G.F., Jorajuría, D., Balbuena, R., Ressia, M. & Ferrero, C. 2006a. Light tractor traffic frequency on soil compaction in the rolling pampas region of Argentina. *Soil and Tillage Research* **86**, 9–14.

Botta, G.F., Jorajuría, D., Balbuena, R., Ressia, M., Ferrero, C., Rosatto, H. & Tourn, M. 2006b. Deep tillage and traffic effects on subsoil compaction and sunflower (*Helianthus annus* L.) yields. *Soil and Tillage Research* **91**, 164–172.

Botta, G.F., Antille, D.L., Bienvenido, F., Rivero, D. & Contessotto, E.E. 2019a. Energy requirements for alleviation of subsoil compaction and the effect of deep tillage on sunflower (*Helianthus annus* L.) yield in the western region of Argentina's Rolling Pampa. *Engineering for Rural Development* **18**, 174–178.

Botta, G.F., Bienvenido, J.F., Antille, D.L., Rivero, E.R.D., Contessotto, E.E., Ghelfi, D.G. & Nistal, A.I. 2019b. Effect of traffic with a light-weight tractor on physical properties of an Aridisol soil in Almeria, Spain. *Revista de la Facultad de Ciencias Agrarias* **51**, 270–279.

Botta, G.F., Jorajuria, D. & Draghi, L.M. 2002. Influence of the axle load, tyre size and configuration on the compaction of a freshly tilled clayey soil. *Journal of Terramechanics* **39**, 47–54.

Botta, G.F., Tolón Becerra, A., Lastra-Bravo, X. & Tourn, M. 2010. Tillage and traffic effects (planters and tractors) on soil compaction and soybean (*Glycine max* L.) yields in Argentinean Pampas. *Soil and Tillage Research* **110**, 167–174.

Bouyoucos, G.J. 1962. Hydrometer method improved for making particle size analyses of soils. *Agronomy Journal* **54**, 464–465.

Burt, E.C. & Wood, R.K. 1987. Three-dimensional tire deformation on deformable surfaces. *Transactions of the ASAE* **30**, 601–604.

Carter, M.R. 1990. Relative measures of soil bulk density to characterize compaction in tillage studies on fine sandy loams. *Canadian Journal of Soil Science* **70**, 425–433.

Chamen, T. 2015. Controlled traffic farming – from worldwide research to adoption in Europe and its future prospects. *Acta Technologica Agriculturae* **18**, 64–73.

Chamen, W.C.T., Moxey, A.P., Towers, W., Balana, B. & Hallett, P.D. 2015. Mitigating arable soil compaction: a review and analysis of available cost and benefit data. *Soil and Tillage Research* **146**, 10–25.

Chyba, J., Kroulík, M., Krištof, K., Misiewicz, P.A. & Chaney, K. 2014. Influence of soil compaction by farm machinery and livestock on water infiltration rate on grassland. *Agronomy Research* **12**, 59–64.

Cresswell, H.P., Smiles, D.E. & Williams, J. 1992. Soil structure, soil hydraulic properties and the soil water balance. *Soil Research* **30**, 265–283.

Dang, Y.P., Balzer, A., Crawford, M., Rincon-Florez, V., Hongwei, L., Melland, A.R., Antille, D., Kodur, S., Bell, M.J., Whish, J.P.M., Lai, Y., Seymour, N., Carvalhais, L.C. & Schenk, P. 2018. Strategic tillage in conservation agricultural systems of north-eastern Australia: why, where, when and how? *Environmental Science and Pollution Research* **25**, 1000–1015.

Desbiolles, J., Saunders, C., Barr, J., Riethmuller, G., Northover, G., Tullberg, J. & Antille, D. 2019. Chapter 6: *Machinery evolution for conservation agriculture*, pp. 81–105. In: Pratley, J. & Kirkegaard, J. (Eds.). 'Australian Agriculture in 2020: From Conservation to Automation'. Wagga Wagga, NSW, Australia: Agronomy Australia and Charles Sturt University. ISBN 13: 978–0–6485819–0–1.

Dexter, A.R. 1991. Amelioration of soil by natural processes. *Soil and Tillage Research* **20**, 87–100.

Earl, R. 1997. Prediction of trafficability and workability from soil moisture deficit. *Soil and Tillage Research* **40**, 155–168.

Ejsmont, J., Taryma, S., Ronowski, G. & Swieczko-Zurek, B. 2016. Influence of load and inflation pressure on the tyre rolling resistance. *International Journal of Automotive Technology* **17**, 237–244.

Etana, A. & Håkansson, I. 1994. Swedish experiments on the persistence of subsoil compaction caused by vehicles with high axle load. *Soil and Tillage Research* **29**, 167–172.

Fernández, P.L., Alvarez, C.R. & Taboada, M.A. 2011. Assessment of topsoil properties in integrated crop-livestock and continuous cropping systems under zero tillage. *Soil Research* **49**, 143–151.

Franzluebbers, A.J. & Stuedemann, J.A. 2008. Soil physical responses to cattle grazing cover crops under conventional and no tillage in the Southern Piedmont USA. *Soil and Tillage Research* **100**, 141–153.

Galambošová, J., Macák, M., Rataj, V., Antille, D.L., Godwin, R.J., Chamen, W.C.T., Žitňák, M., Vitázková, B., Ďuďák, J. & Chlpík, J. 2017. Field evaluation of controlled traffic farming in Central Europe using commercially available machinery. *Transactions of the ASABE* **60**, 657–669.

Godwin, R., Misiewicz, P., White, D., Smith, E., Chamen, T., Galambošová, J. & Stobart, R. 2015. Results from recent traffic systems research and the implications for future work. *Acta Technologica Agriculturae* **18**, 57–63.

Håkansson, I. & Petelkau, H. 1994. Benefits of limited axle load. In: Soane, B.D. & van Ouwerkerk, C. (Eds.). 'Soil compaction in crop production', pp. 684. *Developments in Agricultural Engineering* **11**, 479–500. ISBN: 9780080934006.

Håkansson, I. & Reeder, R.C. 1994. Subsoil compaction by vehicles with high axle load-extent, persistence and crop response. *Soil and Tillage Research* **29**, 277–304.

Hussein, M.A., Antille, D.L., Chen, G., Kodur, S. & Tullberg, J.N. 2018. Agronomic performance of sorghum (*Sorghum bicolor* (L.) Moench) and fertilizer use efficiency as affected by controlled and non-controlled traffic of farm machinery. ASABE Paper No.: 1800250. St. Joseph, MI.: 2018 *ASABE Annual International Meeting, American Society of Agricultural and Biological Engineers*. https://doi.org/10.13031/aim.201800250

Inns, F.M. & Kilgour, J. 1978. *Agricultural tyres*, London, U.K.: Dunlop Ltd., 70 pp.

INTA. 2009. Monitoreo de la cobertura y el uso del suelo a partir de sensores remotos: Resultados 2006-2009. Programa Nacional de Ecoregiones. Informe Técnico Unificado PNECO-1643, pp. 31. Buenos Aires, Argentina: Instituto Nacional de Tecnología Agropecuaria.

Little, T.M. & Hills, F.J. 1978. Agricultural experimentation: design and analysis. John Wiley & Sons Inc. ISBN 10 0471023523.

Luhaib, A.A.A., Antille, D.L., Tullberg, J.N., Hussein, M.A. & Chen, G. 2017. Effect of controlled traffic farming on energy saving in Australian grain cropping systems. ASABE Paper No.: 1700583. St. Joseph, MI.: 2017 ASABE Annual International Meeting, *American Society of Agricultural and Biological Engineers*. https://doi.org/10.13031/aim.201700583

MAFF. 1986. *The analysis of agricultural materials*. Reference Book No.: 427, 3rd Edition. London, U.K.: The Stationery Office, Ministry of Agriculture, Fisheries and Food.

Melland, A.R., Antille, D.L. & Dang, Y.P. 2017. Effects of strategic tillage on short-term erosion, nutrient loss in runoff and greenhouse gas emissions. *Soil Research* **55**, 201–214.

Mendez, M.J. & Buschiazzo, D.E. 2015. Soil coverage evolution and wind erosion risk on summer crops under contrasting tillage systems. *Aeolian Research* **16**, 117–124.

Misiewicz, P.A., Blackburn, K., Richards, T.E., Brighton, J.L. & Godwin, R.J. 2015. The evaluation and calibration of pressure mapping system for the measurement of the pressure distribution of agricultural tyres. *Biosystems Engineering* **130**, 81–91.

Ngo-Cong, D., Mai-Duy, N., Antille, D.L. & van Genuchten, M.T. 2020. A control volume scheme using compact integrated radial basis function stencils for solving the Richards equation. *Journal of Hydrology* **580**, Article number: 124240.

Panigatti, J.L. 1964. Aspectos del uso y conservación de suelos en el Departamento Castellanos, Santa Fe. *Revista IDIA* **13**, 25–36.

Parr, J.F. & Bertrand, A.R. 1960. Water infiltration into soils. *Advances in Agronomy* **12**, 311–363.

Paz, A. & Guérif, J. 2000. Influence of initial packing density, water content and load applied during compaction on tensile strength of dry soil structural units. *Advances in Geoecology* **32**, 22–31.

Peiretti, R. & Dumanski, J. 2014. The transformation of agriculture in Argentina through soil conservation. *International Soil and Water Conservation Research* **2**, 14–20.

Pollard, F. & Webster, R. 1978. The persistence of the effects of simulated tractor wheeling on sandy loam subsoil. *Journal of Agricultural Engineering Research* **23**, 217–220.

Pulido-Moncada, M., Munkholm, L.J. & Schjønning, P. 2019. Wheel load, repeated wheeling, and traction effects on subsoil compaction in northern Europe. *Soil and Tillage Research* **186**, 300–309.

Quiroga, A.R., Buschiazzo, D.E. & Peinemann, N. 1999. Soil compaction is related to management practices in the semi-arid Argentine Pampas. *Soil and Tillage Research* **52**, 21–28.

Raper, R.L., Bailey, A.C., Burt, E.C., Way, T.R. & Liberati, P. 1995a. The effects of reduced inflation pressure on soil-tire interface stresses and soil strength. *Journal of Terramechanics* **32**, 43–51.

Raper, R.L., Bailey, A.C., Burt, E.C., Way, T.R. & Liberati, P. 1995b. Inflation pressure and dynamic load effects on soil deformation and soil-tire interface stresses. *Transactions of the ASAE* **38**, 685–689.
Raper, R.L., Reeves, D.W., Burt, E.C. & Torbert, H.A. 1994. Conservation tillage and traffic effects on soil condition. *Transactions of the ASAE* **37**, 763–768.
Smith, D.L. & Dickson, J.W. 1990 Contributions of weight and ground pressure to soil compaction. *Journal of Agricultural Engineering Research* **46**, 13–29.
Soane, B.D. & van Ouwerkerk, C. 1995. Implications of soil compaction in crop production for the quality of the environment. *Soil and Tillage Research* **35**, 5–22.
Soane, B.D., Dickson, J.W. & Campbell, D.J. 1982. Compaction by agricultural vehicles: a review III. Incidence and control of compaction in crop production. *Soil and Tillage Research* **2**, 3–36.
Spoor, G. & Voorhees, W.B. 1986. Deep tillage. *Soil and Tillage Research* **8**, 317–319.
Swan, T., Kirkegaard, J. & Bowden, P. 2018. Grazing benefits stubble-retention cropping systems as well as livestock. In: GroundCover™ Supplement Issue: **135**, July-August 2018. GRDC. Available at: https://grdc.com.au/resources-and-publications (Date: 18 Jan. 2020).
Taylor, H.M. & Ratliff, L.F. 1969a. Root growth pressures of cotton, pea and peanuts. *Agronomy Journal* **61**, 398–402.
Taylor, H.M. & Ratliff, L.F. 1969b. Root elongation rates of cotton and peanuts as a function of soil strength and soil water content. *Soil Science* **108**, 113–119.
Taylor, J., Trouse, C., Burt, E. & Bailey, A. 1982. Multipass behavior of a pneumatic tire in tilled soils. *Transactions of the ASAE* **25**, 1229–1236.
Tollner, E.W., Calvert, G.V. & Langdale, G. 1990. Animal trampling effects on soil physical properties of two Southeastern U.S. Ultisols. *Agriculture, Ecosystems and Environment* **33**, 75–87.
Unger, P.W. 1996. Soil bulk density, penetration resistance, and hydraulic conductivity under controlled traffic conditions. *Soil and Tillage Research* **37**, 67–75.
Usman, H. 1994. Cattle trampling and soil compaction effects on soil properties of a Northeastern Nigerian sandy loam. *Arid Soil Research and Rehabilitation* **8**, 69–75.
van den Akker, J.J.H. 1998. Prevention of subsoil compaction by defining a maximum wheel load bearing capacity. In: Märländer, B., Tijink, F.J., Hoffman, C. & Beckers, R. (Eds.). 'Soil compaction and compression in relation to sugar beet production', pp. 104. *Advances in Sugar Beet Research IIRB* **1**, 43–54. ISBN: 2960018206.
Vero, S.E., Antille, D.L., Lalor, S.T.J. & Holden, N.M. 2014. Field evaluation of soil moisture deficit thresholds for limits to trafficability with slurry spreading equipment on grassland. *Soil Use and Management* **30**, 69–77.
Walkley, A. & Black, I.A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science* **37**, 29–38.
Way, T.R., Erbach, D.C., Bailey, A.C., Burt, E.C. & Johnson, C.E. 2005. Soil displacement beneath an agricultural tractor drive tire. *Journal of Terramechanics* **42**, 35–46.
Way, T.R., Kishimoto, T., Burt, E.C. & Bailey, A.C. 1997. Tractor tire aspect ratio effects on soil stresses and rut depths. *Transactions of the ASAE* **40**, 871–881.
Wood, R.K. & Burt, E.C. 1987. Thrust and motion resistance from soil-tire interface stress measurements. *Transactions of the ASAE* **30**, 1288–1292.

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Theory of motion of grain mixture particle in the process of aspiration separation

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Abstract. The paper describes the development of a mathematical model for the motion of a seed mixture particle in the aspiration channel of the separator after the particle passes the cone-shaped spreader and enters the workspace of the aspiration channel in the pneumatic dynamics and vibration unit devised by the authors. The unique feature of the proposed new design is the presence of the central pipe with sail members in the aspiration channel. The sail members in the air stream generate the self-oscillatory mode of motion of the central pipe, which results in the efficient separation of the grain seed mixture into the required fractions. On the basis of the prepared equivalent schematic model, the differential equations of the motion of a seed mixture particle in the process of aspiration separation have been generated. Basing on the results of the PC-assisted numerical modelling of the motion paths, on which the material particles (seeds) of the heavy and medium fractions travel, it has been established that they move on different courses, and the course of the heavy fraction seeds is such that, after they pass the cone-shaped spreader and advance further in the air stream through the space of the aspiration channel, they move closer to the pipe of the aspiration channel. Also, their velocities and accelerations are greater than the same kinematic parameters of the medium fraction seeds. The seeds of the light fraction move upwards under the action of the air stream and leave the aspiration separator at its top.

Key words: air flow, aspiration channel, grain seed mixture, material particle, separation.

INTRODUCTION

The necessary condition for the reliable storage of grain and seeds of various crops is the high quality of their postharvest handling, including the cleaning of the grain and seeds from the impurities that accompany them after the harvesting as well as the division of the heap into the required fractions. It should be noted that the separation of grain and seeds and their division into fractions in the state-of-the-art seed-cleaning machines is often done with the use of air flows. Also, this process is performed in aspiration channels of various designs, which, according to the data of experimental investigations that have been conducted, feature a number of shortcomings.

The original design for the aspiration channels did not ensure the uniform feeding of bulk grain inside the channel. Due to their intrinsic properties, the seeds of various forms of grain were difficult to separate into fractions using this design. Yields were negligible, so the separation of seed mixtures using these separators was mainly ineffective. Attempts to make aspiration channels of larger diameters also failed to solve the problem because, in that particular case, significant power was required to create the air flow for suction. In addition to this significant increase in power requirements for the process, fluctuations and losses of air pressure began to occur. That is why the use of such separators for ordinary seed mixtures was ineffective. Subsequently, the aspiration separation method was used only when separating seed material for planting and then only where the seeds as separate entities have better aerodynamic qualities, a higher sail factor, and so on. The shortcomings in the use of aspiration separators in separating such types of bulk grain also include a lack of precise separation of the seeds into fractions and the lack of their effective transportation out of the separation zone.

After the thorough analysis of numerous scientific papers (Panasiewicz, 1999; Panasiewicz et al., 2012; Kharchenko et al., 2017; Stepanenko, 2017; Khamyev et al., 2018; Badretidinov et al., 2019) on the topic of separating grain and seeds of various crops, the authors have come to a conclusion that the highest quality of the separation process is achieved in a vertical aspiration channel with the division of the fractions taking place at its bottom. In this case, high performance can be achieved in the classification of free-flowing dry masses and the possibility of effectively handling multiple-fraction mixtures is implemented.

In order to justify the rational design and kinematic parameters of the grain mixture separator devised by the authors, it is necessary to carry out a number of theoretical investigations, in particular, to develop the theory of motion of a grain mixture particle during aspiration separation.

The scientific research into the process of separating grain and grain crop seeds is represented in the papers (Proturayev & Franchuk, 1970; Burkov 1991; Kotov, 2002; Brăcăcescu et al., 2012; Kyurchev & Kolodiy, 2012; Kroulik et al., 2016; Brăcăcescu et al., 2019) The theoretical basis for the improvement of the process of the vibration separation of dry loose masses has been laid in the papers (Kotov, 2002; Saitov et al., 2016 and 2018; Bedretidinov et al., 2019), in which the process of pneumatic separation in the vertical version of the apparatus is investigated with the use of the principles of the nonlinear dynamics of biphasic media and also the said process is studied with the use of the equation of the seed's motion in an aerodynamic medium (Leshcenko et al., 2009; Kyurchev & Kolodiy, 2015).

The further studies on the said process have proved that the directional flow of air acts on the motion path of the particle (seed) mostly at the moment of its movement from the internal wall to the external one: in the central part of the aspiration channel the velocity of the air is maximal, while near the walls it is reduced, which is detrimental to the separation conditions (Bernik & Palamarchuk, 1996; Vasilkovsky et al., 2007). At the same time, the lower zone of the air stream is not used as a seed material separation factor, although the different soaring velocities of the grain fractions in this section of the separator provide a possibility of obtaining an additional effect of the improved sharpness of separation.

In view of the above-said, this paper presents a theoretical study on the process of the seed material separation in a vertical aspiration channel with bottom discharge, where the self-oscillatory motion of the separator's central pipe is generated in order to separate with high quality the motion paths of different fractions of the seed material.

The aim of the study is to improve the throughput capacity and the quality of loose grain mixture separation by means of theoretically substantiating the rational parameters of the aspiration seed separator.

MATERIALS AND METHODS

Based on the foregoing, some considerable changes can be made in the technological process that is involved in aspiration separation, making it possible to increase its effectiveness. The authors have developed a new design of the seed separator for grain and oil crops, in which the aspiration separation with the bottom separation of fractions is performed. The design and process schematic model of the separator is presented in Fig. 1 a, while Fig. 1 b provides the general view of the separator (Bulgakov et al., 2020).

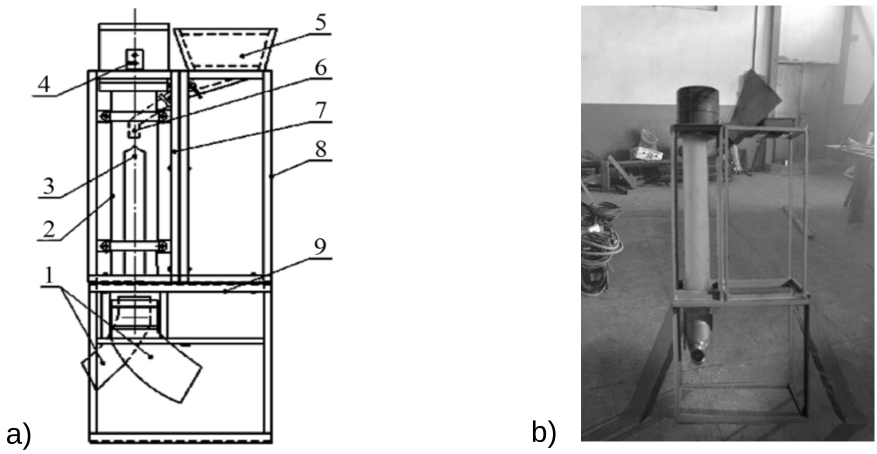

Figure 1. Aspiration seed separator: a – design and process schematic model: 1 – receiving bins for separated seeds, 2 – aspiration channel, 3 – cone-shaped seed spreader, 4 – fan, 5 – grain mixture hopper, 6 – feeding pipe, 7 – aspiration channel mounting frame, 8 – main frame, 9 – frame base plate (Kyurchev & Kolodiy, 2015); b – general view.

The primary operating device of the proposed vibration and aspiration separator is the aspiration channel 2 with a central pipe inside it. The top of the central pipe is equipped with the seed spreader 3. The aspiration channel 2 is mounted in the centre of the frame 7 contained inside the main frame 8. In the upper part of the separator, the fan 4 that induces the flow of air is installed. The air is sucked in at the bottom and moves upwards. Also, on top of the main frame 8, the grain mixture hopper 5 is situated, from which the mixture of seeds moves along the feeding pipe 6. In its bottom part, the main frame 8 contains the base plate 9, to which the receiving bins 1 for separated seeds are attached. The receiving bins 1 have two arched channels for discharging the seeds of the medium and heavy fractions in different directions.

The pneumatic and gravity separator operates as follows. In the process of separation, the grain mixture moves from the hopper 5 via the feeding pipe 6 mounted with a slope in order to facilitate the sliding of the seeds from the hopper 5 to the upper part of the central pipe of the aspiration channel 2. In this way, the grain mixture flows from above to the central pipe of the vertical aspiration channel 2 directly onto the cone-shaped seed spreader 3. The seeds of grain or oil crops are uniformly distributed by the spreader 3 on the radial directions of the cone, move downwards and, at the base of the cone, slip off, leaving the spreader 3, and enter the ring-shaped internal space around the central pipe of the aspiration channel 2. The exhaust fan 4 generates the air updraft, which splits the oncoming mass of seeds into different motion paths depending on their specific gravity. Lighter seeds (and light impurities) are entrained by the air and move upwards, while the seeds in the medium and heavy fractions sink downwards. In their sinking, the medium fraction seeds slightly change their course and arrive into one of the receiving bins 1, while the heaviest fraction seeds fall along such lines of motion that allow them to concentrate around the central pipe of the aspiration channel 2 most closely to its surface.

The shortcomings of the above pneumatic and gravity seed separator include its low throughput capacity, which is due to the absence of any means for intensifying the movement of seeds inside the aspiration channel 2 in order to facilitate the faster advancement of the already separated seeds towards the bottom under the action of external forces.

The vibration and pneumatic dynamics separator with a movable central pipe contained in the vertical aspiration channel represented by a fixed pipe of greater diameter, in which the central pipe is connected to the fixed pipe with elastic members, is an improved version of the above design. The movable pipe is equipped with sail members, which make the pipe perform self-oscillations under the action of the air flow in the aspiration channel and that, in its turn, results in the efficient separation of the grain seed mixture into the required fractions.

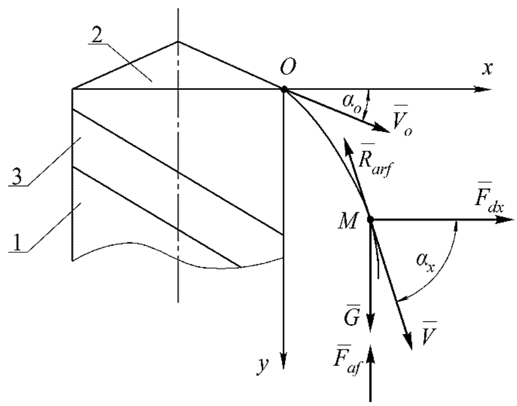

Figure 2. Equivalent schematic model of motion of material particle (seed) after its departure from cone-shaped seed spreader: 1 – separator central pipe; 2 – cone-shaped seed spreader; 3 – sail member.

The initial stage of the theoretical investigation is the analysis of the seed's motion dynamics after its departure from the cone-shaped seed spreader. The following assumptions have been admitted in the mathematical modelling of the separation processes in the above-mentioned section of the separator:

– seed material is fed into the vertical air flow that is uniformly distributed throughout the internal space of the aspiration channel at an initial angle of α_0 and an initial velocity of V_0;

– it is assumed that the case under consideration involves the motion of isolated seeds (material particles), the sizes and masses of which determine their soaring velocity;

– seeds do not change their symmetry axis alignments with respect to the direction of the air flow;

– air flow has a vertical upward direction.

The first step is to generate an equivalent schematic model of the material particle's (seed's) motion after its departure from the cone-shaped seed spreader. Such a schematic model is presented in Fig. 2.

The presented schematic model features the forces acting on the material particle (grain seed). These forces include, first of all: $\bar{F}_{af}$ – force applied by the air flow, $\bar{F}_{dx}$ – perturbing force component generated by the self-oscillatory motion of the separator's central pipe equipped with sail members.

SEPARATING PROCESS MODEL

In the previously completed research (Kotov, 2002), it has been proved that the force F_{dx} can be determined with the use of the following expression:

$$F_{dx} = \frac{l_x \cdot F_{af} \cdot \sin \omega t}{\sqrt{2} \cdot l}, \tag{1}$$

where l_x – displacement of the centre of the separator central pipe cross-section from the vertical axis; ω – angular velocity of the rotation of the separator central pipe during its self-oscillations with respect to the vertical axis; l – maximum possible displacement of the separator central pipe cross-section from the vertical axis; t – arbitrary instant of time.

Further, the expression has to be written down for the air resistance force $\bar{R}_{arf}$ that acts during the motion of the seed particle in the air flow. Subject to the air flow velocity staying at a sufficiently moderate level, that is, below or at 8.0 m s^{-1}, the force under consideration can be determined with the use of the following well-known expression:

$$R_{arf} = \xi \cdot S_M \cdot \frac{V^2}{2} \cdot \rho, \tag{2}$$

where S_m – area of the midsection of the particle; ξ – medium resistance factor: for an ellipsoid shape of the seed ξ = 0.04; ρ – air density; V – velocity of the particle in the air flow.

It must be noted that, upon entering the air space of an aspiration channel, and after leaving the cone-shaped seed spreader, a material particle (a seed) starts its journey at a speed of V_0 in a counter-flowing air current and, as a result, its movement speed V starts to decrease. The direction of the velocity vector $\bar{V}$ of the material particle (seed) motion in the air space of the aspiration channel is defined by the angle a_x between the vector under consideration $\bar{V}$ and the Ox axis.

As is known, the force vector $\bar{R}_{arf}$ has a line of action opposite to the direction of the velocity vector $\bar{V}$.

The material particle (seed) is also under the action of the particle weight force $\bar{G}$ which, as is known, has the following magnitude:

$$G = mg, \tag{3}$$

where m – mass of the particle; g – acceleration of gravity.

The next step is to analyse the motion of the particle (seed) in the absolute coordinate system *Oxy*, the origin of which (point *O*) is situated at the place, where the material particle (seed) leaves the cone-shaped seed spreader and enters the stream of air, the *Ox* axis is directed horizontally to the right, the *Oy* axis – vertically downwards.

In accordance with the schematic model of forces presented in Fig. 1 and on the basis of Newton's second law, a system of differential equations can be generated, which will represent the motion of the material particle (seed) in the air flow after its departure from the cone-shaped seed spreader in the projections on the *Ox* and *Oy* coordinate axes:

$$\left.\begin{aligned} m\ddot{x} &= -R_{arf}\cdot\cos\alpha_x + F_{dx}, \\ m\ddot{y} &= -R_{arf}\cdot\sin\alpha_x - F_{af} + G, \end{aligned}\right\} \tag{4}$$

where m – mass of the particle, $\ddot{x}$, $\ddot{y}$ – components of the particle's acceleration along the *Ox* and *Oy* axes, respectively.

In view of the fact that:

$$V^2 = \dot{x}^2 + \dot{y}^2, \tag{5}$$

and

$$\cos\alpha_x = \frac{\dot{x}}{V} = \frac{\dot{x}}{\sqrt{\dot{x}^2+\dot{y}^2}}, \tag{6}$$

$$\sin\alpha_x = \frac{\dot{y}}{V} = \frac{\dot{y}}{\sqrt{\dot{x}^2+\dot{y}^2}}, \tag{7}$$

and taking into account the expression (2), the following is arrived at:

$$R_{arf}\cdot\cos\alpha_x = \xi\cdot S_M\cdot\rho\cdot\frac{\left(\dot{x}^2+\dot{y}^2\right)}{2}\cdot\frac{\dot{x}}{\sqrt{\dot{x}^2+\dot{y}^2}}, \tag{8}$$

or

$$R_{arf}\cdot\cos\alpha_x = \frac{1}{2}\xi\cdot S_M\cdot\rho\cdot\sqrt{\dot{x}^2+\dot{y}^2}\cdot\dot{x} \tag{9}$$

Similarly, the following is obtained:

$$R_{arf}\cdot\sin\alpha_x = \frac{1}{2}\xi\cdot S_M\cdot\rho\cdot\sqrt{\dot{x}^2+\dot{y}^2}\cdot\dot{y}, \tag{10}$$

After the expressions (1), (3), (9) and (10) are substituted into the system of differential equations (4) and both sides of the equations are divided by the particle's mass *m*, the result is:

$$\left.\begin{aligned} \ddot{x} &= \frac{l_x\cdot F_{af}\cdot\sin\omega t}{\sqrt{2}\cdot m\cdot l} - \frac{1}{2m}\xi\cdot S_M\cdot\rho\cdot\sqrt{\dot{x}^2+\dot{y}^2}\cdot\dot{x}, \\ \ddot{y} &= -\frac{F_{af}}{m} + g - \frac{1}{2m}\xi\cdot S_M\cdot\rho\cdot\sqrt{\dot{x}^2+\dot{y}^2}\cdot\dot{y}. \end{aligned}\right\} \tag{11}$$

In order to simplify the writing of the system of differential Eqs (11), the following designations are introduced:

$$B_1 = \frac{\xi\cdot S_M\cdot\rho}{2}, \qquad B_2 = \frac{l_x\cdot F_{af}}{\sqrt{2}\cdot l}. \tag{12}$$

By substituting the expressions (12) into the system of differential Egs (11), the following is obtained:

$$\left.\begin{aligned} \ddot{x} + \frac{B_1}{m} \cdot \sqrt{\dot{x}^2 + \dot{y}^2} \cdot \dot{x} = \frac{B_2}{m} \sin \omega t, \\ \ddot{y} + \frac{B_1}{m} \cdot \sqrt{\dot{x}^2 + \dot{y}^2} \cdot \dot{y} = g - \frac{F_{af}}{m}. \end{aligned}\right\} \quad (13)$$

The system of differential Egs (13) is a system of nonlinear differential equations of second order, which, as is known, cannot be solved by quadrature. It can only be solved with the use of numerical methods (for example, the Runge-Kutta method), by operating the PC in the MathCAD problem-solving environment. The numerical solving provides a result in the form of the graphical relations between the motion of the particle along the axes *Ox* and *Oy* and the time *t*.

The system of Egs (13) has to be solved under the following initial conditions:

at $t = 0$:

$$\dot{x}_o = V_o \cdot \cos\alpha_o,\ \dot{y}_o = V_o \cdot \sin\alpha_o,\ x_o = 0,\ y_o = 0. \quad (14)$$

The initial movement speed $V_o$of a material particle (seed) is determined by its speed of movement along the side of the cone-shaped spreader, which is where it immediately ends up. Therefore, while moving along the cone-shaped spreader's side, the particle is not influenced by anything at all because the entering air flow does not affect it (the particle). But, immediately after the particle leaves the cone-shaped spreader and even under the conditions of a negligible deviation from the central pipe, the vector of the particle's initial speed $\bar{V}_o$ is strictly parallel to the cone's side and has a deviation from the horizontal plane at an angle of α_o. Upon any further movement taking place, the particle acquires the speed *V* as stated above. In projections to the accepted coordinate axes, the value of the initial speed is determined from expressions (14).

However, by using the experimental data, the system of differential Eqs (13) can be reduced to the linear form and solved by quadrature. That will result in obtaining the analytical solution that is sufficient for practical purposes.

On the basis of experimental investigations, the following has been arrived at:

$$V_x = \dot{x} = (0.15...0.20)\ \text{m}\cdot\text{s}^{-1},$$

$$V_y = \dot{y} = (0.70...0.80)\ \text{m}\cdot\text{s}^{-1}.$$

Then:

$$V = \sqrt{0.15^2 + 0.70^2}...\sqrt{0.20^2 + 0.80^2} = \sqrt{0.513}...\sqrt{0.680} =$$

$$= (0.72...0.82)\ \text{m}\cdot\text{s}^{-1}.$$

The next step is to substitute the left (minimal) values of the intervals obtained for $\dot{x}$, $\dot{y}$ and *V* into the system of equations (13), then to do the same with the right (maximal) values and, by solving the generated systems of equations by quadrature, the respective value ranges are obtained for the displacements of the particle along the *Ox* axis and the *Oy* axis.

Consequently, assuming that $\dot{x} = V_x = \text{const}$, $\dot{y} = V_y = \text{const}$ and $V = \text{const}$, the following system of equations is obtained instead of the system (13):

$$\left.\begin{aligned} \ddot{x} &= -\frac{B_1}{m}\cdot V\cdot V_x + \frac{B_2}{m}\sin\omega t, \\ \ddot{y} &= -\frac{B_1}{m}\cdot V\cdot V_y + g - \frac{F_{af}}{m}. \end{aligned}\right\} \quad (15)$$

The result of the first integration is:

$$\left.\begin{aligned} \dot{x} &= -\frac{B_1}{m}\cdot V\cdot V_x\cdot t - \frac{B_2}{m\cdot\omega}\cos\omega t + C_1, \\ \dot{y} &= \left(-\frac{B_1}{m}\cdot V\cdot V_y + g - \frac{F_{af}}{m}\right)\cdot t + L_1, \end{aligned}\right\} \quad (16)$$

where C_1 and L_1 – arbitrary constants.

The result of the second integration is:

$$\left.\begin{aligned} x &= -\frac{B_1}{2m}\cdot V\cdot V_x\cdot t^2 - \frac{B_2}{m\cdot\omega^2}\sin\omega t + C_1\cdot t + C_2, \\ y &= \left(-\frac{B_1}{m}\cdot V\cdot V_y + g - \frac{F_{af}}{m}\right)\cdot\frac{t^2}{2} + L_1\cdot t + L_2, \end{aligned}\right\} \quad (17)$$

where C_2 and L_2 – arbitrary constants.

Therefore the authors have rendered the nonlinear system of differential equations into a more simple scheme of differential equations (15) with constant coefficients. The solution for the system (15) - the first integral (16) and the second integral (17) - made it possible to undertake the necessary transformations and to achieve simple equations, describing with sufficient precision the movement of a material particle (seed) under the influence of the forces being applied to it inside the aspiration channel.

The above arbitrary constants can be found using the initial conditions (14):

$$C_1 = V_o\cos\alpha_o + \frac{B_2}{m\cdot\omega},\; L_1 = V_o\cdot\sin\alpha_o,\; C_2 = 0 \text{ and } L_2 = 0. \quad (18)$$

After (18) is substituted into (16), the following is obtained:

$$\left.\begin{aligned} \dot{x} &= -\frac{B_1}{m}\cdot V\cdot V_x\cdot t - \frac{B_2}{m\cdot\omega}\cos\omega t + V_o\cdot\cos\alpha_o + \frac{B_2}{m\cdot\omega}, \\ \dot{y} &= \left(-\frac{B_1}{m}\cdot V\cdot V_y + g - \frac{F_{af}}{m}\right)\cdot t + V_o\cdot\sin\alpha_o. \end{aligned}\right\} \quad (19)$$

The result of substituting (18) into (17) is:

$$\left.\begin{aligned} x &= -\frac{B_1}{2m}\cdot V\cdot V_x\cdot t^2 - \frac{B_2}{m\cdot\omega^2}\sin\omega t + \\ &+\left(V_o\cdot\cos\alpha_o + \frac{B_2}{m\cdot\omega}\right)\cdot t, \\ y &= \left(-\frac{B_1}{m}\cdot V\cdot V_y + g - \frac{F_{af}}{m}\right)\cdot\frac{t^2}{2} + V_o\cdot\sin\alpha_o\cdot t. \end{aligned}\right\} \quad (20)$$

The systems of Eqs (19) and (20) represent the laws of variation for the velocity and the displacement of the material particle (seed), respectively, as functions of time with due account for the design, kinematic and dynamic parameters of the aspiration separator.

This way, the transformations that have been carried out made it possible to reach systems that involved differential Eqs, (19) and (20), which are the most suitable equations for solving the problem digitally, on a PC.

In order to carry out the PC-assisted numerical calculations, it is necessary to define the initial and boundary conditions. For example, the initial velocity V_o of the motion of the material particle (seed) at the instant of its departure from the surface of the cone-shaped seed spreading unit was determined by the authors on the basis of the experimental data for various types of seeds and was found to be within the range of $V_o = 0.70$–0.80 m s^{-1}. For the numerical calculation purposes, it is assumed that $V_o = 0.75$ m s^{-1}.

This initial speed value was determined by the authors for individual sunflower seeds after decoding the video recording of the seed's movement along the side of the cone-shaped spreader and processing it using the appropriate software on a PC. The initial deflection angle of the initial velocity vector V_o, that describes the motion of the material particle (seed) at the instant, when it leaves the surface of the cone-shaped seed spreader, is taken to be equal to $a_o = 20°$. This angle is exactly determined by the factual dimensions of the cone-shaped seed spreader that was used by the authors, ie. the angle α_o is determined by the inclination angle of the side of the cone-shaped seed spreader to the horizon.

Further, the force generated by the air stream F_{af} is to be determined. Basing on the air compressor power rating N, the force generated by the air stream F_{af} is equal to:

$$F_{af} = \frac{N}{V_{af}} \tag{21}$$

where $V_{af} = (4.5$–$5.5)$ m s^{-1} – velocity of the air flow, which is taken to be equal to $V_{af} = 5.0$ m s^{-1} for the calculation purposes; $N = 150.0$ W.

Hence, the air flow force value equal to $F_{af} = 30.0\ N$ is assumed for the numerical calculations.

The angular velocity ω of the aspiration separator pipe rotation, which is determined with the use of the expression $\omega = V_{af} \cdot (r_D)^{-1}$, under the condition that the pipe radius r_d is equal to 0.10 m, has the following magnitude: $\omega = 50.0$ s^{-1}. The other parameters used in the numerical calculations are assigned the following values:
$m = 0.068$ g – mass of the material particle (seed); $L_x = 0.010$ m; $l = 0.020$ m – linear displacements of the central pipe of the aspiration separator.

RESULTS AND DISCUSSION

Basing on the analytic expressions (19) and (20), PC-assisted numerical calculations have been carried out and the graphical relations have been obtained for the material particle (seed) motion paths, the velocity and acceleration of the particle (seed) as functions of time t at specific values of the design, kinematic and dynamic parameters of the aspiration grain heap separator. When carrying out the PC-assisted numerical calculations, the following values were assumed by the authors for the mass of a single grain crop seed: 0.020 grams in the light fraction; 0.040 grams in the medium fraction; 0.080 grams in the heavy fraction. These single grain crop seed masses are the most effectively-separated fractions when using the aspiration separator that has been developed by the authors. The above-mentioned graphical relations are presented in Fig. 3–6.

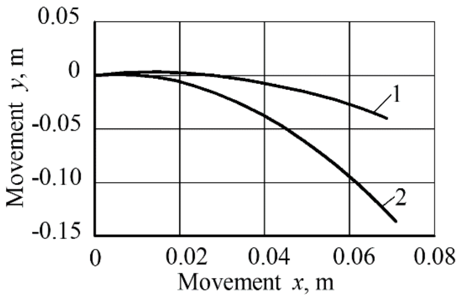

Figure 3. Material particle (seed) motion path in plane *Oxy*: 1 – medium fraction seeds; 2 – heavy fraction seeds.

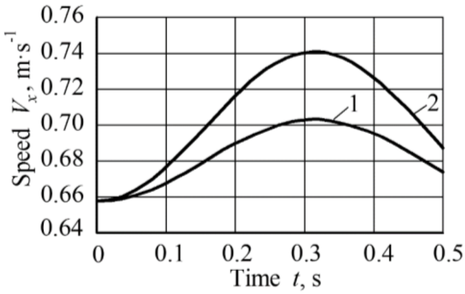

Figure 4. Variation of material particle (seed) velocity V_x as function of *t* relative to *Ox* axis: 1 – medium fraction seeds; 2 – heavy fraction seeds.

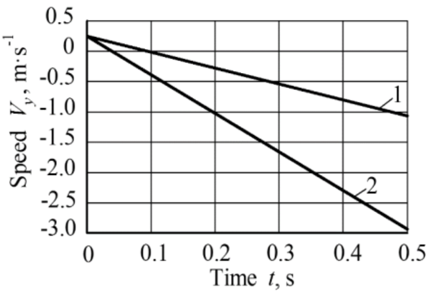

Figure 5. Variation of material particle (seed) velocity V_y as function of *t* relative to *Oy* axis: 1 – medium fraction seeds; 2 – heavy fraction seeds.

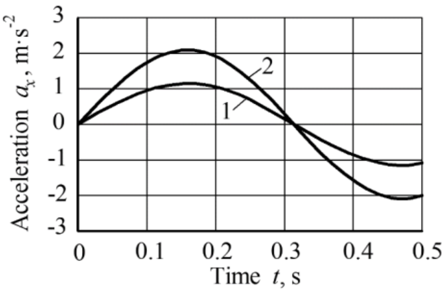

Figure 6. Variation of material particle (seed) acceleration α_x as function of *t* relative to *Ox* axis: 1 – medium fraction seeds; 2 – heavy fraction seeds.

As is obvious from the presented graphs, the motion path of the material particle (seed), the diagram of which is shown in Fig. 3a, has such a curvilinear shape that the heavy fraction seeds virtually do not translate along the *x* axis, but concentrate in their downward movement close to the central pipe of the aspiration channel. Moreover, within the time interval under consideration, the increase of the values of the *y* coordinate is accompanied by only insignificant variation of the x coordinate for both the masses. The projections of the velocities on the respective coordinate axes *x* and *y* also demonstrate different behaviours (Figs 4 and 5). For example, at the initial instant of the departure of the particle (seed) from the cone of the seed spreader, the variation of the particle's motion along the *y* axis is slow, while the variation of the velocity projection on the *x* axis is more intensive. The pattern of variation followed by the acceleration of the particle (seed), as shown in Fig. 6, is similar to that followed by the velocity projection on the same axis *x*. After that, the process of the free fall of the material particle (seed) in the space of the aspiration channel under the action of the air flow starts together with the very process of the seeds being separated along different motion paths in the bottom part of the aspiration channel, depending on the fraction of the seeds. Thus, as is seen in the presented graphic relations, the velocities and accelerations of the heavy fraction seeds are greater after their departure from the cone-shaped seed spreader and during their further movement in the air flow in the space of the aspiration channel. As a result of that, the heavy fraction seeds move securely closer to the aspiration channel central pipe, while the medium fraction seeds – farther from it. At the same time, the light fraction seeds move upwards and the stream of air carries them away outside the aspiration separator.

CONCLUSIONS

1. The mathematical model has been developed for the motion of the particle after its departure from the cone-shaped seed spreader and the system of differential equations has been generated for the motion of the material particle (seed) at this stage of movement.

2. The analytical solution has been obtained for the system of differential equations of the motion of the particle in the vertical air flow of the aspiration channel, which has enabled obtaining with the assistance of the PC the graphical relations for the particle's motion path, its velocity and acceleration after its departure from the cone-shaped seed spreader as functions of time at specific values of the design, kinematic and dynamic parameters of the aspiration separator.

3. As has been shown by the results of the PC-assisted numerical modelling of the motion paths of the material particles (seeds) in the heavy and medium fractions, they move on different paths, that is, the heavy fraction seeds, after their departure from the cone-shaped seed spreader and during their further advancement in the air flow in the space of the aspiration channel, move closer to the pipe in the aspiration channel. Also, their velocities and accelerations are greater, than the same kinematic characteristics of the medium fraction seeds. At the same time, the light fraction seeds travel under the action of the air flow upwards and outside from the aspiration separator.

REFERENCES

Badretidinov, I., Mudarisov, S., Tuktarov, M., Dick, E. & Arslanbekova, S. 2019. Mathematikcal modelling of the grain material separation in the pneumatic system of the grain cleaning machine. *Journal of Applied Engineering Scienve* **17**(4), 529–534. doi: 10.5937/jaes17-22640

Bernik, P.S. & Palamarchuk, I.P. 1996. Conveyor vibration machines for finishing and handling treatment. Kiev, Võshaja škola, 237 pp.

Brăcăcescu, C., Pirna, I., Sorica, C., Popescu, S. & Stan, O. 2012. Experimental researchon influence of functional parametrs of gravity separator on quality indicators of separation process with application on cleaning of wheat seeds. *Engineering for Rural Development* **11**, 16–21.

Brăcăcescu, C., Tenu, I., Mircea, C. & Bunduchi, G. 2019. Experimental research on influence of functional parameters of combined installations designed at separating the impurities out of cereal seeds. E35 Web of Conferences, Vol 112, Article no 03004, 8th *Int Conf on Thermal Equipment, Renewable Energy and Rural Development*. doi: 10.1051/e3sconf/201911203004

Bulgakov, V., Nikolaenko, S., Holovach, I., Adamchuk, V., Kiurchev, S., Ivanovs, S. & Olt, J. 2020. Theory of grain mixture particle motion during aspiration separation. *Agronomy Research* **18**(1), 18–37. doi.10.15159/AR.20.057

Burkov, A.I. 1991. Research into work process of closed pneumatic system in seed-cleaning machine. Thesis for degree of Candidate of Sciences. Kirov, 217 pp.

Khamyev, V., Gulyev, A. & Boiko, A. 2018. Justification of the design of pneumatic sorting machine for the preparation of selection seeds. *MATEC Web of Conferences* **224**, 6 pp. doi: 10.1051/matecconf/201822405008

Kharchenko, S. Borshch, Y. & Abduev, M. 2017. Efficiency of stratification of grain mixes at their cleaning in the pneumo separating channels of grain separators. *National interagency scientific and technical collection. Design, production and operation of agricultural machines* **47**(1), Kropyvnytskyi: TsNTU, pp. 253–260.

Kotov, V.V. 2002. Determining velocity of grain in air flow. *Technologies and Mechanical Means in Field Husbandry*, Zernograd, pp. 137–140.

Kroulik, M., Hula, J. & Rybka, A. 2016. Pneumatic conveying characteristics of seeds in a vertical ascending airstream. *Research in Agricultural Engineering* **62**(2), 56–63.

Kyurchev, S.V. & Kolodiy, A.S. 2012. Analysis of methods to improve agricultural crop capacity and requirements to separated material. In: *Proceedings of Scientific Papers,* Vinnitsa National Agrarian University, **11**(2), pp. 322–327.

Kyurchev, S.V. & Kolodiy, A.S. 2015. Multi-criterion analysis of existing seed separators with different operating devices. In: *Bulletin of Kharkiv National Technical University of Agriculture* **156**(1), 86–92.

Leshchenko, S.M., Vasilkovsky, A.N., Vasilkovsky, M.I. & Gontšarov, V.V. 2009. Raising efficiency of pre-treatment of grain mixtures. In: *Agricultural Machinery*, Lutsk, **18**, pp. 230–234.

Panasiewicz, M. 1999. Analysis of the pneumatic separation process of agricultural materials. *International Agrophysics* **13**(2), 233–239.

Panasiewicz, M., Sobczak, P., Mazur, J., Zawiślak, K. & Andrejko, D. 2012. The technique and analysis of the process of separation and cleaning grain materials. *Journal of Food Engineering* **109**(3), 603–608. doi: 10.1016/j.jfoodeng.2011.10.010

Poturayev, V.N. & Franchuk, V.P. 1970 Selected theoretical prerequisites for research into mechanics of motion of thick loose load layer under the action of vibration. Kiev, *Naukova Dumka*, pp. 173–181.

Saitov, V.E., Farafanov, V.G., Gataullin, R.G. & Saitov, A.V. 2018. Research of a diametrical fan with suction channel. *IOP Conf. Series: Materials Science and Engineering* **457**, 6 pp, doi: 10.1088/1757-899X/457/012009

Saitov, V.E., Kurbanov, R.F. & Suvorov, A.N. 2016. Assessing the Adequacy of Mathematical Models of Light Impurity Fractionation in Sedimentary Chambers of Grain Cleaning Machines. *Procedia Enginering* **150**, 107–110. doi: 10.1016/j.proeng.2016.06.728

Stepanenko, S. 2017. Research pneumatic gravity separation grain materials. In: *Scientific Proseedings V International scientific-technical conference 'Agricultural machinery'*, **2**, 143–145.

Vasilkovsky, M.I., Goncharova, S.J. & Leshchenko, S.M. 2007. Substantiation of parameters for grain separation in inclined air flow. Design, Production and Operation of Agricultural Machines: *Nationwide Interagency Research and Technology Collected Papers*. Kirovograd National Technical University, Kirovograd, **37**, 132–137.

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Assessment of the methane emission for different typologies of fattening swine facilities in the department of Antioquia – Colombia

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Abstract. The explosive growth of swine production at high stocking densities in confinement farming worldwide, has raised concerns the environmental impact, health and livestock productivity and the production of associated gases in this type of large-scale farms. The aim of this paper was to study the methane gas concentration and emissions of ten different typologies of swine production installations. The facilities were in the department of Antioquia - Colombia, they were located between 800–2,300 meters above sea level (m.a.s.l.) of heights, they mainly employed natural ventilation as refrigeration strategy and they were used for pigs in fattening stage. Methane measurements were taken at animal height. Sensors were located at intermediate points of the ventilation inlet and outlet areas. The behaviour of methane concentration and emission of the facilities were analysed along with the correlation and temporal evolution of climatic variables, comfort indices and construction typologies. The information was analysed using *descriptive statistics*, *analysis of variance (ANOVA)* and *principal component analysis (PCA)*. Were found an *average* of CH_4 Emission Rate (ER) per facility (kg year^{-1}) of 607.9, Global Warming Potential (GWP) per facility (kg year^{-1}) of 15,197.42 and significant correlations between ER and cleaning frequency (CF), animal unit (AU), air flow (Q), animal density(AD) and relative humidity (RH) were evidenced. This is the first research reported in Colombia, that will be important to create some governmental policies.

Key words: typologies of construction, methane emissions, natural ventilation, greenhouse gases, swine production.

INTRODUCTION

The agricultural sector is regarded as the highest user and administrator of natural resources, and as such, is considered to have a high impact on the environment due to its capability of reducing greenhouse gasses emissions (GHG) (Lenerts et al., 2019). GHG emissions represent losses of energy, nitrogen and organic matter for the livestock sector. Consequently, there is a strong link between emission intensity and effective use of resources, and most mitigation interventions will lead to improved efficiency in the use of resources throughout the sector's supply chains (Gerber et al., 2013). developing countries still have to take measures to improve the use of natural resources the potential to reduce 70% of GHG (Gitz et al., 2016) . According to the third national statement of climate change of Colombia, activities such as agriculture, forestry and other uses of the land provide an approximate of 43% of the GHG (70 $MtCO_2eq$), waste 8%, industrial processes and product use 5% and energy 44% (IDEAM, PNUD, MADS, DNP 2015). Livestock production under confinement optimizes the land usage, however, increases the volume of residues (Briukhanov et al., 2017) that are produced and transformed into gases. These gases are grouped according to their importance in climate change and human health (Petersen et al., 2016). According to the Food and Agricultural Organization (FAO) the livestock sector is estimated to provide 6.2 Gt of CO_2 equivalent (CO_2eq) per year, which represents 14.5% of human induced, of the total emissions of the livestock sector. Swine production generates 0.7 Gt of CO_2eq, which sums up to 9% (Gerber et al., 2013). At the livestock sector, the swine production is at the third-place following by beef and dairy products, a trend that follows the European model, where swine production represents 26% of livestock GHG emissions (Noya et al., 2016). There are other authors who affirm that pig production could have a greater contribution of GHG than milk production, as is the case of De Vries & de Boer, (2010) who consider it should be in second place after beef, with an emission rate between 3.9 to 10 kg of CO_2eq per kilogram of animal. Likewise Noya et al. (2017) stated that swine products are second highest GHG generators amongst meat produce. This has become evident by studies on life cycles developed by Reckmann et al. (2013), Noya et al. (2017) and Noya et al. (2016) in which CO_2eq production per kilogram of meat produced in Germany is 3.22, 3.42 and a last rank of 2.30–3.30 kg, respectively. Monogastric animals as poultry and pigs have had a considerable growth in consumption with a 2.8% annual increase (Steinfeld & Gerber 2010), being the highest meat product consumed in European regions (Noya et al., 2016) and overall worldwide with a growth projection reaching 40% (Nations Food and Agriculture - FAO 2011), which represents a big challenge.

According to de Vries & de Boer (2010) monogastric animals have a higher Global Warming Potential- GWP (It is a relative measure of how much heat can be trapped by a given greenhouse gas, compared to a reference gas, usually carbon dioxide), generally determined by N_2O and CH_4 emissions due to manure management. N_2O and CH_4 are important contributors considering their GWP in the lapse of a century is 298 and 25 times, respectively, higher than CO_2 (IPCC. 2007). GHG generation according to the chain of production go as follows: 1) Animal feed production with 48% of emissions, 2) Manure management and storage with 27.4%, most of it as CH_4 (19.2%), 3) Livestock and livestock feed transportation with a moderate contribution of 5.7% and lastly 4) Farming energy consumption with barely 3.5% of the total emissions. (Gerber et al., 2013). For Gert J. M., Bannink, A. & Chadwick, D. (2006), in monogastric animals, such as pigs, the CH_4 is produced mostly in the large intestine, however, the stockpiled manure in underground pits, outdoors and on the shed floors is also relevant source under the following conditions: 1) The temperature and ventilation rate increase the work of methanogenic bacteria which transform acetate, CO_2 and H_2 into methane in a thermophilic environment, 2) Storage time, 3) Optimum pH close to neutral (Philippe et al., 2011), 4) High levels of degradable organic matter, and 5) High amounts of humidity which facilitate methanogenesis in both the liquid and solid phases of manure. Likewise

Philippe & Nicks (2015) provides that emissions of CO_2, CH_4 and N_2O contribute 81, 17 and 2% of the total emissions in housing, representing 3.87, 0.83, 0.17 kg of CO_2eq per carcass, respectively. The value of CH_4 generation in housing by an animal in the fattening stage is 16.7 g CH_4 per day, a period that makes for 70% of the total emissions, while periods of gestation, lactation and weaning contribute approximately 10% of the total emissions.

There are currently regulations for toxic gases such as ammonia, however, it was not possible to find evidence of a regulation that can limit emissions of GHG from animal housings. Additionally, the existing legislation is limited to requesting reports, such is the case of the European Union, where producers are requested to report yearly CH_4 emissions higher than 100,000 kg and Israel when it's higher than 10,000 kg a year (Bjerg et al., 2019). There are few studies worldwide regarding the measurement of CH_4 concentrations in swine population in tropical climates, in which the animal housing works on natural ventilation most of the year. The studies, that have been made to focuses on typologies of construction in slurry pits, as shown in the study of Petersen et al. (2016), where 7 housing types were evaluated in Denmark and where it was discovered that the current estimates of CH_4 emissions from pig and cattle manure management sit at 0.032 and 0.015 kg CH_4, respectively, indicating that liquid manure pits in animal confinements are a significant source. Another frequent analysis is the evaluation of the types of floor in the animal housing and their GHG generation, such is the case in Belgium, where evaluated fattening pigs' production on deep litter system and in the straw-flow system (Philippe et al., 2012). One more study developed in North Carolina by Sharpe et al. (2001) in controlled ventilation farms and liquid manure pits under animal confinements. Their results showed that during the cold winter measurement period, CH_4 fluxes averaged 6.9 g CH_4 animal^{-1} d^{-1} and during summer measurement periods, CH_4 fluxes were much greater and averaged 33 g CH_4 animal^{-1} d^{-1}. Several studies have been carried out for NH_3 emissions in Latin America, in Antioquia, Colombia studies have been made on poultry houses (Osorio-Saraz et al., 2014), while in Brazil the focus has been on swine and fishing facilities (Cecchin et al., 2017). None of these reports were made for CH_4 studies. Within this context, this paper had the goal of studying the concentrations and emissions of CH_4 in fattening pigs' facilities, where is difficult to separate GHG emissions made directly by animals from the ones made by manure. As a result, the emissions of animals and their housing are grouped in one category: Emissions of housing (total emissions from livestock and manure within the facilities) (Sedorovich et al., 2007). This paper is one of the first to make such approach in the country.

MATERIALS AND METHODS

This study was conducted in Antioquia, Colombia, where most of the country´s swine production is made (30.7%), in fattening livestock alone the percentage is 35.5%, which equals about 1,394,769 animals, this according to the last survey made by the National Administrative Department of Statistics (Departamento Administrativo Nacional de Estadística (DANE) 2016). The design of the investigation takes an observational approach, given that no variables were affected on the studied units. We evaluated a total of 10 commercial farms that produce pigs in fattening stage in different towns and altitude variables of Antioquia. All of the typologies of construction observed had natural ventilation and their individual characteristics are represented in Table 1.

Table 1. Characteristics of each typology

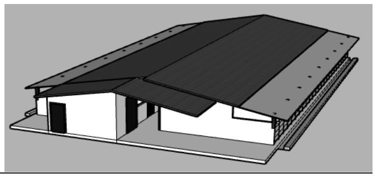
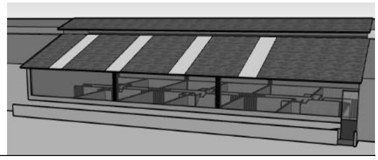
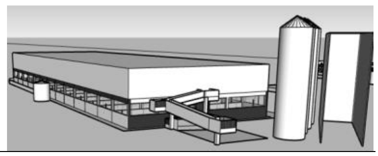
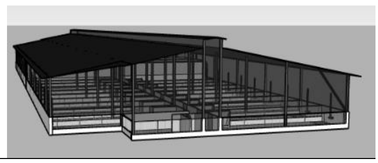
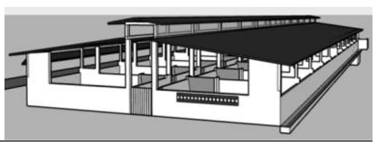
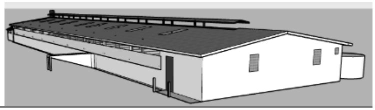
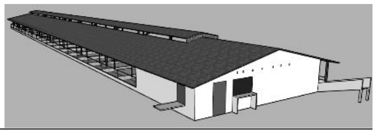
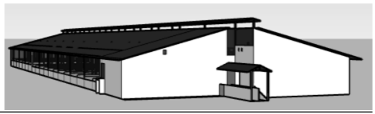
Typology 1	Climate classification: Cold Height above sea level: 2,174 m Temperature: 21.2 °C Relative Humidity: 76% Animal Units: 18.7 Cleaning Frequency: 0	
Typology 2	Climate classification: Mild Height above sea level: 1,179 m Temperature: 27.8 °C Relative Humidity: 67.8% Animal Units: 31.0 Cleaning Frequency: 1	
Typology 3	Climate classification: Mild Height above sea level: 1,481 m Temperature: 25.9 °C Relative Humidity: 65.8% Animal Units: 30.2 Cleaning Frequency: 1	
Typology 4	Climate classification: Cold Height above sea level: 2,202 m Temperature: 22.7 °C Relative Humidity: 64.4% Animal Units: 23.9 Cleaning Frequency: 2	
Typology 5	Climate classification: Mild Height above sea level: 1,504 m Temperature: 26.0 °C Relative Humidity: 63.0% Animal Units: 137.8 Cleaning Frequency: 2	
Typology 6	Climate classification: Warm Height above sea level: 816 m Temperature: 29.1°C Relative Humidity: 62.2% Animal Units: 66.5 Cleaning Frequency: 2	
Typology 7	Climate classification: Mild Height above sea level: 1,732 m Temperature: 25.0 °C Relative Humidity: 64.3% Animal Units: 73.8 Cleaning Frequency: 2	

Table 1 (continued)

Typology 8	Climate classification: Cold Height above sea level: 2,236 m Temperature: 21.8 °C Relative Humidity: 66.7% Animal Units: 72.1 Cleaning Frequency: 1	
Typology 9	Climate classification: Mild Height above sea level: 1,408 m Temperature: 26.2 °C Relative Humidity: 62.2% Animal Units: 49.5 Cleaning Frequency: 1	
Typology 10	Climate classification: Mild Height above sea level: 1,112 m Temperature: 28.4 °C Relative Humidity: 60.6% Animal Units: 164.9 Cleaning Frequency: 2	

* Cleaning Frequency = 0) When the floor is completely evacuated, 1) Daily and 2) Every two days.

Climate description:

Given the varied topography of Antioquia, most of the climate variants are represented, the observed farms were located between 800 and 2,300 meters above sea level, which in turn offers thermal samples of cold, mild and warm climates according to the climate classification from Caldas-Lang. Table 1 shows details of the location of each typology.

Technical Management of the farms:

The research objects correspond to commercial farms of pig fattening, animals were a cross between Landrace and Yorkshire breeds, genetically known as F1 (First generation of a cross of pure breeds). All the observed farms had the same nutritional management of balanced feed made of: 1) Energy sources such as corn, oils, fats and agricultural subproducts, 2) Proteins of vegetal origins, which includes mostly soy and animal flour made of fish, meat, bones and dairy derivatives, and 3) Vitamin and mineral supplements.

Characteristics of the Facilities

Ventilation of these installations is natural procured through windows. procured through windows. In cool weathers lateral curtains were employed, while in mild and warmer conditions half-wall and wall less structures were employed respectively.

Additionally, Seven Typologies presented over roof, all floors were made of smooth concrete in each facility. In Table 1 are presented the average values of some variables and the characteristics of each typology.

Table 2. Typology classification according to Climate classification

Climate classification (masl)	Typology
Warm (0–1,000)	6
Mild (1,000–2,000)	2, 3, 5, 7, 9, 10
Cold (2,000–3,000)	1, 4, 8

The different typologies were classified by thermal variations as shown in Table 2, where most of the farms were located in mild climates, which concurs with the reality of swine production in Colombia.

Timeframe for measurements

The fieldwork for this paper was conducted during the months of May, June and July of 2019. It was reported that the first trimester in which April-May had abundant and frequent rains, with a value higher than 300 millimeters, this according to the information provided by the Institute of Hydrology, Meteorology and Environmental Studies (Instituto de Hidrología, Metereología y Estudios Ambientales – IDEAM) and the agriculture ministry of Colombia (Ministerio de Agricultura y Desarrollo Rural (MADR) 2019).

Features of equipment used

A custom-made low cost CH_4-gas measurement system (MGMS) was developed for monitoring purposes. Employing the sensors detailed in Table 3, an Adruino based gas monitoring system was implemented, as can be seen in Fig. 1. Metal oxide semiconductor sensors were employed for measuring CH_4, along with air temperature, relative humidity, atmospheric pressure and air velocity. Data was saved every 5 minutes in a micro SD memory card by the date and time. Also were employed: Bidirectional Anenometer (range 0–60 m s^{-1}, accuracy ± 2%), used to estimate the prevailing wind direction) and an instrument for the analysis of the WetBulb Globe Temperature (**WBGT**) Index (HD 32.2) (working temperature -5 to 50 °C, storage temperature -25 to 65 °C, working relative humidity 0–90% RH no condensation; instrument uncertainty ± 1%).

Table 3. Reference, range and accuracy of low-cost (*LC*) sensors

Sensor	Reference	Range
Air temperature	*SHT31*	-40 to 90 °C
Relative humidity	*SHT31*	0 to 100%;
Air velocity	*Wind sensor Rev P*	0 to 67 m s^{-1}
CH_4 gas	*MQ4*	300 to 10,000 ppm
Pressure	*BMP280*	300–1,100 hPa

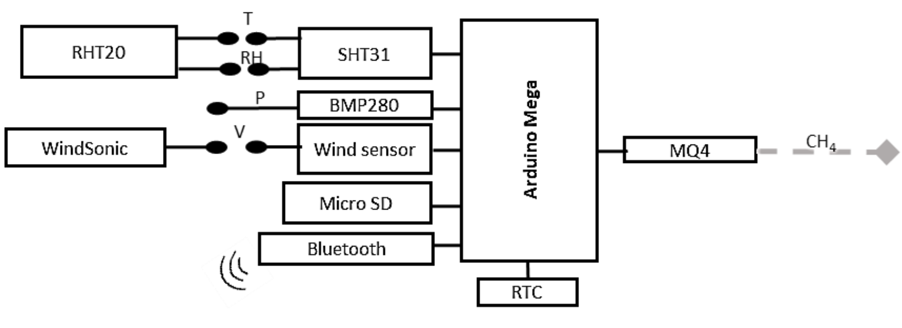


Figure 1. Sketch of the measured variables setup: stand-alone sensors. Measured variables: temperature (T), relative humidity (RH), air velocity (V) and atmospheric pressure (P) and methane (CH_4).

Location of the collector devices and measurement frequency

The research object was the animal housing. The data collection was made continuously during 24 hours in each farm. 4 sensor kits were installed in the middle of the ventilation area, to determine predominant direction of winds, inlets and outlets of air flow. The location for each of the sensor boxes was altered according to each of the typologies of construction evaluated.

Emission factors determination and other variables

The monitored variables using the sensor systems were:

- Temperature (T) = °C;
- Relative humidity (RH) = %;
- Atmospheric pressure (P) = mbar;
- Air velocity (V) = $m^{-1}h$;
- Black globe temperature (Tg)= °C;
- Dew point temperature (Dp) = °C;
- Sensor box area for air flow (BA) = 0.00456 m^2.

The relevant variables of each typology of construction were:

- Height above sea level (hasl);
- climate classification (CC) = Caldas -Lang methodology;
- average animals' weight (AW) = kg;
- Total amount of animals at the facility (AA);
- Total facility area (A) = m^2;
- Effective ventilation area (EVA)= m^2;
- Cleaning frequency (CF) = 0 when the facility is completely evacuated, 1 daily and 2 every two days.

The calculations made to find the variables to analyse were:

a) Animal unit (**AU**): The emissions in the housing facilities are represented as kg AU^{-1}, in which one AU is represented in 500 kg of animal mass.

b) animal density (**AD**) equation:

$$AD = (AW * AA)/A \quad (1)$$

*Expressed in m^2.

c) Ventilation airflow rate (**Q**) equation:

$$Q = V * BA \quad (2)$$

*Expressed in m^3h^{-1}.

d) Factor **K** to converter concentration CH_4 ppm in kg m^3 in function of the following variables: P = Pressure; n = Moles of gas; T = Temperature; R = Universal constant of ideal gases.

e) Concentration of CH_4 (**C**) =

$$C = C_0 - C_i \quad (3)$$

were C_0 = outlet gas concentration, ppm; C_i = inlet gas concentration, ppm; *Expressed in ppm.

f) Emission Rate (ER) equation:

$$ER = KQ(C_0 - C_I) \quad (4)$$

were C_0 = outlet gas concentration, ppm; C_i = inlet gas concentration, ppm; *Expressed in ppm; *Expressed in kg year^{-1}.

g) **THI Index** temperature humidity index (Machado et al., 2016):

$$THI = (0.8 * T) + RH \left[\frac{T - 14.3}{100}\right] + 46.3 \quad (5)$$

were T = air temperature (°C); RH = relative humidity (%).

h) **WBGT index** wet bulb and black globe temperature index (de Oliveira Júnior et al., 2018):

$$WBGT = 0.7\,\mathrm{wbT} + 0.2\,\mathrm{Tg} + 0.1\,\mathrm{dbT} \quad (6)$$

were wbT = wet-bulb temperature; Tg = black globe temperature and dbT = dry bulb temperature.

i) Global Warming Potential (GWP) equation:

$$ECO_2eq = 25\,ECH_4 \quad (7)$$

* Expressed in kg year^{-1} per farm; * taking into account that the warming potential of CH_4 over a100-year period are 25 times that of CO_2 (Salomon et al., 2007). This estimation considered the emissions from the building.

Statistical design

To process the information, the *Principal Components Analysis (PCA)* method was used to obtain an efficient model for emission rates. A model of 12 characterization variables was obtained from the typologies. Additionally, the calculations were developed correlating the variables of emission rates. Descriptive statistical tools were used alongside *analysis of variance (ANOVA)*. All of the calculations were developed in the R Software (RStudio: Integrated Development for R. RStudio, Inc., Boston, MA).

RESULTS AND DISCUSSION

The average values, confidence intervals and standard deviation for the studied typologies are presented in Table 4, where shows the ER (kg year^{-1}) average of 607.90 ± 588.13, THI 74.95 ± 4.67 and WBGT 73.21 ± 4.95. Table 5 and 6 shows the Emission Rates (ER) and the GWP by typologies of construction. According to the meta-analysis developed by Philippe et al. (2013), CH_4 ER in pig production is in the range of 5 to 60 g day^{-1} animal^{-1}, additionally a study developed in breeding farms with deep litter system and controlled ventilation reports emission values between 9.9 to 12.8 g day^{-1} animal^{-1} (Philippe et al., 2013). Another study developed under the same typological characteristics, but in the fattening period by Philippe et al. (2012) found ranges between 7.2 to 25.1 g d^{-1} animal^{-1}. At the present study, values between 1.22 to 20.98 g day^{-1} animal^{-1} (Table 6) were obtained, which are in the range of those found in other studies. However, the average that was found (6.14 g day^{-1} animal^{-1}) is below than the others studies. The difference can be attributed to the fact that the studied farms have natural ventilation and the average values with which they are being compared come from farms with other refrigeration systems and typology of construction. In addition, it is important to highlight that the floors of the evaluated farms corresponded to concrete floors to which a dry removal of the excrement or frequent washing is normally carried out. Therefore, less fermentation processes should be expected and as a consequence lower CH_4 emissions.

Table 4. Average values, confidence intervals and standard deviation of each variable

	Mean	*confidence intervals*		*std*
ER (kg year^{-1})	607.90	440.75	775.04	± 588.13
EVA(m^2)	157.55	128.88	186.21	± 100.88
P(mbar)	854.81	839.62	870.00	± 53.45
THI	74.95	73.62	76.28	± 4.67
WBGT(°C)	73.21	71.80	74.61	± 4.95
T (°C)	25.43	24.26	26.59	± 4.10
CF	1.50	1.31	1.69	± 0.68
AU	66.85	53.45	80.26	± 47.17
Q	6.88	5.69	8.07	± 4.20
AD	55.46	50.58	60.34	± 17.17
Hals (masl)	1.584.40	1449.70	1719.10	± 473.98
RH (%)	64.99	62.66	67.31	± 8.18

Table 5 shows CH_4 ER by typologies of construction. It was found that the emissions rate (ER) of typology 1 has a significant difference due to the low values that could be related to the low average temperature (21.24 °C), high average relative humidity (76%) and the low density of animals (27 kg m^{-2}). Typology 5 is highlighted with a significant difference between the high emission values of CH_4 respect to the average, both typologies show significant differences in the result of the mean value of ER in relation to the others. The typologies that presented greater ER AU are 2 and 4 (Table 6) which are located in cold and mild climates, this high emission could be explained in part by the area of natural ventilation (96 and 24.8 m^2) smaller compared to the average typologies as show in Table 4 (*mean* 157.5 m^2), animals density (70.5–76.3 kg m^{-2}) above average (55.46 kg m^{-2}), and animals mass (90–110 kg) above the average (83.3 kg). Typology 1 (Table 6) exhibit value for ER AU year^{-1} (2.77 kg year^{-1}) below the average (12.5 kg year^{-1}), which could be due to a low density of animals (27.86 kg m^{-2}). Among the other typologies there were no significant differences. A study developed in North Carolina by Sharpe et al. (2001) shows CH_4 fluxes averaged 6.9 g CH_4 animal^{-1} d^{-1} in winter, results close to the average presented in this study (6.14 g day^{-1} animal^{-1}).

Table 5. CH_4 ER by typologies of construction multiple comparison test

Typology	ER facility (kg year^{-1})	*std*	*Group
1	51.90	± 48.00	*b*
2	947.26	± 531.01	*ab*
3	217.24	± 127.24	*ab*
4	1,018.29	± 808.57	*ab*
5	1301.98	± 916.15	*a*
6	315.21	± 410.72	*ab*
7	354.65	± 298.39	*ab*
8	563.48	± 361.83	*ab*
9	507.75	± 436.70	*ab*
10	801.20	± 285.28	*ab*
Mean	607.90		

* Results of Tukey's multiple comparison test.

GWPs are used as a relative index to standardize emissions of GHGs for comparing how efficiently each gas traps heat in the atmosphere (Sedorovich et al., 2007). The index attempts to integrate the overall climate impacts of a specific action, it relates the impact of emissions of a gas to an equivalent emission of a CO_2 mass. The average found of GWP AU among the 10 typologies presented a value of 312.39 kg year^{-1} of CO_2 eq in a GWP time horizon of 100 years (Table 6). It was not possible to find estimated GWP values in swine per facility at literature review, nevertheless, to have an estimate of the magnitude, the facilities emission results of a study in a dairy farm Sedorovich et al. (2007) found average values of GWP AU 1,242 kg year^{-1} CO_2 eq, ratify the lower GWP of pig production, where these results validate those described by Noya et al., (2016) and Vries & de Boer, (2010) who determined that CH_4 emissions in pigs are below cattle. GWP data can be compared with those presented by a review research papers where the median of emission factors for dairy cows (302.5 g day^{-1}·LU-^{1}) was more than three times higher than the value for pigs (85 g day^{-1}·LU^{-1}) (Rzeźnik & Mielcarek 2016).

Table 6. ER per animal, animal unit (AU) and GWP AU

Typology	ER animal (kg year^{-1} animal^{-1})	ER animal (g day^{-1} animal^{-1})	ER AU (kg year^{-1})	GWP AU (kg year^{-1})
1	0.44	1.22	2.77	69.31
2	6.72	18.41	30.54	763.43
3	0.86	2.36	7.18	179.60
4	7.66	20.98	42.54	1,063.38
5	2.17	5.96	9.45	236.26
6	0.66	1.82	4.74	118.50
7	1.10	3.03	4.80	120.09
8	1.09	3.00	7.82	195.38
9	0.92	2.53	10.26	256.44
10	0.76	2.08	4.86	121.47
Mean	2.24	6.14	12.50	312.39

Table 7. Anova and Tukey's multiple comparison test Concentrations of CH_4 (ppm) and thermal comfort indices for each typology

Typology	C (ppm)	*std*		WBGT	*std*		THI	*std*	*Group
1	103.6	± 66.58	*b*	69.4	± 1.81	*a*	72.4	± 2.50	*ab*
2	704.4	± 391.26	*a*	73.4	± 2.88	*a*	76.6	± 3.20	*ab*
3	63.8	± 33.27	*b*	72.4	± 3.04	*a*	73.2	± 2.28	*ab*
4	266.4	± 130.10	*ab*	68.8	± 2.28	*a*	68.6	± 3.43	*b*
5	267.4	± 215.40	*ab*	73.4	± 2.79	*a*	76.0	± 3.24	*ab*
6	66.2	± 64.01	*b*	77.6	± 3.50	*a*	79.2	± 2.48	*a*
7	161.4	± 131.26	*b*	75.4	± 6.46	*a*	75.4	± 5.72	*ab*
8	179.6	± 129.39	*b*	69.0	± 2.91	*a*	72.2	± 2.94	*ab*
9	105.2	± 78.8	*b*	74.8	± 6.22	*a*	76.6	± 5.41	*ab*
10	184.6	± 57.2	*b*	78.0	± 5.43	*a*	79.2	± 4.54	*a*

* Results of Tukey's multiple comparison test.

To evaluate the relationship of thermal comfort indices with CH_4 gas, was used the concentration variable (C) obtained with equation 4 described in materials and methods, the results can be seen in Table 7 as well as those of THI and WBGT indices. Among the typologies 1, 3, 6, 7, 8, 9 and 10 there were no significant differences, among the 1, 3 and 6 were the lowest concentrations. The highest concentration occurred in facility 2, followed by 4 and 5, located in cold and mild climates. Typology 2 presented very high concentration values of CH_4 (C) respect to the mean and median; which could be explained by the adverse positions in most of the measured variables; high temperatures T (27.8 °C), high animal density AD (70.5 kg m^{-2}), cleaning frequency (CF) of 2 days and one of the lowest air velocity (V) (0.17 m s^{-1}). In the same way values of THI and WBGT reached are shown in Table 7. It can be seen that the typological facilities 1, 3, 4 and 8 were below THI ≤ 74, which suggests that the farms are most of the day in a situation of thermal comfort, similar results with those of the WBGT index with values under 72. Facilities that present an environment of greater thermal discomfort are Typologies 2, 5, 6, 7, 9 and 10, having a greater relationship with ventilation type than for its location at height above sea level. The highest values of WBGT and THI were found in typologies 6, 7 and 10, which are located in mild and warm climates mainly.

Fig. 2 and Table 8 presents the results of a Principal Component Analysis *(PCA)*, developed to find correlations between ER and the other measured variables. There are records that production levels of CH_4, can be altered by several factors, such as housing conditions, manure management and diet composition (Philippe & Nicks, 2015). This study can support these alterations with the findings of significant correlations between ER y CF, AU, Q, AD y RH, results are presented in Table 8.

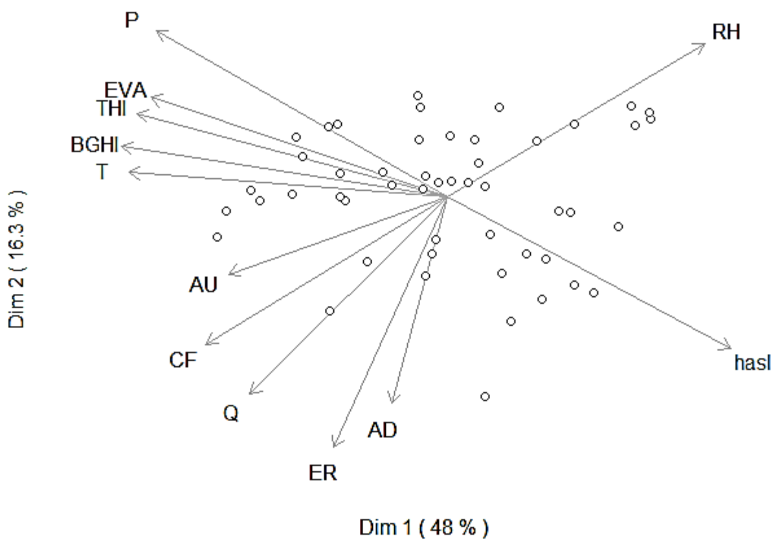


Figure 2. *Principal Component Analysis (PCA)*

According to Gabriel et al. (2014) and Petersen et al. (2016) there is evidence that the ER CH_4 emitted from stored slurry declines with decreasing temperature, as also was found by Sharpe et al. (2001) their results showed that during the cold winter measurement period, CH_4 fluxes averaged 6.9 g CH_4 animal^{-1} d^{-1}

Table 8. Variables with *significant correlations* with ER

	correlations	*pvalue*	*confidence intervals*	
CF	0.309	0.029	0.034	0.541
AU	0.292	0.040	0.015	0.527
Q	0.463	0.001	0.212	0.657
AD	0.279	0.050	0.001	0.517
RH	-0.372	0.008	-0.589	-0.105

and during summer measurement periods, CH_4 fluxes were much greater and averaged 33 g CH_4 animal^{-1} d^{-1}. However, it was not possible to find in this study a direct relationship between T and ER. Although Fig. 2 does not indicate a direct relationship between the height above sea level (hals) and ER, it was found that typologies 6 and 10 located in warm weather, as well as typologies 7 and 9 located in temperate climates also have high indices of thermal discomfort, as shown in Table 7.

CONCLUSIONS

This study represents a Colombia first approach to estimate emission factors (ER) of CH_4 of the housing in fattening pigs' facilities. The results obtained from this study are added to others developed in different countries that have shown evidence that the emissions generated in the facilities are important and should be incorporated into national greenhouse gas inventories, with an average CH_4 Emission Rate (ER) per facility of 607.9 kg year^{-1}. Even though the data gathered in this work exhibit high variability, due to the climatic and typology of construction diversity, they can be used to guide further research. The present research highlights that the estimation of ER is a multivariable problem, where the correlations between, environmental, physiological, structural and animal management variables must be considered. It is shown that at different typologies of construction used in Colombia that operate with natural ventilation, significant levels of ER are presented, finding a higher ER in cold and mild climates where 80% of Antioquia state pig farming is concentrated, That could be, due to typologies in the cold and mild climates facilities have less natural ventilation than the warm climate. However, it is necessary to go deeper into this type of study, especially in typologies located in warmer weathers. In addition, it shows how these typologies in summer months, which are the majority of the year in tropical countries, it should use systems that improve animal thermal comfort, because they have adverse conditions according to the THI and WBGT indexes founded.

It is hoped that this work will serve as a basis for advancing a national inventory of gas emissions in the pig sector and guiding policies and strategies to minimize the impact that the swine sector can produce on the environment in the country.

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REFERENCES

Bjerg, B., Demeyer, P., Hoyaux, J., Didara, M., Grönroos, J., Hassouna, M., Amon, B., Bartzanas, T., Sándor, R., Fogarty, M., Klas, S., Schiavon, S., Juskiene, V., Radeski, M., Attard, G., Aarnink, A., Gülzari, Ş., Kuczyński, T., Fangueiro, D., Marin, M., Mihina, S., Verbič, J., Calvet, S., Jeppsson, K., Menzi, H., Sizmaz, O. & Norton, T. 2019. Review of Legal Requirements on Ammonia and Greenhouse Gases Emissions from Animal Production Buildings in European Countries. In *2019 ASABE Annual International Meeting*, **23**.

Briukhanov, A., I. Subbotin, R., Uvarov. & E. Vasilev. 2017. Method of Designing of Manure Utilization Technology. *Agronomy Research* **15**(3), 658–663.

Cecchin, D., Cecchin, D., Campos, A., Cruz, V., Sousa, F., Amaral, P. & Yanagi J, T. 2017. Air Quality in Swine Growing and Finishing Facilities with Different Building Typologies TT - Qualidade Do Ar Em Instalações Para Suínos Em Crescimento e Terminação Com Diferentes Tipologias Construtivas. *Revista Brasileira de Engenharia Agrícola e Ambiental* **21**(5), 339–343.

Departamento Administrativo Nacional de Estadística (DANE). 2016. *3rd National Agricultural Survey, Colombia*. Bogotá, Colombia.

Gabriel, D., Allen, A., Bastviken, D., Conrad, R., Gudasz, C., St-Pierre, A., Thanh-Duc, N Del Giorgio, PA. 2014. Methane Fluxes Show Consistent Temperature Dependence across Microbial to Ecosystem Scales. *Nature* **507**(7493), 488–491.

Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A. & Tempio, G. 2013. Facing Climate Change through Livestock. Organización de las naciones unidas para la alimentación y la agricultura (FAO), Roma.

Gert, J.-M., Bannink, A. & Chadwick, D. 2006. Greenhouse Gas Abatement Strategies for Animal Husbandry. *Agriculture, Ecosystems and Environment* **112**(2–3), 163–170.

Gitz, V., Meybeck, A., Lipper, L., Young, C. & Braatz, S. 2016. Food and Agriculture Organization of the United Nations *Climate Change and Food Security: Risks and Responses*. http://www.fao.org/3/a-i5188e.pdf.

IDEAM., PNUD., MADS., DNP., CANCILLERÍA. 2015. *National Inventory of Greenhouse Gases (GHG) Colombia 2012*.

IPCC, 2007. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment R*eport of the Intergovernmental Panel on Climate Change* [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 996 pp.

Lenerts, A., Popluga, D. & Naglis-Liepa. 2019. Benchmarking the GHG Emissions Intensities of Crop and Livestock–Derived Agricultural Commodities Produced in Latvia. *Agronomy Research* **17**(5), 1942–1952.

Machado, S., Nääs, I., Dos Reis, J., Caldara, F. & Santos, R. 2016. Sows and Piglets Thermal Comfort: A Comparative Study of the Tiles Used in the Farrowing Housing. *Engenharia Agricola* ***36***(6): 996–1004.

Ministerio de Agricultura y Desarrollo Rural (MADR). 2019. 53 Ministerio de Agricultura y Desarrollo Rural (MADR) *National Agroclimatic Report May 2019*.

Nations Food and Agriculture - FAO. 2011. FAO *World Livestock 2011 - Livestock in Food Security World*.

Noya, I., Villanueva-Rey, P., González-García, S., Fernandez, M.D., Rodriguez, M.R. & Moreira, M.T. 2017. Life Cycle Assessment of Pig Production: A Case Study in Galicia. *Journal of Cleaner Production* **142**, 4327–4338.

Noya, I., Aldea, X., Gasol, C., González-García, S., Amores, M., Colón, J., Ponsá, S., Roman, I., Rubio, M., Casas, E., Moreira, M. & Boschmonart-Rives, J. 2016. Carbon and Water Footprint of Pork Supply Chain in Catalonia: From Feed to Final Products. *Journal of Environmental Management* **171**, 133–43.

de Oliveira, J.A., de Oliveira Júnior, A., de Souza, S., da Cruz, V., Vicentin, T. & Glavina, A. 2018. Development of an Android APP to Calculate Thermal Comfort Indexes on Animals and People. *Computers and Electronics in Agriculture* ***151***(October 2017), 175–84. https://doi.org/10.1016/j.compag.2018.05.014.

Osorio-Saraz, J., Osorio-Saraz, J., Ferreira-Tinoco, I., Gates, R., Oliveira-Rocha, K., Combatt-Caballero, E. & Campos-de-Sousa, F. 2014. "Adaptation and Validation of a Methdology for Determing Ammonia Flux Generated by Litter in Naturally Ventilated Poultry Houses." *Dyna* **81**(187), 137–143.

Petersen, O., Olsen, A., Elsgaard, L., Triolo, J. & Sommer, S. 2016. Estimation of Methane Emissions from Slurry Pits below Pig and Cattle Confinements. *PLoS ONE* **11**(8), 1–16.
Philippe, F.X., Laitat, B., Nicks, B. & Cabaraux, J.F. 2012. Ammonia and Greenhouse Gas Emissions during the Fattening of Pigs Kept on Two Types of Straw Floor. *Agriculture, Ecosystems and Environment* **150**, 45–53. http://dx.doi.org/10.1016/j.agee.2012.01.006
Philippe, F X., Laitat, M., Wavreille, J., Nicks, B. & Cabaraux, J.F. 2013. Influence of Permanent Use of Feeding Stalls as Living Area on Ammonia and Greenhouse Gas Emissions for Group-Housed Gestating Sows Kept on Straw Deep-Litter. *Livestock Science* **155**(2–3): 397–406. http://dx.doi.org/10.1016/j.livsci.2013.05.005
Philippe, F.X. & Nicks, B. 2015. Review on Greenhouse Gas Emissions from Pig Houses: Production of Carbon Dioxide, Methane and Nitrous Oxide by Animals and Manure. *Agriculture, Ecosystems and Environment* **199**, 10–25. http://dx.doi.org/10.1016/j.agee.2014.08.015
Philippe, F., Cabaraux, J. & Baudouin, N. 2011. Ammonia Emissions from Pig Houses: Influencing Factors and Mitigation Techniques. *Agriculture, Ecosystems and Environment* **141**(3–4), 245–260.
Reckmann, K., Traulsen, I. & Krieter, J. 2013. Life Cycle Assessment of Pork Production: A Data Inventory for the Case of Germany. *Livestock Science* **157**(2–3), 586–596. http://dx.doi.org/10.1016/j.livsci.2013.09.001
RStudio Team. 2015. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/.
Rzeźnik, W. & Mielcarek, P. 2016. Greenhouse Gases and Ammonia Emission Factors from Livestock Buildings for Pigs and Dairy Cows. *Polish Journal of Environmental Studies* **25**(5), 1813–1821.
Sedorovich, D., Rotz, A. & Richard, T. 2007. Greenhouse Gas Emissions from Dairy Farms. In *2007 ASABE Annual International Meeting, Technical Papers*, 14.
Sharpe, R.R., Harper, L.A. & Simmons, J.D. 2001. Methane Emission from Swine Houses in North Carolina. *Chemosphere Global Change Science* **3**, 1–6.
Steinfeld, H. & Gerber, P. 2010. Livestock Production and the Global Environment: Consume Less or Produce Better? *Proceedings of the National Academy of Sciences* **107**(43), 18237–38. http://www.pnas.org/cgi/doi/10.1073/pnas.1012541107
de Vries, M. & de Boer, I.J.M. 2010. Comparing Environmental Impacts for Livestock Products: A Review of Life Cycle Assessments. *Livestock Science* **128**(1–3). 1–11. http://dx.doi.org/10.1016/j.livsci.2009.11.007

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https://doi.org/10.15159/AR.20.028

Association between body condition and production parameters of dairy cows in the experiment with use of BCS camera

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Abstract. Dairy production effectiveness in the farm depends on many technical, technological and biological factors. State of the dairy cow condition constitutes one of the most important element in the assessment of dairy herd and production indices. Including access to modern technical solution to assess body condition of dairy cows, i.e. BCS camera, some results of observation in the herd with 362 cows were collected to find any relationship between BCS index and milk yield per cow including 5 lactation groups and cows differed in age as well as four seasons. Basing on data collected in the period of 11 months it was found that cows with the BCS index lower than 2.9 showed the highest daily milk production. Increase in BCS index was associated with decrease in amount of produced milk per day. The statistical analysis showed significant effect of lactation period, age of animals and season on BCS results in the considered dairy cow production cycle.

Key words: BCS, camera, cow, index, milk yield.

INTRODUCTION

Factors that affect the condition of cows are age, housing system, physiological state, production cycle and season (Borkowska, 2000). That is why it is important to constantly monitor the condition of dairy cows at various stages of the production cycle (Bewley & Schutz, 2008). A tool that can be included as a valuable solution for farmers in cow assessment is body condition monitoring. This tool allows farmer to assess the balance of feed and the adequacy of nutrition in relation to the physiological state of the animal. Excessive fatness as well as emaciation complicate the accumulation of metabolic energy in adipose tissue and muscle tissue. In practice, a fat cow is doomed to emaciation because the body is too overloaded (Januś et al., 2012).

Problems most often occur in the perinatal period, i.e. about 100 days after calving, when feed intake is not able to cover energy needs. At that time, fat reserves are not sufficient enough. This may cause metabolic diseases such as ketosis, acidosis, fatty liver, or labour paralysis. The results of conducted research have shown that in the case of these animals milk production does not bring sufficient results compared to the results of cows in good condition and reproduction efficiency is also reduced (Sablik et al., 2014).

The method most commonly used to monitor the condition of cows is the non-invasive method based on the five-point BCS scale (Guliński, 1996). The main purpose of this assessment is to attempt to visually determine the thickness of the fat layer under the skin. Many scientific studies have been conducted and these studies showed a significant relationship between the condition of animals and their productivity, fertility and overall health (Berry et al., 2002).

Due to genetic conditions, dairy cows at the peak of lactation release most of their energy. This is due to high milk production (Wójcik et al., 2012). Early stage of lactation is a period in which the animals have a negative energy balance, the energy consumed with the feed is unable to fully cover the demand. In the early lactation phase, due to the negative energy balance, the animal can produce up to 7 kg of milk using fat reserves. This phenomenon has a negative effect on the health and condition of the cows. In order to maintain proper milk production, energy deficiencies are supplemented with adipose tissues, which means that the mass of dairy cows can be dramatically reduced to even 2 kg per day (Delaby et al., 2009). Therefore, many factors indicate that proper management of energy reserves significantly affects the level of milk production, animal health and dry matter intake.

In the research carried out by Contreras et al. (2004) it was found that dairy cows with a low BCS (body condition score) index, i.e. below 3 points during the dry period produced significantly more milk after calving in comparison with animals characterized by the condition rated above 3 points. Moreover, the milk fat content was also higher. This thesis was also confirmed by Waltner et al. (1993). During the tests, a decrease in milk production by about 230 kg was observed in group of cows whose condition exceeded 4 points on the BCS scale.

Based on the research of many authors, it can be concluded that the level of milk production and lactation efficiency significantly affect the condition of cows (c McCarthy et al., 2007).

The purpose of the research was to find a relationship between the condition of cows and season, lactation number, lactation period and milk yield, using a BCS camera system.

MATERIALS AND METHODS

Research on the assessment of the body condition of dairy cows was carried out within the period from April 15, 2018 to March 15, 2019 on a farm in the Lower Silesian region (Poland). On April 15, 2018 the cattle herd included 623 animals, while on the day of the end of the research period there were 571 heads of cattle. The animals were kept in a free-stall barn with individual stalls covered with rubber mats. There was a side-by-side milking parlor (2×16). Cows were milked three times a day. A group of 362 Holstein-Friesian cows was evaluated in the research. In 2018 the average annual milk yield per cow was just over 10,000 kg.

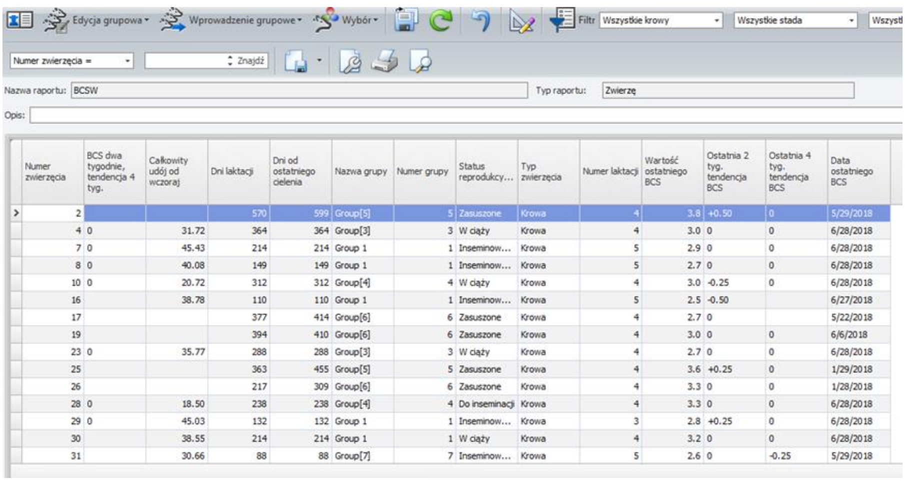
The animals were fed complete feeds in the TMR system. Feed doses were prepared separately for each technological group according to the feeding standards corresponding to daily milk production.

Each day during the research period assessment of the condition of cows on a five-point scale with an accuracy of 0.1 point was carried out using a DeLaval BCS 3D camera (Fig. 1). The camera was mounted above the selection gate (Fig. 2) and as a result it was possible to take three-dimensional photos of each milked cow after leaving the milking parlour. The software calculated the cow body condition based on photography. The three-dimensional camera analyzed 32,000 extreme points of the cow's back profile. The specific places included in the assessment there were: base of the tail, sciatic tumors, lumbar vertebrae, cruciate ligaments, and short ribs. The used 3D camera took 30 photos per second. The animal health status was assessed using DelPro software cooperating with the camera with a low error rate below 0.1% of the standard deviation.

Figure 1. DeLaval BCS camera.
Source: www.delaval.com and own photo.

The processed data support the decision-making process regarding herd management, and also facilitate consultations with a nutritional advisor and veterinarian. The system also generates extensive reports (Fig. 3) that the farmer can individually modify and select only the data that interests him.

Figure 2. DeLaval BCS camera mounted above the selection gate.
Source: Lesiakowski, 2016.



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Numer zwierzęcia = | Znajdź

Nazwa raportu: BCSW | Typ raportu: Zwierzę

Opis:

Numer zwierzęcia	BCS dwa tygodnie, tendencja 4 tyg.	Całkowity udój od wczoraj	Dni laktacji	Dni od ostatniego cielenia	Nazwa grupy	Numer grupy	Status reprodukcy...	Typ zwierzęcia	Numer laktacji	Wartość ostatniego BCS	Ostatnia 2 tyg. tendencja BCS	Ostatnia 4 tyg. tendencja BCS	Data ostatniego BCS
2			570	599	Group[5]	5	Zasuszone	Krowa	4	3.8	+0.50	0	5/29/2018
4	0	31.72	364	364	Group[3]	3	W ciąży	Krowa	4	3.0	0	0	6/28/2018
7	0	45.43	214	214	Group 1	1	Inseminow...	Krowa	5	2.9	0	0	6/28/2018
8	0	40.08	149	149	Group 1	1	Inseminow...	Krowa	5	2.7	0	0	6/28/2018
10	0	20.72	312	312	Group[4]	4	W ciąży	Krowa	4	3.0	-0.25	0	6/28/2018
16		38.78	110	110	Group 1	1	Inseminow...	Krowa	5	2.5	-0.50		6/27/2018
17			377	414	Group[6]	6	Zasuszone	Krowa	4	2.7	0		5/22/2018
19			394	410	Group[6]	6	Zasuszone	Krowa	4	3.0	0	0	6/6/2018
23	0	35.77	288	288	Group[3]	3	W ciąży	Krowa	4	2.7	0	0	6/28/2018
25			363	455	Group[5]	5	Zasuszone	Krowa	4	3.6	+0.25	0	1/29/2018
26			217	309	Group[6]	6	Zasuszone	Krowa	4	3.3	0	0	1/28/2018
28	0	18.50	238	238	Group[4]	4	Do inseminacji	Krowa	4	3.3	0	0	6/28/2018
29	0	45.03	132	132	Group 1	1	Inseminow...	Krowa	3	2.8	+0.25	0	6/28/2018
30		38.55	214	214	Group 1	1	W ciąży	Krowa	4	3.2	0	0	6/28/2018
31		30.66	88	88	Group[7]	7	Inseminow...	Krowa	5	2.6	0	-0.25	5/29/2018

Figure 3. Example report generated via DeLaval DelPro system.
Source: Own study.

All collected data were generated in the form of reports using the DelPro system, then saved in Excel. The results. of the BCS (body condition score) assessment were recorded every 30 days for each animal individually Daily and monthly milk productivity values were also assigned to the appropriate cows.

The time when the condition of dairy cows was assessed in the research included four periods corresponding to the seasons of the year:

- Spring (W): months April - June,
- Summer (L): months July - September,
- Autumn (J): months October - December,
- Winter (Z): months January - February.

In each analyzed season, cows of different ages were identified including their lactation number. The next factor grouping the animals was the lactation period (Table 1).

On the basis of the collected and compiled data, the following indices were calculated: average BCS values, daily milk production and milk production for three months for cows in each group (including season, lactation number and lactation period). Then the test results obtained from the DelPro system were used to statistical analysis. Correlation coefficients between variables were determined. Using the ANOVA test, the significance of the influence of various factors on the condition of cows characterized by the BCS value was determined. The effect of season, cow age, and lactation period on the BCS score was assessed. Changes in the average milking test were also assessed depending on the condition of the animals. Results were statistically analyzed using Statistica v.13 software (StatSoft Polska, Cracow, Poland).

Table 1. The reproduction status of cows included in particular lactation period

Period of lactation	Stage of lactation
0–50 days	After calving
51–90 days	Beginning of lactation
91–200 days	Middle of lactation
201–300 days	End of lactation
> 300 days	Dried

Source: Own study.

RESULTS AND DISCUSSION

Basing on the collected data during the experimental period it was found that the distribution of dairy cow condition assessments on the BCS five-point scale was equivalent to the normal distribution (Fig. 4). The average assessment of the condition of the animals in the analyzed period was 2.99 points, whereas the standard deviation amounted to 0.15 points.

Analyzing the distribution of dairy cow condition assessments, it was found that 34% of all BCS values are grades between 3.01 and 3.25 points, while 32% of the observations were grades in the range of 2.76–3.00

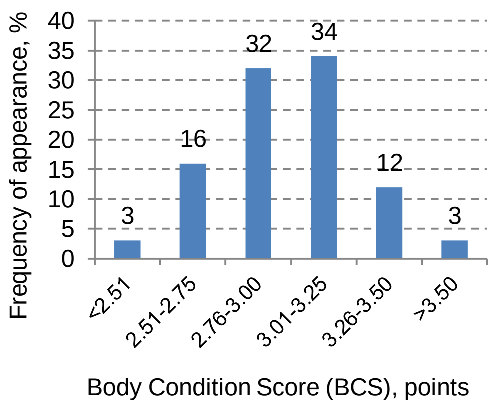

Figure 4. Distribution of dairy cow condition assessments on the BCS scale.

Source: Own study.

points. In other studies, which consisted of visual assessment of the condition of dairy cows, the most frequently used assessment was 3.25 points and this result constituted 33.14% of the total BCS values (Gołębiewski, 2017). Percentage of animals with a BCS condition < 2.5 points was 3%. The same result was achieved in the case of cows that showed high energy reserves. According to Markusfeld et al. (1997) excessively emaciated animals are characterized by condition on the BCS scale not higher than 2.5 points, while excessively fat cows reach BCS values > 3.5 points. In the research carried out by Van Os et al. (2019) cows scoring ≤ 2.0 were considered thin. The vast majority of the condition assessments, i.e. 78% were in the range of 2.76–3.5 points.

Analyzing results obtained in the research, it is possible to indicate some differences in the BCS index value depending on the age of the tested animals, the season of the year and the lactation period of cows in the analyzed period. Based on the correlation matrix, Table 2 summarizes the correlation coefficients for the BCS index given in points.

Table 2. Correlation coefficient between the BCS index and the variables ($p < 0.05$)

Variable	Season	Lactation number	Lactation period	Daily milk productivity	Milk production
BCS	r = - 0.18	r = - 0.17	r = 0.54	r = - 0.66	r = - 0,67

Source: Own study.

The carried out statistical analyses showed some relationships between considered variables. Correlation coefficients were calculated for five variables. It is possible to indicate that daily milk yield and milk production were correlated to the highest degree with the BCS index. The correlation coefficient between the mentioned variables was r = -0.66 for average daily milk productivity and r = -0.67 for milk production, respectively. The average BCS indices were negatively correlated with these variables, i.e. higher milk yield resulted in a decrease in the BCS index (given in points) and a deterioration in the condition of cows. The difference between the minimum and maximum value of the features for the average daily milk production was 34 kg, while in the case of milk production the result was 1,015 kg. The lactation period was also characterized by a significant correlation with the result of r = 0.54. In the case of the lactation number, a low relationship was found at r = -0.17. The analyzed features were significant at $p < 0.05$. The lactation number connected with the age of dairy cows and the season of the year were poorly correlated with BCS.

Significant differences in the assessment of the BCS index between heifers after first calving and older cows were observed in the analyzed period (Fig. 5). The animals in first lactation showed significantly higher values of the BCS index in comparison witholder cows. The diversity of average values of animal condition assessments in the first lactation was about 0.1 point, whereas for older cows the amplitude of diversity increased to 0.2 points. The curve of changes concerning cow condition during the examined period for animals in fifth and higher lactation decreased the fastest way and reached the lowest value amounted to 2.85 points in the winter. In the study carried out by Gołębiewski (2017), a significantly higher level of body condition was also observed for heifers after first calving in comparison with older cows. It was indicated that heifers after first calving were characterized by a significantly higher fat content than older cows. The obtained results of observations are similar with reports given by other authors

(Ruegg & Milton, 1995; Domecq et al., 1997; Dechow et al., 2003: Mao et al., 2004; Lee & Kim, 2006; Friggens et al., 2007; Roche et al., 2007). The condition of dairy cows tends to decrease with subsequent lactation (Waltner et al., 1993; Coffey et al., 2002) and such changes were recorded in the presented own studies.

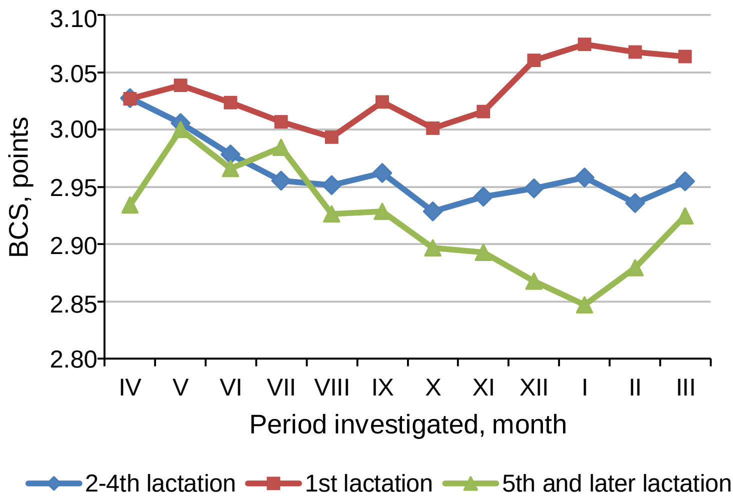

Figure 5. Changes in the BCS index for cows in first lactation and more lactations.
Source: Own study.

Table 3. Analysis of variance of the factors influencing the body condition score (BCS)

Factor	Sum of Squares	Degrees of freedom	Mean Square	F-Ratio	P-Value
Main variables					
Season	0.1112	3	0.0371	5.4	0.002667
Lactation number	0.2335	4	0.0584	8.6	0.000027
Period of lactation	1.5451	4	0.3863	56.6	0.000000
Interactions					
Season*Lactation number	0.0553	12	0.0046	0.7	0.765308
Season*Period of lactation	0.0758	12	0.0063	0.9	0.528741
Lactation number* Period of lactation	0.1544	16	0.0096	1.4	0.175204
Error	0.3273	48	0.0068	-	-

Source: Own study.

Based on the analysis of variance (Table 3), the significance of the impact of the lactation period on the BCS value, i.e. the fat level of dairy cows was demonstrated. Changes in the condition of cows and the average daily performance in relation to the lactation period are shown in Fig. 6. Including increase in milk yield per cow, a decrease in the condition of animals was observed. The average BCS value of cows after calving was 2.95 points, while the average daily milking reached 41.1 kg of milk per cow. In the early lactation, i.e. up to 90 days after calving, there was an increase in daily milk production by 2.18 kg per cow at the decreased body condition, which drop to 2.85 points. Kulpys et al. (2009) recorded the similar trends. Studies carried out by Januś &

Borkowska (2005) showed that after calving, there is a sudden increase in the cow's demand for nutrients. This effect is caused by an increase in milk production in the first days of lactation. Losses of body condition during this period are caused by the phenomenon of fat concentration as a result of not preparing the body to collect such large amounts of feed to meet nutritional needs.

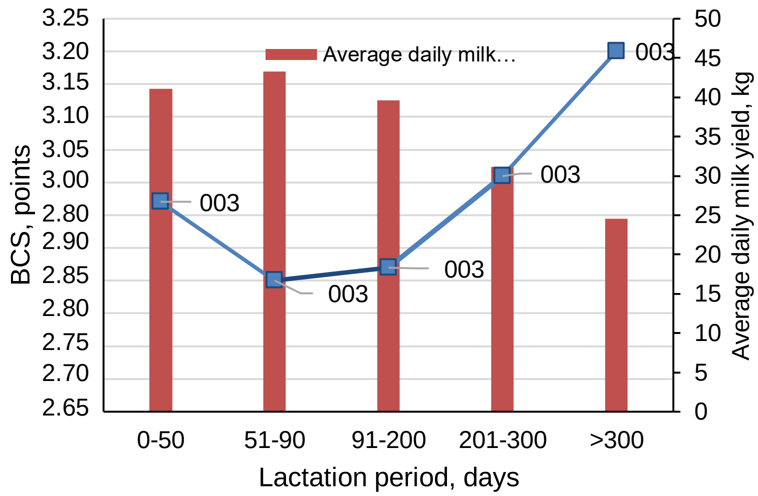

Figure 6. Changes in the condition of cows and daily milk production in relation to the lactation period.

Source: Own study.

After 90 days there was observed a slow increase in body condition state. During the middle part of lactation cows supplemented their energy reserves, which indicated a positive balance. Milk production gradually decreased. After 200 days of lactation, the BCS index value increased rapidly to 3.2 points during the dry period. The average daily milk production decreased by almost half in comparison with the early lactation period.

Based on the analysis of variance (Table 3), the significance of the effect of lactation number on the average condition state of dairy cows was indicated. The condition state of dairy cows decreased with subsequent lactation. Waltner et al. (1993) and Coffey et al. (2002) recorded similar trends. The research results showed that the cows produced less milk at the highest BCS index. Analysis of variance also showed a significant impact of the season on the condition state of cows. There was a downward trend in the BCS score from April to December. In the experiment carried out by Jankowska & Sawa (2004) it was shown that the condition of heifers after first calving was slightly worse than older cows in the spring, while in the other periods the tendency was reversed. These observations were similar with the presented own investigation (Fig. 5). The season and the associated thermal conditions, especially heat stress are among the important factors determining the physiological state and productivity of dairy cows (Herbut et al., 2019).

Variables included in the analysis of cow condition, i.e. lactation number, period of lactation and milk yield per cow constitute also important elements of complex approach to dairy herd efficiency assessment (Luik-Lindsaar et al., 2019), evaluation of lifetime milk productivity (Cielava et al., 2017) and assessment of dairy cow herd indices associated with different milking systems (Gaworski et al., 2018). So there is possible to find common area for further research, where cow condition can be considered in connection with many factors creating dairy system in the farm.

CONCLUSIONS

The use of the system with a BCS camera allows for quick and easy assessment of the condition of cows. The automatic BCS index measurement method saves the time in comparison with performing a visual or tactile tests. It is the system that supports the farmer in daily activities concerning management of a dairy herd.

Basing on the analysis of research results regarding the assessment of the dairy cow condition obtained with help of the BCS camera, the following conclusions can be given:

1. Results of the analysis of variance of the BCS index for cows clearly show that the factors deciding about differentiation in assessment of the cow condition are: lactation period, lactation number and season. No significant effect on the BCS index of cow was found for two considered together factors.

2. Animals in first lactation showed significantly higher BCS values in comparison with older cows. The diversity of average values of assessments of the condition of cows in first lactation was about 0.1 point, while for older cows, the amplitude of diversity increased to 0.2 points.

3. It was confirmed that the cows in first lactation produced smallest amount of milk at the highest BCS score. The average condition was 3.05 points while the daily average milk production was about 35 kg per cow. Older cows in fifth and higher lactation had the lowest condition state with an average value of 2.92 points and milk production amounted to 36 kg. Cows in third lactation were characterized by the highest milk productivity.

4. The carried out investigation showed that cows with low energy reserves obtained significantly better results in daily milk production compared to other animals. Animals with less than 2.9 points were producing on average 44 kg of milk, while the lowest results were achieved by cows with a BCS index less than 3.5 points. High BCS values, regardless of the season of the year, contributed to declines in milk production.

REFERENCES

Berry, D.P., Buckley, F., Dillon, P.G., Evans, R.D., Rath, M. & Veerlmp, R.F. 2002. Genetic parameters for level and change of body condition score and body weight in dairy cows. *Journal of Dairy Science* **85**(8), 2030–2039. doi: 10.3168/jds.S0022-0302(02)74280-X

Bewley, J.M. & Schutz, M.M. 2008. An interdisciplinary review of body condition scoring for dairy cattle. *The Professional Animal Scientist* **24**(6), 507–529. doi: 10.15232/S1080-7446(15)30901-3

Borkowska, D. 2000. The infIuence o seleeted factors on physical condition of cows bred on individual farms. *Medycyna Weterynaryjna* **56**(11), 743–745 (in Polish).

Cielava, L., Jonkus, D. & Paura, L. 2017. Lifetime milk productivity and quality in farms with different housing and feeding systems. *Agronomy Research* **15**(2), 368–375.

Coffey, M.P., Simm, G. & Brotherstone, S. 2002. Energy balance profiles for the first three lactations of dairy cows estimated using random regression. *Journal of Dairy Science* **85**(10), 2669–2678. doi: 10.3168/jds.S0022-0302(02)74352-X

Contreras, L.L., Ryan, C.M. & Overton, T.R. 2004. Effect of dry cow grouping strategy and prepartum body condition score on performance and health of transition dairy cows. *Journal Dairy Science* **87**(2), 517–523. doi: 10.3168/jds.S0022-0302(04)73191-4

Dechow, C.D., Rogers, G.W., Klei, L. & Lawlor, T.J. 2003. Heritabilities and correlations among body condition score, dairy form and selected linear type traits. *Journal of Dairy Science* **86**(6), 2236–2242. doi: 10.3168/jds.S0022-0302(03)73814-4

Delaby, L., Faverdin, P., Michel, G., Disenhaus, C. & Peyraud, J.L. 2009. Effect of different feeding strategies on lactation performance of Holstein and Normande dairy cows, *Animal* **3**(6), 891–905. doi: 10.1017/S1751731109004212

Domecq, J.J., Skidmore, A.L., Lloyd, J.W. & Kaneene, J.B. 1997. Relationship between body condition scores and milk yield in a large dairy herd of high yielding Holstein cows. *Journal Dairy Science* **80**(1), 101–112. doi: 10.3168/jds.S0022-0302(97)75917-4

Friggens, N.C., Berg, P., Theilgaard, P., Korsgaard, I.R., Ingvartsen, K.L., Lovendahl, P. & Jensen, J. 2007. Breed and parity effects on energy balance profiles through lactation: Evidence of genetically driven body energy change. *Journal of Dairy Science* **90**(11), 5291–5305. doi: 10.3168/jds.2007-0173

Gaworski, M., Leola, A., Kiiman, H., Sada, O., Kic, P. & Priekulis, J. 2018. Assessment of dairy cow herd indices associated with different milking systems. *Agronomy Research* **16**(1), 83–93. doi: 10.15159/AR.17.075

Gołębiewski, M. 2017. Study on the suitability of the modified body condition scoring in dairy herd management, with particular emphasis on its impact on production, repro-duction and animal health. *Wydawnictwo SGGW, Warszawa*, 152 pp. (in Polish).

Guliński, P. 1996. Praktyczna ocean kondycji krów mlecznych. *Przegląd Hodowlany* **11**, 4–5 (in Polish).

Herbut, P., Angrecka, S., Godyń, D. & Hoffmann, G. 2019. The physiological and productivity effects of heat stress in cattle – a review. *Annals of Animal Science* **19**(3), 579–593. doi: 10.2478/aoas-2019-0011

Jankowska, M. & Sawa, A. 2004. Kondycja krów czarno-białych z różnym udziałem genów rasy holsztyńsko-fryzyjskiej a ich użytkowość mleczna i rozpłodowa. *Zeszyty Naukowe Przeglądu Hodowlanego* **72**(1), 93–99 (in Polish).

Januś, E. & Borkowska, D. 2005. Zmiany kondycji krów oraz dziennej wydajności i składu mleka w przebiegu laktacji. *Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego* **1**(1), 75–84 (in Polish).

Januś, E., Borkowska, D., Wilgos, A. & Czaplicka, M. 2012. Evaluation of the relationship between body condition of high-yield Black-and-White Polish Holstein-Friesian cows and their productivity. *Annales UMCS Lublin – Polonia* **4**, 34–40.

Kulpys, J., Paulauskas, E., Pilipavicius, V. & Stankevicius, R. 2009. Influence of cyanobacteria *Arthrospira (Spirulina) platensis* biomass additives towards the body condition of lactation cows and biochemical milk indexes. *Agronomy Research* **7**(2), 823–835.

Lee, J.Y. & Kim, I.H. 2006. Advancing parity is associated with high milk production at the cost of body condition and increased periparturient disorders in dairy herds. *Journal of Veterinary Science* **7**(2) 161–166. doi: 10.4142/jvs.2006.7.2.161

Lesiakowski, R. 2016. Obora z pierwszą w Polsce kamerą BCS. *Hodowla i Chów Bydła* **2**, 58–61 (in Polish).

Luik-Lindsaar, H., Põldaru, R. & Roots, J. 2019. Estonian dairy farms' technical efficiency and factors predicting it. *Agronomy Research* **17**(2), 593–607. doi: 10.15159/AR.19.067

Mao, I., Sloniewski, L.K., Madsen, P. & Jensen, J. 2004. Changes in body condition score and in its genetic variation during lactation. *Livestock Production Science* **89**(1), 55–65. doi: 10.1016/j.livprodsci.2003.12.005

Markusfeld, O., Galon, N. & Ezra, E. 1997. Body condition score, health, yield and fertility in dairy cows. *Veterinary Record* **141**(3), 67–72. doi: 10.1136/vr.141.3.67

McCarthy, S., Berry, D.P., Dillon, P., Rath, M. & Horan, B. 2007. Influence of Holstein Fresian strain and feed system on body weight and body condition score lactation profiles. *Journal of Dairy Science* **90**(4), 1859–1869. doi: 10.3168/jds.2006-501

Roche, J.R., Berry, D.P., Lee, J.M., MacDonald, K.A. & Boston, R.C. 2007. Describing the body condition score change between successive calvings: A novel strategy generalizable to diverse cohorts. *Journal of Dairy Science* **90**(9), 4378–4396. doi: 10.3168/jds.2006-729

Ruegg, P.L. & Milton, R.L. 1995. Body condition scores of Holstein cows on Prince Edward Island, Canada: Relationships with yield, reproductive performance and disease. *Journal of Dairy Science* **78**(3), 552–564. doi: 10.3168/jds.S0022-0302(95)76666-8

Sablik, P., Kobak, P., Skrzypiec, A., Klenowicz, A. & Dereziñska, D. 2014. Comparison of body condition scores in polish Holstein-friesian cows of black-and-white variety manager in different housing systems. *Zootechnica* **13**(1), 57–66.

Van Os, J.M.C., Weary, D.M., Costa, J.H.C., Hötzel, M.J. & von Keyserlingk, M.A.G. 2019. Sampling strategies for assessing lameness, injuries, and body condition score on dairy farms. *Journal of Dairy Science* **102**(9), 8290–8304. doi: 10.3168/jds.2018-15134

Waltner, S.S., McNamara, J.P. & Hillers, J. K. 1993. Relationships of body condition score to production variables in high producing Holstein dairy cattle. *Journal of Dairy Science* **76**(11), 3410–3419. doi: 10.3168/jds.S0022-0302(93)77679-1

Wójcik, A., Gołębiewski, M. & Nałęcz-Tarwacka, T. 2012. Kondycja krów mlecznych (BCS) a kształtowanie się parametrów produkcyjnych. *Przegląd Hodowlany* **10-12**, 9–12 (in Polish).

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Estimate of manure present in compost dairy barn systems for sizing of manure storage

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Abstract. Milk production is increasingly modernized as a result of the growing demand for food around the world. Improvements in livestock facilities are observed, with a large increase in the use of feedlot systems such as the Compost Dairy Barn. Increasing milk production in confinement systems has also raised concerns such as the management of wastes (water, faeces and urine) from the system, which has become one of the most important issues in the intensive dairy farms. The aim of this work was to estimate the amount of manure present in compost dairy barn systems in order to size the manure storage. The study was conducted at four compost dairy barns in southern Minas Gerais, Brazil. These compost barns had different bedding materials and dimensions. In each farm, data on milk yield and quality (daily production, fat and protein content), animal weight and amount of feed ingested by the animals were collected. Total-day manure delivered by the cows in the feeding alley and milking parlour was piled up together and weighed. Based on the results, it was observed that, in the compost dairy barns, only part of the total manure produced per day was delivered in the milking parlour (1.6 and 2.0%) and in the feed alley (27.6 to 49.3%). These results are very important for designers for the proper manure management system design of the dairy farms.

Key words: animal facility, compost-bedded pack barn, dairy farming, manure, organic wastes.

INTRODUCTION

The performance improving in dairy farms is related to a large increase in herd confinement, using intensive housing systems such as freestalls and compost barns. Producers tend to look for more pure breed animals with better production rates and increasingly efficient nutrition.

With the increase of milk production in feedlot systems, concerns such as the management of wastes (water, faeces and urine) from the facilities have become one of the most important issues in intensive dairy farms.

In the Compost Dairy Barns (CB) system animals are housed in an open bedding area that allows them to move freely without barriers inside the resting area (Leso et al., 2019). The bedded pack (made up of organic material and cattle excreta) should often be cultivated over to incorporate the excreta into the organic material of the pack to promote the aerobic composting process (Leso et al., 2020). In this confinement system manure waste can be found mainly in the bedding area, feed alley and milking parlour.

Considerable quantities of dirty water, composed of milking parlour wash-water, milk spillages, runoff from cattle yard areas and effluent from silage and manure are produced on dairy farms (Martínez-Suller, 2010). Waste from milk production is produced daily while washing the floor of the facilities. Rinsing waters from milking parlour and milk tanks may also be part of the waste. The amount of waste produced daily will depend on the type of production system, the way the facility is washed and the quality of the workforce. The manure delivered by the herd represents the largest amount of the total waste produced in the dairy farms. Daily manure production of the animals is estimated from 9.0–12.0% of the live weight of the herd (Campos et al., 2002).

The discharge of untreated wastes into the soil, lakes, rivers causes serious environmental problems: groundwater and surface water pollution, soil pollution, proliferation of flies and gas emissions. However, several management and treatment alternatives have been developed and tested to mitigate the effects on the environment (Freitas, 2008).

For the dairy producers, an appropriately sized manure pit storage system gives several advantages, such as: reduction of effluent movement tasks daily, flexibility in the use of manure as fertilizer and more effective utilization of nutrients and water present in the manure.

Thus, estimating the amount of manure produced in dairy cattle facilities allows the correct sizing of manure storage, which is a low cost alternative to prevent the manure percolation or leaching through the soil.

The manure management system requires a correct project design, based on the potential scale of the dairy farm, as well as the potential environmental and climatic issues and the technical and productive management issues. A right sizing of volume and time of storage is related to all these aspects. Therefore, the global investment for CB has to consider the presence of a concrete feeding alley and the manure removal system. The manure storage should be sized to store all the farm wastes according to the waiting period needed for the application of the treated wasted in the soil based on the current legislation. Enough flexibility has to be considered in order to use the manure in the best period of the crops. For Brazilian conditions, Freitas (2008) recommends at least 30 days of storage volume.

In the CB facilities manure pit store usually is built to receive the manure that the cows deliver in the feeding alley while they are standing up eating the ration or drinking water. As part of the dejections is kept in the bedded pack and is composed in the bed, it is enough to manage the part of excreta released in feeding alley. Therefore the manure storage must be sized according to the amount of manure (faeces plus urine) released in the feeding alley. The ratio between manure released in the feeding alley and in bedded pack is the main question when the design of the barn is starting. In fact it is important to calculate with good precision the storage size. Janni et al. (2007) cited that farmers estimated about 25% to 30% of manure released in the feeding alley, and used a mini pit to store manure for about 2–3 days. However the right sizing of the manure storage has to be based on technical criteria.

The aim of this work was to estimate the amount of manure present in compost dairy barns in order to size properly the manure storage.

MATERIALS AND METHODS

The study was conducted in four compost dairy barns (CB) in southern Minas Gerais, Brazil. In each farm two herds, one with high milk yield (HMY) and one with low milk yield (LMY) were evaluated. In CB1 62 cows were housed, 25 LMY (18.2 kg head^{-1} day^{-1}) and 37 HMY (23.7 kg head^{-1} day^{-1}). In CB2, 53 cows were housed, 18 LMY (22.1 kg head^{-1} day^{-1}) and 35 HMY (27.2 kg head^{-1} day^{-1}). In CB3 58 cows were housed, 22 LMY (20.8 kg head^{-1} day^{-1}) and 36 HMY (25.4 kg head^{-1} day^{-1}). In CB4 68 cows were housed, 28 LMY (25.8 kg head^{-1} day^{-1}) and 40 HMY (29.2 kg head^{-1} day^{-1}). In all CB the cows were milked twice a day and fed once a day.

Some characteristics of the four CB are shown in Table 1.

Table 1. Main characteristics of Compost Dairy Barn (CB) evaluated in this study

					Bedding area	Feeding alley	Stocking density
CB	Herd Production	Milk Yield (kg head^{-1} day^{-1})	Number animals	Length (m)	Width (m)	Width (m)	(m^2 head^{-1})
1	Low	18.2	25	54.5	15.3	4.0	13.4
	High	23.7	37				
2	Low	22.1	18	50.0	14.0	4.0	13.2
	High	27.2	35				
3	Low	20.8	22	48.0	14.2	3.8	11.7
	High	25.4	36				
4	Low	25.8	28	55.0	15.0	4.2	12.1
	High	29.2	40				

The CB facilities have different dimensions. The bedding area is 832.32 m^2 (54.4×15.3 m) in CB1, 700 m^2 (50.0×14 m) in CB2, 681.6 m^2 (48×14.2 m) in CB3 and 825 m^2 (55×15 m) in CB4. As bedding material, CB1, CB2 and CB3 used sawdust and CB4 used wood shavings.

In all the four CB the feeding alley is 4.0 m width, and the surface floor made of concrete.

In each CB the following data were collected: milk yield (litres for herd divided by the total number of cows), milk quality (bulk tank fat and protein content), animal weight (weight of the animals of the herd), amount of feed ingested by the animals (kg of dry matter offered less the refusal dry matter for total herd). The amount of manure produced by LMY and HMY cows over a 24-hour period was measured. All the manure delivered by the cows in the feeding alley and milking parlour was piled up, placed in plastic buckets and weighed with digital scales. Before collecting the manure from the floor of the feeding alley, scraping was performed to remove solid manure present from the previous day.

Eq. 1 was used to estimate the amount of total manure produced by cow per day (ASAE, 2005). The amount of manure delivered in the bedding area was calculated by subtracting from the total manure the manure collected and weighed in feeding alley and in milking parlour.

$$D_{total} = (P_{Milk} \cdot 0.172) + (C_{ms} \cdot 2.207) + (G_{Milk} \cdot 171.83) + (PT_{Milk} \cdot 505.31) - 8.17 \qquad (1)$$

where D_{total} is the mass of total manure (manure excretion by cow per day); P_{Milk} is milk production by cow (kg of milk head^{-1} day^{-1}); C_{MS} is the dry matter intake (kg of dry matter by head^{-1} day^{-1}); G_{Milk} is the fat in milk (g g_{milk}^{-1}); and PT_{Milk} is the protein in milk (g g_{milk}^{-1}).

For sizing of the manure storage, the volume ($V_{est,}$ in m^3) of tanks must be calculated using Eq. 2 (Palhares, 2019).

$$V_{est} = T_a \cdot V_{res} \cdot Ft \qquad (2)$$

where T_a is the storage time (days); V_{res} is the total volume of waste produced per day (m^3); and Ft is the value of the factor dependent on the type of water diversion system (rain, sprinkler and water cooler) that exists in the facilities (feeding alley).

About Factor (Ft), the following values are used. Ft equal to 1.0 is used when no water flows into the manure storage, because no sprinklers are placed in the feeding alley and the facility is covered and has gutters on all roofs with a rainwater drainage system that falls to the floor. Ft is equal to 1.20 when part volume of goes into the manure storage. The environment has gutters on all roofs, but the rain that falls on the floor goes to the pond. Ft is equal to 1.35 when all water arrives to the manure storage (rain falls on the roofs and is collected in the concrete floors of the barn and sprinklers are placed in the feeding alley).

It was also considered that the manure has a density of 600 kg m^{-3} (Freitas, 2008) and that a storage time of 30 days is required.

RESULTS AND DISCUSSION

Table 2 shows the Milk yield (L – low and H – high), values of body weight average (BW), milk protein (PT_{milk}), fat in milk (G_{milk}), dry matter intake (C_{MS}) and total mass of total manure (D_{total}) evaluated in this study.

Body weight (BW) was from 609.4 to 672.5 kg average cow^{-1} (Table 2). According to laboratory analysis, total milk protein (PT_{Milk}) and milk fat (G_{Milk}) ranged from 3.18 to 3.39 g g_{milk}^{-1} and 4.16 to 4.50 g g_{milk}^{-1}, respectively. Dry matter intake per day (C_{MS}) ranged from 7.4 to 20.7.4 kg cow^{-1} day according the estimation results by Eq. 1, the total manure mass (D_{total}) ranged from 35.6 to 66.0 kg head^{-1} day^{-1}.

Table 3 shows the amount of manure delivered by the cows in the different places of the facilities (milking parlour, feeding alley and bedding area) evaluated in this study for L and H MY levels. No significant differences (P < 0.05) in the distribution between MY levels were found.

Table 2. Amount of manure delivered in different places of the facilities (milking parlour, feeding alley and bedding area)

CB	Milk yield	BW kg cow^{-1}	PT_{Milk} g g_{Milk}^{-1}	G_{Milk} g g_{Milk}^{-1}	C_{MS} kg cow^{-1} day^{-1}	D_{total} kg cow^{-1} day^{-1}
1	L	609.4	3.39	4.16	7.4	35.6
	H	625.7			10.0	42.3
2	L	652.7	3.42	4.50	18.8	62.3
	H	672.5			19.4	64.4
3	L	632.4	3.18	4.25	13.5	48.6
	H	652.3			13.7	49.9
4	L	652.3	3.18	4.25	16.8	56.7
	H	672.4			20.7	66.0

BW is the body weight average cow^{-1}; PT_{Milk} is the milk protein (g g_{milk}^{-1}); G_{Milk} is the fat in milk (g g_{milk}^{-1}); C_{MS} is the dry matter intake (kg of dry matter cow^{-1} day^{-1}); D_{total} is the total mass of total manure (kg of manure by cow per day).

The results show that the amount of manure present in milking parlour ranged between 1.6 and 2.0% of the total manure produced per cow per day. In literature the values considered for the effluents produced in milking parlour are between 10 and 15% (Chai et sl., 2016; White et al., 2001). However, the effluents comes from teat washing (4%), wash water (86%) and excreta (10%) (Minogue et al., 2015). White et al. (2001) found a high correlation between the time spent in a location and the proportion of manure released on it, and measured 4.6% of the faeces and 3.1% of the total urine produced in the holding area near the milking parlour and in the milking parlour for grazing systems. Most of the manure (84.1% urine and 84.7% faeces) occurred in the paddock, suggesting that the storage capacity and cost of manure handling facilities for pasture-based dairy systems could be greatly reduced.

Table 3. Average and standard deviation of manure delivered in different places of the facilities (milking parlour, feeding alley and bedding area) by MY level

Milk yield	Milking parlour (kg cow^{-1} day^{-1})	Feeding alley (kg cow^{-1} day^{-1})	Bedding area (kg cow^{-1} day^{-1})
L	0.93 ± 0.25	16.90 ± 0.16^{a}	32.92 ± 11.24
H	1.01 ± 0.21	22.52 ± 1.35^{b}	32.05 ± 10.23

L – Low milk yield; H – High milk yield. Different letter between rows shows significate differences ($P < 0.05$).

In the feeding alley, the amount of manure released ranged between 27.6 to 49.3% of the total manure per cow per day. The manure delivered in the bedding area (estimated as total manure per cow per day less milking parlour and feeding alley manure collected) ranged between 49.0 to 70.6% of the total amount of manure produced per cow per day. The amount of manure in the bedding area in LMY of CB2 and LMY of CB4 (70% and 68.3% respectively) was similar to values reported by Janni et al. (2007).

The highest amount of manure delivered in the feeding alley was in the HMY of CB1 (49.3%). Samer (2011) found that in corral systems about 75–80% of the waste is gathered from the feeding places and in free-stalls about 100% of the waste is gathered from the manure canals.

In this study, it was considered that all the manure delivered in the feeding alley would be stored for a period of at least 30 days (T_a) in a pit storage system (Freitas, 2008), in order to have good flexibility in the application of manure on the soil (Table 4).

In all the evaluated compost barns, manure and rainwater were deposited in the manure storage. So, considering the design of the four facilities, Ft was equal to 1.2. Based on these parameters, the total volume of manure produced per day (V_{res}), estimated volume of manure produced (V_{est}) and total volume of manure produced (V_{total}) were calculated.

Table 4 shows the volume for 100 cows with the mean values obtained in the present study for different MY levels. In addition, the maximum value was used to calculate the volume requirement with a security margin.

Table 4. Total volume (V_{total}) required for 30 days (T_a) to store manure of 100 cows by milk yield level (MY) from feed alley of the compost barn. Average manure density (D_d) = 600 kg m^{-3}, water contribution factor (F_t) = 1.2

MY	Variable	Feeding alley (kg cow^{-1} day^{-1})	V_{res}(m^3 day^{-1})	V_{est} (m^3)	V_{total} (m^3)
L	Mean	16.9	84.5	25.0	101.4
	Maximun	17.1	85.5	30.2	102.6
H	Mean	22.5	112.6	52.4	135.2
	Maximun	24.1	120.5	60.5	144.6

Considering the results showed in the Tables 2, 3 and 4, it was observed that the lowest portion of the manure produced per day (V_{res}) was delivered in the milking parlour and the highest in the in the feeding alley and bedding area (up to ~50%). These results are in line with those reported by Priekulis & Āboltiņš (2015), which found that approximately a half, or even more, of the manure from cattle was represented by farmyard manure.

Therefore, based on the estimated and calculated values for 100 cows, it was observed that the total volume of manure (V_{total}) ranges between 101.4 to 144.6 m^3 for 30 day of storage.

CONCLUSIONS

According to the results of this study, it was possible to estimate the amount of manure present in compost dairy barns and how manure is distributed in the different areas of the facility. These data allow to design the appropriate manure storage system. The correct estimation of manure production per day is the key for sizing the manure storage system according the waiting period required by legislation.

The volume of the manure storage for compost dairy barn system is calculated based on the amount of the manure delivered by the cows. On the basis of the results of this study, in the feeding alley the maximum measured value should be considered (49.3%). Considering 30 days as the minimum waiting time period before to apply the manure on the soil, the volume of storage per cow required is 1.209 m^3 cow^{-1} on average, but it ranges from 1.014 m^3 cow^{-1} for the lowest value measured for the low milk yield (LMY) to 1.446 m^3 cow^{-1} for the highest value measured for the high milk yield (HMY).

These values give a very important contribution to the designer of compost barns because the manure storage system is a key part of the facility. A consistent, flexible and safe system of storage allows the strategic use of manure as fertilizer and reduces the risks of environmental impact.

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REFERENCES

ASAE. 2005. *Manure Production and Characteristics*. American Society of Agricultural Engineers, St. Joseph, MI 49085-9659, USA, 20 pp.

Campos, A.D., Ferreira, W.A., Paccola, A.A., Lucas Júnior, J.D., Ulbanere, R.C., Cardoso, R.M. & Campos, A.T. 2002. Aerobic biological treatment and recycling of bovine manure in intensive system of milk production. *Ciencia e agrotecnologia* **26**, 426–438 (in Portuguese).

Chai, L., Kröbel, R., MacDonald, D., Bittman, S., Beauchemin, K.A., Janzen, H.H., Mc Ginn, S.M. & Vanderzaag, A. 2016. An ecoregion-specific ammonia emissions inventory of Ontario dairy farming: Mitigation potential of diet and manure management practices. *Atmos. Environ.* **126**, 1–14.

Freitas, J.Z. 2008. *Manure storage for Cattle Waste.* Niterói, Programa Rio Rural. 12 pp. (in Portuguese).

Janni, K.A., Endres, M.I., Reneau, J.K. & Schoper, W.W. 2007. Compost dairy barn layout and management recommendations. *Appl Eng Agric.* **23**(1), 97–102.

Leso, L., Pellegrini, P. & Barbari, M. 2019. Effect of two housing systems on performance and longevity of dairy cows in Northern Italy. *Agronomy Research* **17**(2), 574–581.

Leso, L., Barbari, M., Lopes, M.A., Damasceno, F.A., Galama, P., Taraba, J.L. & Kuipers, A. 2020. Invited review: Compost-bedded pack barns for dairy cows. *J. Dairy Sci.* **103**(2), 1072–1099.

Martínez-Suller, L., Provolo, G., Carton, O.T., Brennan, D., Kirwan, L. & Richards, K.G. 2010. The composition of dirty water on dairy farms in Ireland. *Irish J. Agr. Food Res.* **49**, 67–80.

Minogue, D., French, P., Bolger, T. & Murphy, P.N. 2015. Characterisation of dairy soiled water in a survey of 60 Irish dairy farms. *Irish J. Agr. Food Res.* **54**(1), 1–16.

Palhares, J.C. 2019. Management of wastes in dairy production. *https://www.agencia.cnptia.embrapa.br* (in Portuguese). Accessed 8.7.2019.

Priekulis, J. & Āboltiņš, A. 2015. Calculation methodology for cattle manure management systems based on the 2006 IPCC guidelines. In *Nordic View to Sustainable Rural Development*, Proc 25th NJF Congress, Riga, Latvia, 16-18 June 2015, pp. 274–280.

Samer, M. 2011. How to construct manure storages and handling systems? *IST Transactions of Bio-systems and Agricultural Engineering* **1**(1), 1–7.

White, S.L., Sheffield, R.E., Washburn, S.P., King, L. D. & Green, J.T. 2001. Spatial and time distribution of dairy cattle excreta in an intensive pasture system. *J. Environ. Qual.* **30**(6), 2180–2187.

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A sustainable approach to boosting liquid biofuels production from second generation biomass resources in West Africa

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Abstract. West African region has abundant second generation biomass resources consisting of agricultural residues, forest resources; municipal solid wastes; and animal wastes that could be harnessed to produce liquid biofuels. A number of countries in the region have developed energy policies to foster bioenergy production. Despite the national intent expressed in various countries' bioenergy policies, development of bioenergy facilities and liquid biofuels production from cellulosic sources in the region are essentially at the research and development stage. This study, through comprehensive reviews of various bioenergy policies, news reports, related journal articles and development reports, examined the reasons for the delay in the development of bio-refineries in the region. The study then articulated feasible solutions to address the challenges. Among the discovered causes of the delay are over-dependence on fossil fuels and defective energy policy implementation manifesting in the form of lack of continuity. Other issues include poor private sector's involvement and inadequate incentives necessary for private investors' participation. This study concludes that boosting liquid biofuels production in West Africa would require public-private collaboration that is built from bottom-up. Successful bioenergy facilities' development in the region would need to be community level scaled rather than being mega projects, and it would need to involve participation of communities as collaborators. In addition, to ensure sustainable production, it would be necessary to incorporate public enlightenment, and grant tax incentives to investors. Moreover, it would need to include a sustainable technology training package that would empower local engineers and technicians to not only develop bioenergy facilities that are suitable for the locality but also to maintain and improve them. Furthermore, Continuity and consistency in policy implementation and financing prioritization are essential to boosting liquid biofuel production in the West African region and to enable West African region to occupy its rightful place in the global bioeconomy.

Key words: bioeconomy, bioenergy, liquid biofuels, sustainability, West Africa.

INTRODUCTION

There is an increasing demand for transition from fossil fuel based energy source to biofuels. The clamor for the change is essentially driven by three main factors, namely: 1) Climate change trigger by greenhouse gas emissions from fossil fuel combustion; 2) impending depletion of natural resources, especially the fossil fuels being non-renewable natural resources, and 3) an increased worldwide population with increasing need for energy supply. Other important reasons while transitioning to advanced biofuel production and use include the need to eliminate various negative consequences of using solid biofuels. For example, quoting World Health Organization, Ndiaye (2018), stated that 'over 4 million people die prematurely from illness attributable to the household pollution from cooking with solid fuels'. He further stated that 'More than 50% of premature deaths due to pneumonia among children under 5 are caused by the particulate matter (soot) inhaled from household air pollution'. There is therefore a need for the development of advanced biofuel technologies to produce liquid biofuels. Production of liquid biofuels from second generation biomass have been hailed as a viable renewable energy source that would not only enable us to satisfy the increasing energy demand, it is also seen as a carbon-neutral source of energy that would enable us reduce the impending climate change danger. Such endeavor is also expected to help eliminate or at least reduce other negative consequences of using petroleum products and solid biofuels. Second generation biomass sources of liquid biofuel production are derived from cellulosic sources such as forest and agricultural residues, and market/household organic wastes (Jokiniemi & Ahokas, 2013).

Second generation biomass availability in West Africa

The West African region is blessed with abundant cellulosic biomass resources. That is why according to Ndiaye (2018), 'Wood accounts for more than 80% of all primary energy in West African countries'. The West African regional vegetation zones range from the dense tropical forest in the south to the savanna grassland in the north (Fig. 1). The amount of residues from harvesting and processing these abundant woody resources in the form of logging and saw-milling are enormous and they are readily available for use as feedstock for the production of liquid biofuels.

Figure 1. West African vegetation zones.
Source:https://eros.usgs.gov/westafrica/sites/default/files/inlineimages/160823_Climate _zones.jpg

Liquid biofuels and necessity to boost production of liquid biofuels

All over the world, due to its being a carbon-neutral source of energy, there is an increasing demand from liquid biofuels. Consequently, there is also an increasing emphasis on the production of liquid biofuels, especially from cellulosic sources rather than from first generation biomass sources like grains and other food crops. While Brazil and the USA are the largest producers of liquid biofuels, many other countries like China and India have developed aggressive programs of liquid biofuels production. However, the production of liquid biofuels in the West African region is still at an infancy stage. Most of the countries in the region depend mainly on petroleum fuels for transportation, for cooking and for electric energy generation. Apart from seasonal scarcity of the petroleum fuels in some countries, the use of fossil fuels is causing a lot of environmental pollution. It is thus necessary that having been endowed with abundant sources of lignocellulossic biomass, the region has a lot to benefit by pursuing aggressive liquid biofuel production and utilization like other regions of the world (van Zyl, 2011).

The aim of this paper was to review the status of liquid biofuels production in West Africa, to articulate the driving factors and challenges to its abundant production in the region, and to provide some insights on how to boost sustainable production of liquid biofuels in the region.

MATERIALS AND METHODS

The methodology of this study involved a comprehensive review of bioenergy literature and reports to identify commitments made by a number of West African countries and by the Economic community of West African States to the development of bioenergy sector, especially the production of liquid biofuels. The study also reviewed various liquid biofuels production initiatives in the region with the aim of articulating the driving motivations behind the investments and various sources of support received. The levels of success of the initiatives, encountered development challenges as well as other limiting factors were also evaluated. Steps were then taken to articulate what can be done to remove the obstacles to the development of bioenergy in the region. Various models of possible pathways to boost liquid biofuels production in the region were then developed and analyzed before drawing conclusion on sustainable approach to boosting liquid biofuels in West Africa.

RESULTS AND DISCUSSION

This section provides the outcomes of our review on global trends in the production of liquid biofuels. We also provided a summary of liquid biofuel production policies of a number of countries in the region and of ECOWAS. Furthermore, we articulated various liquid biofuel production initiatives in the region. We also discussed various challenges of producing liquid biofuels from lignocellulosic biomass, and concerns raised regarding potential environmental and socio-economic impacts of bioenergy development in West Africa. We then presented a model of possible pathways to boosting liquid biofuels and the requisite enabling infrastructure in a way that the problems are appropriately addressed. Finally, we analyzed the potential impacts of implementing the framework in achieving some sustainable development goals in West Africa.

Liquid biofuels production methods

Bioethanol, biodiesel and bio-oil are the three liquid biofuels of interests. Methods of processing lignocellulosic biomass to liquid biofuels can be divided into two categories, namely: biochemical processes and thermochemical processes. Biochemical methods are used for the production of biogas, bioethanol and biodiesel from lignocellulosic biomass while thermochemical methods are used to convert biomass to bio-oil. Enzymatic fermentation method is the most commonly used biochemical method. Biochemical methods generally consist of three main steps, namely: pre-treatment, enzymatic pyrolysis and fermentation. Torrefaction, thermal liquefaction, pyrolysis and gasification are the common thermochemical methods. Bio-oils are the most preferred pyrolysis fuel types for transportation purpose while biochar and flue gases are pyrolysis by-products produced along with bio-oil. Bio-oils are in most cases unsuitable for direct use, they often require refining and further processing steps just like in petroleum refineries. There has been a lot of scholarly works on the liquid biofuels, biomass feedstocks and the bioprocessing methods. This review is focused on boosting liquid biofuels, readers are directed to the following literatures for further details on biofuels production methods: Balat et al., 2009; Zhang et al., 2010; Panwara et al., 2012; Yılmaz & Selim, 2013; Kumar et al., 2015, and Kan et al., 2016.

Global trends in the production of liquid biofuels

The production and use of liquid biofuels is growing globally. According to WBA (2019), 'In 2017, 138 billion litres of biofuels were produced including bioethanol, biodiesel, HVO (Hydrogenated Vegetable Oil) etc'. It accounts for about 3% of the transport sector energy use in 2017. IRENA (2020), projected that liquid biofuels in the form of ethanol and biodiesel could account for 10% of transport sector energy use by 2030. The reason for the growth is due to the many advantages of biofuels as carbon neutral alternatives to fossil fuels, its renewability, and its promotion of rural development (von Maltitz et al., 2009; Ghobadian, 2012; Oyedepo et al., 2019). The global change from fossil fuels to biofuels is led by the EU with its legislative support which required member countries to ensure the blend of conventional fossil fuels with a certain percentage of bioethanol. Many countries, including the US, Brazil, China and India have since followed suite (Ghobadian, 2012; IEA, 2019a). Global production of biofuels in 2018 was 154 billion litres. Total biofuel output is forecast to increase 25% by 2024. According to the report, China is set to have the largest biofuel production growth of any country while the United States and Brazil still provide two-thirds of total biofuel production in 2024. However, biofuel production in the United States and EU member states is not on track to meet the projected 2030 demand (IEA, 2019a and 2019b; Nyström, 2019).

West Africa's biofuels production potentials

West Africa has a lot of potential for significant contribution to global biofuels production as it is bequeathed with abundant biofuels feedstocks, especially from lignocellulosic sources. Onuoha (2010), Ishola et al. (2013); Iye & Bilsborrow (2013); Simonyan & Fasina (2013), Ben-Iwo et al. (2016), Adewuyi (2020), and Gnansounou et al. (2020) provided extensive reviews of various types of biomass resources and biofuels production potentials in Nigeria. Baumert et al. (2018) and Bridge builders (2019) discussed the growing of Jatropha and its use for biofuel production systems of Burkina Faso. According to Dianka (2012), 'West African region has a large potential to become a leading producer of many types of biofuels given the availability of raw materials, climate conditions, land availability and production costs'. He further opined that 'the economical and political stability of the Union favour the development of a common biofuels market that can become a substantial exporter of biofuels to the EU'. Table 1 shows the projected biofuels production potentials from some West African countries. Simonyan and Fasina (2013) also stated that 'Nigeria is capable of producing 2.01 EJ (47.97 MTOE) of energy from the 168.49 million tonnes of agricultural residues and wastes that can potentially be generated in a year.' In another discuss, Onuoha (2010) indicated that Nigeria has the capacity to produce 61 million tonnes of animal waste per year; 83 million tonnes of crop residue per year, and 13,071,464 hectares of forest land to produce woody biomass. In another report, quoting Iwayemi (2008), Dunmade (2017) stated that Nigerian biomass endowment stands at 144 million tonnes per year, a large percentage of which can be harnessed for energy production. Tables 1a and 1b show the projected amount of residues for each crop from some West African countries by 2050.

Table 1a. Total amount of residues for each crop and country in 2050 (Gnansounou et al., 2020)

Countries	Residues in 2050 (tons year^{-1})			
	Banana	Cassava	Maize	Oil palm fruit
Benin	9,234	819,256	7,685.674	3,077.327
Burkina Faso	-	973	8,918.250	-
Cote d'Ivoire	129,339	501,689	3,715.272	10,684.203
Gambia	-	2,419	166,548	242,021
Ghana	33,892	3,119,461	11,614,328	11,937,521
Guinea	93,332	249,527	3,771,572	4,045,498
Liberia	56,800	117,975	-	1,102,467
Mali	104,809	10,079	9,656,257	-
Niger	-	30,143	60,527	-
Nigeria	-	9,918,856	65,349,629	30,153,631
Senegal	15,077	35,143	1,304,100	971,578
Sierra Leone	-	810,805	272,168	1,347,265
Togo	10,652	212,098	4,425,680	584,178
Total	453,135	15,828,423	116,940,006	64,145,689

Table 1b. Total amount of residues for each crop and country in 2050 (Gnansounou et al., 2020)

Countries	Residues in 2050 (tons year^{-1})				
	Rice	Cotton	Sorghum	Soybeans	Sugarcane
Benin	1,065,856	1,472,230	989,713	59,640	30,021
Burkina Faso	1,674,142	2,827,218	14,391,713	93,638	374,608
Cote d'Ivoire	902,837	1,387,922	366,398	3,412	1,620,026
Gambia	35,543	2,949	207,549	-	-
Ghana	348,962	74,947	2,208,393	-	121,572
Guinea	1,195,235	228,402	284,269	-	115,888
Liberia	167,959	-	-	11,262	119,738
Mali	1,874,119	2,628,206	9,565,583	13,338	295,313
Niger	11,585	48,853	9,418,996	-	106,378
Nigeria	3,061,685	1,631,367	45,238,144	2,448,622	1,544,025
Senegal	452,933	186,605	880,192	-	879,523
Sierra Leone	747,960	-	266,739	-	37,117
Togo	72,804	493,664	2,186,686	14,621	-
Total	11,611,622	10,982,362	86,004,374	2,644,424	5,244,209

Table 2 shows the projected amount of cellulose, hemicellusoe, and lignin that could be produced by each country by 2050 while Table 3 shows the West African biofuels production potentials and sources. Within the last two decades, oilpalm and jathropa have gained a lot of attention as energy crops of focus for biofuels production (Torrey, 2010). Looking at the foregoing data, it is clear that West African region has a great potential to make significant contribution to global liquid biofuels production.

Table 2. Amount of cellulose, hemicellulose and lignin available in the selected WA area by 2050 considering that 50%residues were left on the field. Energy content in the selected biomass (Gnansounou et al., 2020)

Countries	Cellulose (tons year^{-1})	Hemicellulose (tons year^{-1})	Lignin (tons year^{-1})	Total Energy content (GJ year^{-1})
Benin	2,435,313	1,463,603	1,341,186	93,989,852
Burkina Faso	4,602,408	2,882,498	2,164,508	172,284,720
Cote d'Ivoire	3,259,012	1,997,339	1,904,670	126,688,100
Gambia	106,951	67,504	54,160	4,000,678
Ghana	4,899,719	3,009,908	2,692,610	190,514,168
Guinea	1,484,255	911,400	828,349	60,235,161
Liberia	260,013	160,925	153,594	10,195,012
Mali	3,593,196	2,223,739	1,742,045	135,486,934
Niger	1,671,345	1,104,608	660,506	60,474,290
Nigeria	25,635,444	16,035,878	12,817,400	981,005,286
Senegal	731,502	469,398	384,690	27,583,543
Sierra Leone	480,114	290,291	274,929	19,140,877
Togo	1,262,812	781,084	629,165	47,632,777

Table 3. West African biofuels production potentials and sources (Dianka, 2012)

Country	Biofuel type production potential and sources
Benin	20,000 m^3 year^{-1} ethanol based on cassava
Burkina Faso	20,000 m^3 year^{-1} ethanol based on sugarcane
Ivory Coast	19,000 m^3 year^{-1} ethanol based on molasses
Guinea-Bissau	~10,000 m^3 year^{-1} ethanol based on cashew tree apples
Mali	18,000 m^3 year^{-1} ethanol based on molasses
Niger	Biodiesel based on jatropha
Senegal	15,000 m^3 year^{-1} ethanol based on molasses
Togo	10,000 m^3 year^{-1} biodiesel based on jatropha
Total	Ethanol (93,000 m^3 year^{-1}) and biodiesel (20,000 m^3 year^{-1})

Status of liquid biofuels production in West Africa

ECOWAS and its member countries, with the support of their partners, have been working hard to boost the production of liquid biofuels as a component of its regional policy on renewable energy. ECOWAS Renewable Energy Policy (EREP) and ECOWAS Energy Efficiency Policy (EEEP) policies outline Renewable Energy and Energy Efficiency targets to be achieved by ECOWAS countries by 2030. Auth and Musolino (2014) stated that, 'he goals of the policies correspond precisely to the poles of the UN Secretary General's initiative on Sustainable Energy for All and have led to new developments in the renewable energy and energy efficiency sectors throughout the region'. FAO (2019) also hinted that 41 countries out of the 54 African countries mentioned in their policies that they will implement at least one modern bioenergy measure to mitigate their GHG emissions. Liquid biofuels and biogas are among the top five policies and measures that African countries are focusing on to combat GHG emissions (ECOWAS, 2013; Dunmade, 2017; IEA, 2019a and 2019b).

In West Africa, fourteen countries indicated the need to develop modern bioenergy to mitigate their GHG emissions. Focusing on Liquid biofuels, ECOWAS member countries adopted biofuel-blending targets of 5 percent for ethanol and biodiesel in 2013 and a Bioenergy Strategy in 2016. Majority of the ECOWAS countries are focusing on liquid biofuel production to be used for transport except Cabo Verde that wants to generate electricity from biodiesel in remote islands. Mali also intends to develop 4 MW through hybrid systems (solar and jatropha) for rural electrification while Niger focuses on the production of biofuel and biogas for cooking purposes (FAO, 2019). Nigeria is promoting a blending mandate of 10 percent ethanol with gasoline (E10) and 20 percent of biodiesel with diesel (B20). Similarly, Liberia hopes to reduce its emissions by 58 ktCO2e per year by blending its diesel fuel with 5 percent of biodiesel from palm oil. Furthermore, Burkina Faso is also promoting the creation of biofuel plants with a blending mandate of 10 percent ethanol and 5 percent biodiesel. Other West African countries like Togo, Gambia, Guinea and Sierra Leone are also advocating production of liquid biofuels from various feedstocks such as sugarcane, corn and rice husk (Abila, 2012; Jokiniemi & Ahokas, 2013; Popoola et al., 2015).

Jathropa and oilpalm

Jathropa and oilpalm have received a lot of investment attention in the last two decades as energy crops with great potentials in many West African countries. There has also been a lot of discussions about the pros and cons regarding their cultivation and utilization as feedstocks for biofuel production. Jatropha is a hardy tree that thrives in hot, dry climates. A number of jathropa species are widely grown as an energy crop across West Africa especially in Burkina Faso, Ghana, Mali, Niger, and Nigeria. Jatropha curcas is particularly recommended for cultivation as energy crop. It does well in quite extreme conditions of semi-arid climate and marginal soil, however, to yield plenty of fruit for producing oil, it needs sufficient water, fertilizer and care (The Research Council of Norway, 2011). There are concerns that its intensive cultivation would lead to 'converting arable land' for the cultivation of energy crops and that could affect staple food production, lead to increased food prices and food insecurity. Anderson (2007) was of the opinion that jatropha is a crop that can be used for biofuels without causing the aforementioned problems because it is used by farmers as a border around their crops. She stated that its appalling scent and inedible seeds keep animals away from the food crops inside. In addition, the oil from jatropha seed can be easily refined into biodiesel. Anderson (2007) also opined that jatropha may have a future in sub-Saharan Africa once crop improvements have been devised and each country adapted modern management practices are established.

Oil palm (Elaeis guineensis) is another crop of importance that originated and widely grown in various parts of West Africa. It is a versatile crop in that all parts of the plant, 'from the roots to the flowers to the by-products, are used for food, traditional medicine, and for their important sociocultural value' (Yombouno, 2014). Nigeria is the largest producer of palm oil, with a global market share of 3%. It is followed by Cote d'Ivoire (Duku et al., 2011). The last two decades have witnessed rapid growth in commercial oilpalm plantations, many of which are supported by governments and by foreign investors. However, there are also some concerns regarding environmental and socioeconomic impacts of commercial oilpalm plantations. For example, Cernansky (2019) stated that there are concerns about impacts on local water supplies, wildlife populations, biodiversity, climate change as well as loss of farmlands by the locals to commercial oilpalm plantations. Additional impacts include communities displacement as well as their cultural/traditional institutions alterations. All stakeholders especially local communities involvement as participants in large scale oilpalm development would be necessary for its sustainable growth.

Major investors in West African energy crop cultivation and biofuels production

Although there are lots of enthusiasm regarding transition to the production and utilization of advanced liquid biofuels in West Africa but only limited number of the initiatives have been started. Those that were started are essentially focused on blending petroleum fuels with varying percentages of ethanol imported largely from Brazil. There are also some indigenous and foreign companies in West Africa that started commercial production of energy crops and/or biofuels production. Annuanom Industrial Project Limited, KITE-Ghana (Kumasi Institute of Technology, Energy, and Environment), the Gratis Foundation, New Energy-Ghana, and Biofuel Africa Ltd are some of the major players in Ghana with investment focus on jathropa (Antwi-Bediako, 2019). For example, Biofuel Africa Ltd. started its first working farm operation in Ghana in 2007. Its activities involve growing and selling jatropha fruits and seeds to production and sale of jatropha oil on a commercial scale (Torrey, 2010). There were also some government-private participation programmes aimed at fostering biofuels production. An example is the case of Nigerian National Petroleum Corporation's partnership with state governments and other industry partners (Dianca, 2012; Dunmade, 2018a). Tables 4 and 5 are Ghana based bioenergy/biofuels investment projects based jathropa and oilpalm serving as picturesque of the trends in West Africa. While there are robust bioenergy production policies by many West African countries, the majority of the initiatives focusing on local production of liquid biofuels are still on the drawing boards awaiting technical partnerships and funding. Although the delay in the take-off of these laudable projects are of concern, it however provides opportunities for proper learning from current producers. It should afford the ECOWAS countries ample time to learn, and develop/utilize lifecycle based liquid biofuel production facility design principles. Adopting sustainable design approach would not only result in minimum ecological footprint but also ensure economically sustainable and socially acceptable production systems (Cristobal-Sarramian & Atzmuller, 2018; Dafrallah, 2010; van Zyl, 2011; Popoola et al., 2015; Ndiaye, 2018; Dunmade, 2019; Nyström et al., 2019).

Table 4. Major investments in Jatropha curcas plantations in Ghana (Duku et al., 2011)

Name of institution/company	Land area under cultivation (ha)	Funding sources
B1 Ghana	700	Private investment
ADRA/UNDP	800	UNDP/GEP/ADRA
New Energy	6	Donor funding
Gbimisi Women Group	4	UNIPEM/UNDP-GEP
AngloGold Ashanti Ltd	20	Corporate Fund
Valley View University	4	University Funds
Total	1,534	

Table 5. Major investments in the oil palm industry in Ghana (Duku et al., 2011)

Company	Location
Benso Oil Palm Plantation (BOPP) Ltd.	Benso
Twifo Oil Palm Plantation (TOPP) Ltd.	Twifo Praso
Ghana Oil Palm Development Corporation Ltd (GOPDC); known as the Okumaning Oil Palm Plantation Development Programme	Akim Oda
National Oil Palm Plantation	New Juaben
Norpalm Ghana Ltd (formerly called Prestea Oil Palm Plantation)	Presten
National Oil Palm Plantation Ltd	Ayiem

Sustainable pathway to boosting liquid biofuels production and utilization in West Africa

It has been projected that Africa has the potential to be the world's largest bioenergy producer by 2050. It was estimated that the continent has the capacity to produce up to a quarter (317 EJ per annum) of the projected total world potential of 1272 EJ per annum (Auth & Musolino, 2014). However, production of advanced liquid biofuels in commercial quantities is still at the infancy stage in many West African countries (Adjovi, 2012; Afrane, 2012; Dunmade, 2020). There are therefore a number of things to be put in place in order to boost West African liquid biofuels production in a way that would enable it to occupy its rightful place in the world. There is a need to develop a program of identifying, selecting and focusing on massive production of a few number of high yield locally adaptable bioenergy crops that would serve as feedstock for the biofuels sub-sector (Dioha & Kumar, 2020). Although significant progress has been made in this regard, as one can see a rise in foreign investment in the production of biofuel crops like jatropha, cassava, oilpalm, cashew and sugarcane. However, jatropha is a bioenergy crop with future potential for liquid biofuel production because cassava and sugarcane are important staples in the sub-region. Another good step that has been taken in that direction is the enabling policies that have been put in place by many West African countries to enhance the optimum production and utilization of Jatropha for biofuel production in the sub-region. Further progress would include expanding the sources of feedstock for the industry. Among the areas that could be explored include putting policies and logistic infrastructure in place for the gathering and marketing of forest residues, sawmilling wastes, household and market organic wastes (Auth & Musolino, 2014; Oyedepo et al., 2019).

FAO (2019) reported that there is increasing liquid biofuel use in Western African countries like Nigeria and Ghana. The growing trend has been attributed to the adoption of two policies in the region, namely: the 5 percent biofuel-blending target for ethanol and biodiesel in 2013 and a bioenergy strategy in 2016. However the growing demand needs to be met with adequate supply of locally produced liquid biofuel. Hence the need to boost the local production as doing so would lead to the development of the local economy, increased rural jobs, improved public health and the overall improvement in standard of living of the citizens. In addition, boosting liquid biofuels production in West Africa would involve providing an enabling environment for steady growth in local demand as well as expansion to regional and international consumer market for the production outputs (Smeets et al., 2007; ECOWAS, 2013; Popoola et al., 2015; FAO, 2019). Fig. 2 illustrates a model pathway to boosting liquid biofuels production in West Africa. It shows the need for a participative approach, that involves all the stakeholders.

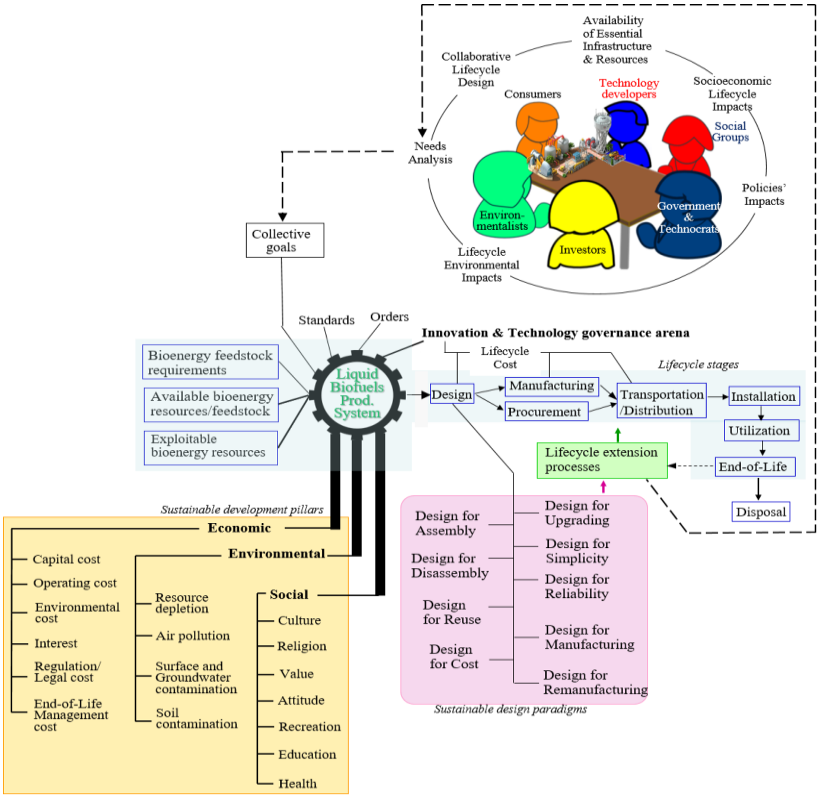

Figure 2. Diagrammatic illustration of a model sustainable pathway to boosting liquid biofuel production in West Africa.

It also shows the need to consider the availability of requisite infrastructure for stable and sustainable operation of biofuels production facilities on a long run (Dunmade, 2010 and 2020). Furthermore, the framework advocates the use of lifecycle engineering principles both in the facility design and management of the operations of such liquid biofuel production systems (Dunmade, 2012 and 2013a). It sees a necessity to ensure that available bioenergy resources match the requirements of the developed liquid biofuels production systems. In addition, it shows the need for progressive capacity building for the evolution/proliferation of local or hybrid liquid biofuel production technologies. Moreover, improved biofuel production would require tailoring the capacity to manageable scale at the beginning and making progressive production and market expansion as the technical-knowhow increases. Involvement of local communities and other stakeholders as participants, right from the beginning throughout the lifecycle of the project would be necessary for the success and continuous improvement/expansion of liquid biofuels production in the region (Dunmade, 2013b and 2018b).

Bioenergy development challenges and solutions

Although there are several benefits in producing liquid biofuels from second generation biomass, there are also many challenges to its implementation (FAO and

PISCES, 2009). Challenges to liquid biofuel production from lignocellulosic biomass can be divided into three major categories, namely: Feedstock, technology and policy issues.

According to Nigam & Singh (2011), ’production of second-generation biofuel requires most sophisticated processing production equipment, more investment per unit of production and larger-scale facilities to confine and curtail capital cost scale economies. To achieve the potential energy and economic outcome of second-generation biofuels, further research, development and application are required on feedstock production and conversion technologies’.

Furthermore, there are many concerns raised regarding potential environmental and socio-economic impacts of bioenergy development in West Africa (Dunmade, 2019). Issues raised regarding bioenergy production in Africa include the requirement of large expanse of land to grow energy crops on a commercial scale, loss of land rights of local communities, economic viability of large scale energy crops production, negative impacts on climate change, water pollution, soil erosion, deforestation, food security, human health and social conflicts, and loss of biodiversity (The Research Council of Norway, 2011; UNU-IAS, 2012; Kgathi et al., 2017). ECOWAS (2014) stated that ‘collecting these resources also exposes women-mostly in rural areas-to the risk of injury, rape, or harassment, and limits the time available for education, commercial activities, or leisure.’ The use of firewood for cooking also exposes women to ‘household air pollution from indoor smoke, small particle pollution, carbon monoxide, and nitrogen oxides’ (ECOWAS, 2014).

It is also argued that large scale energy crops production will deprive farmers the land to grow crops required for their subsistence, and that this could lead to food scarcity and food price hike (Anderson, 2007) Consequently, many people at the economic fringes may not be able to afford to buy nutritional food. Energy crops can thus worsen food insecurity in poor countries (Adjovi, 2012). Another concern is that large bioenergy development programs can lead to deforestation. It is also believed that it will worsen the current energy provision burden placed primarily on women, which is not only depriving them of engaging in gainful employment but also responsible for widespread respiratory diseases resulting from their cooking with charcoal and firewoods (Forest cover, 2019). All the aforementioned concerns are genuine and worth consideration in bioenergy development programs. However, those concerns can be addressed through adequate planning and supportive policies. For example, according to Simonyan & Fasina (2013), ‘lignocellulosic feedstocks (such as trees, shrubs, grasses, agricultural and forest residues) are potentially more abundant and cheaper than feedstock from conventional agriculture because they can be produced with fewer resources and on marginal and poor lands. Also, agricultural and forest residues are currently available from current harvesting activities without the need for additional land cultivation’. They further stated that about 50% of the residue are burnt on cropland before the start of the next growing season.’ Such burning of residue is not only a waste of resources but it also contributes to global warming, photochemical smog and associated health problems. Agrifood process residues, which are currently being sent to landfills, are also potential energy sources that can be harnessed to boost biofuels production in West Africa. The effort being made in Benin, Burkina Faso, Ivory Coast, Mali and Togo at promoting a community based intercropping of improved Jatropha seeds with food crops is helping to stop soil erosion, restoring degraded lands, and providing a significant increase and sustainability on food crops yields (Kotrba, 2013; Bridge builders, 2019). These goals will be easily realized under a more efficient plant breeding and improved agronomic management (Abila, 2014; Baumert et al., 2018). In addition, community scale bioenergy development programs involving the use of improved energy and food crops seedling, efficiently managed biofuel processing and marketing with adequate supportive governmental policies would eliminate or at least minimize most of the aforementioned concerns. Kgathi et al. (2017) buttressed this point by reporting ‘positive environmental impacts such as high energy return on investment and high GHG savings when Jatropha is cultivated on abandoned agricultural fields in some parts of West Africa’.

Furthermore, community scale bioenergy programs would lead to increased women employment, improved social status/standard of living of rural dwellers, increase local government revenues, and general rural development. Moreover, it will reduce women’s dependence on using firewood for cooking and thereby reduce the associated respiratory diseases. Anderson (2007) recommended slow implementation of jathropa as a biofuel to allow time for farmers to adapt to new modern farming practices/technology. Jatropha, according to Anderson (2007), would be an ideal supplement to small farmers’ income since it doesn't require irrigation, requires very little fertilizer and very little care.

CONCLUSIONS

This paper presented the current trend in global liquid biofuels production, with a focus on the status of biofuels production in West African sub-region. A number of steps that need to be taken to boost liquid biofuel production in the region were then highlighted. Among the articulated points are the need to expand the current feedstock base, establishment of enabling environment for continuous improvement in consumer market demand, and steps to evolution and proliferation of locally owned advanced lignocellulosic biofuel production technologies. Taking these steps are expected not only to boost liquid biofuel production in the region, but it will foster continuous growth and encourage innovations in the sector. Potential impacts of implementing the framework is that it will ultimately lead to attainment of a number of sustainable development goals in the West African sub-region.

REFERENCES

Abila, N. 2014. Biofuels adoption in Nigeria: Attaining a balance in the food, fuel, feed and fibre objectives. *Renewable and Sustainable Energy Reviews* **35**, 347–355.

Abila, N. 2012. Biofuels development and adoption in Nigeria: Synthesis of drivers, incentives and enablers. *Energy Policy* **43**, 387–395.

Adewuyi, A. 2020. Challenges and prospects of renewable energy in Nigeria: A case of bioethanol and biodiesel production. *Energy Reports* **6**, 77–88.

Adjovi, L. 2012. Is biofuel a solution for Burkina Faso? Accessed on 20 March 2020 at https://www.bbc.com/news/world-africa-20370221

Afrane, G. 2012. Examining the potential for liquid biofuels production and usage in Ghana. *Energy Policy* **40**, 444–451.

Anderson, S. 2007. Biofuels in Mali: Decreasing Food Insecurity. https://www.worldfoodprize.org/documents/filelibrary/images/youth_programs/research_papers/2007_papers/cf_anderson_090CCDCB05518.pdf

Auth, K. & Musolino, E. 2014. ECOWAS renewable energy and energy efficiency status report 2014. https://www.ren21.net/wp-content/uploads/2019/05/ECOWAS_EN.pdf

Antwi-Bediako, R, Otsuki, K, Zoomers, A. & Amsalu, A. 2019. Global Investment Failures and Transformations: A Review of Hyped Jatropha Spaces. *Sustainability 2019*, **11**, 3371, 1–23. doi:10.3390/su11123371

Balat, M., Balat, M., Kırtay, E. & Balat, H. 2009. Main routes for the thermo-conversion of biomass into fuels and chemicals. Part 1: Pyrolysis systems. *Energy Conversion and Management* **50**, 3147–3157.

Baumert, S., Khamzina, A. & Vlek, P. L.G. 2018. Greenhouse gas and energy balance of Jatropha biofuel production systems of Burkina Faso. *Energy for Sustainable Development* **42**, 14–23. https://doi.org/10.1016/j.esd.2017.09.007

Ben-Iwo, J., Manovic, V. & Longhurst, P. 2016. Biomass resources and biofuels potential for the production of transportation fuels in Nigeria. *Renewable and Sustainable Energy Reviews* **63**, 172–192. https://doi.org/10.1016/j.rser.2016.05.050

Bridge builders. 2019. Integrated Jatropha Growing, Burkina Faso. Accessed online on 14 March, 2020 at http://www.bridge-builders.de/integrated-jatropha-growing-burkina-faso/

Cernansky, R. 2019. As palm oil production ramps up in Africa, communities work to avoid problems plaguing other regions. Accessed online on 22 March 2020 at https://ensia.com/features/sustainable-palm-oil-production-west-central-africa/

Cristobal-Sarramian, A. &Atzmuller, D. 2018. Yeast as a production platform in biorefineries: conversion of agricultural residues into value-added products. *Agronomy Research* **16**(2), 377–388. https://doi.org/10.15159/AR.18.066

Dafrallah, T., Edjekumhene, I., Edze, P., Ngom, A. & Ommer, V. 2010. Bioenergy for Rural Development in West Africa: The case of Ghana, Mali and Senegal. Accessed online on 6 February 2020 at http://www.gnesd.org/PUBLICATIONS/Bioenergy-Theme

Dianka, M. 2012. Promoting Biofuels in West Africa: An Engine for Development. *In: Janssen R., Rutz D. (eds) Bioenergy for Sustainable Development in Africa*. Springer, Dordrecht

Dioha, M.O. & Kumar, A. 2020. Sustainable energy pathways for land transport in Nigeria. *Utilities Policy* **64**, 101034.

Duku, M.H., Gua,S. & Hagan, E.B. 2011. A comprehensive review of biomass resources and biofuels potential in Ghana. *Renewable and Sustainable Energy Reviews* **15**, 404–415.

Dunmade, I.S. 2020. Sustainable Production of Value Added Commodities from Biomass Feedstocks. Accepted for publication in "Valorization of biomass to value-added commodities: current trend, challenges and future outlook (MO Daramola & OA Ayeni Eds)" to be published by Springer Nature

Dunmade, I.S. 2019. Potential social lifecycle impact analysis of bioenergy from household and market wastes in African cities. *Agronomy Rese*arch **17**(4), 1599–1616. https://doi.org/10.15159/AR.19.162

Dunmade, I.S. 2018a. Public-private partnership in recycling: an evaluation of its climate change impact reduction benefits. *International Journal of Engineering & Technology* **7**(4), 6698–6702. doi: 10.14419/ijet.v7i4.14547

Dunmade, I. 2018b. The pursuit of circular economy goal in Africa: An exploratory study on the activities of the African Union and its member states. *Journal of Popular Education in Africa*. **2**(2), 78–88.

Dunmade, I.S. 2017. Potentials for Sustainable Power Supply in Nigeria: An Overview of Energy Resources in Western Nigeria. *International Journal of Energy and Power Engineering* **6**(3), 34–39. doi: 10.11648/j.ijepe.20170603.13

Dunmade, I.S. 2013a. "Design for Multi-Lifecycle: A *Sustainability Design Concept." International Journal of Engineering Research and Applications* **3**(2), 1413–1418.

Dunmade, I.S. 2013b. A Case Study on Needs Assessment for Sustainable Rural Development. *World Environment* **3**(4), 127–132. doi: 10.5923/j.env.20130304.01

Dunmade, I.S. 2012. The Use of Lifecycle Management Principles in Biosystems Engineering: A Pragmatic Approach to Solving Agri-Industrial Sustainability Problems. *Journal of Agricultural Science and Technology* A **2**(3), 357–362.

Dunmade, I.S. 2010. Collaborative lifecycle design – A viable approach to sustainable rural technology development. International *Journal of Technology Management and Sustainable Development* **9**(2), 149–158.

ECOWAS (Economic Community of West African States) 2013. ECOWAS Renewable energy policy. Abuja, ECOWAS. 82 pp. Accessed online on 7th February 2020 at http://www.ecreee.org/sites/default/files/documents/ecowas_renewable_energy_policy.pdf

FAO 2019. Energy in and from agriculture in the African Nationally Determined Contributions (NDC) A review. Accessed online on 2 February 2020 at http://www.fao.org/3/ca6359en/ca6359en.pdf

FAO and PISCES. 2009. Small-Scale Bioenergy Initiatives: Brief description and preliminary lessons on livelihood impacts from case studies in Asia, Latin America and Africa. http://www.fao.org/3/a-aj991e.pdf

Forest cover. 2019. Bioenergy in West Africa: impacts on women and forests. Accessed online on 12 Mar 2020 at https://globalforestcoalition.org/wp-content/uploads/2019/09/forestcover-59-EN.pdf

Ghobadian, B. 2012. Liquid biofuels potential and outlook in Iran. *Renewable and Sustainable Energy Reviews* 16, 4379–4384.

Gnansounou, E., Pachón, E.R., Sinsin, B., Teka, O., Togbé, E. & Mahamane, A. 2020. Using agricultural residues for sustainable transportation biofuels in 2050: Case of West Africa. *Bioresource Technology* **305**, 1–9.

IEA. 2019a. Renewables 2019: Market analysis and forecast from 2019 to 2024. Fuel report – October 2019. Accessed online on 2 February 2020 at https://www.iea.org/reports/renewables-2019

IEA. 2019b. "Tracking Transport", IEA, Paris. Accessed online on 2 February 2020 at https://www.iea.org/reports/tracking-transport-2019

IRENA (International Renewable Energy Agency) 2020. Liquid Biofuels. Accessed online on 18 March 2020 at https://www.irena.org/transport/Liquid-Biofuels

Ishola, M.M., Brandberg, T., Sanni, S.A. & Taherzadeh, M.J. 2013. Biofuels in Nigeria: A critical and strategic evaluation. *Renewable Energy* **55**, 554–560.

Iwayemi, A. 2008. Nigeria's Dual Energy Problems: Policy Issues and Challenges. *International Association for Energy Economics* **53**, 17–21.

Iye, E. & Bilsborrow, P. 2013. Cellulosic ethanol production from agricultural residues in Nigeria. *Energy Policy* **63**, 2013, pp. 207–214.

Jokiniemi, I & Ahokas, J. 2013. A review of production and use of first generation biodiesel in agriculture. *Agronomy Research* **11**(1), 239–248.

Kan, T., Strezov, V. & Evans, T.J. 2016. Lignocellulosic biomass pyrolysis: A review of product properties and effects of pyrolysis parameters. *Renewable and Sustainable Energy Reviews* **57**, 1126–1140.

Kgathi, D.L., Mmopelwa, G., Chanda, R., Kashe, K. & Murray-Hudson, M. 2017. A review of the sustainability of Jatropha cultivation projects for biodiesel production in southern Africa: Implications for energy policy in Botswana. *Agriculture, Ecosystems & Environment* **246**, 314–324.

Kotrba, R. 2013. MOU signed to develop jatropha plantations in West Africa. Accessed online on 13 March 2020 from http://www.biodieselmagazine.com/articles/8982/mou-signed-to-develop-jatropha-plantations-in-west-africa

Kumar, A., Kumar, N., Baredar, P. & Shukla, A. 2015. A review on biomass energy resources, potential, conversion and policy in India. *Renewable and Sustainable Energy Reviews* **45**, 530–539.

Ndiaye, L.G. 2018. Biomass to energy an opportunity for Africa. Accessed on 5 February 2020 at https://indico.cern.ch/event/667667/contributions/2878023/attachments/1679366/2697395/Presentation_Latgrand_NDIAYE_ASP2018.pdf

Nigam, P.S. & Singh, A. 2011. Production of liquid biofuels from renewable resources. *Progress in Energy and Combustion Science* **37**, 52–68.
Nyström, I., Bokinge, P. & Franck, P. 2019. Production of liquid advanced biofuels - global status. Accessed online on 2 February 2020 at https://www.miljodirektoratet.no/globalassets/publikasjoner/m1420/m1420.pdf
Onuoha, K.C. 2010. What are the prospects and challenges of biofuels in Nigeria? Accessed online on 18 March 2020 at https://papers.ssrn.com/sol3/papers.cfm?abstract_id=1959778
Oyedepo, S.O., Dunmade, I.S., Adekeye, T., Atabo, A.B., Olawole, O.C., Babalola, P.O., Kilanko, O. & Leramo, R.O. 2019. Bioenergy Technology Development in Nigeria: Pathway to Sustainable Development. *International Journal of Environment and Sustainable Development* **18**(2), 175–205. https://doi.org/10.1504/IJESD.2019.099513
Panwara, N.L., Kotharib, R. & Tyagic, V.V. 2012. Thermo chemical conversion of biomass – Eco friendly energy routes. *Renewable and Sustainable Energy Reviews* **16**, 1801–1816.
Popoola, L., Larwanou, M. & Jimoh, S.O. 2015. Biofuel initiatives in West Africa and the Sahel: potential for success. *International Forestry Review* **17**(3), 136–148. Accessed online on 2 February 2020 at http://www.afforum.org/sites/default/files/English/English_97.pdf
Simonyan, K.J. & Fasina, O. 2013. Biomass resources and bioenergy potentials in Nigeria. *African journal of agricultural research* **8**(40), 4975–4989. doi: 10.5897/AJAR2013.6726
Smeets, E.M.W., Faaij, A.P.C., Lewandowski, I.M. & Turkenburg, W.C. 2007 A bottom-up assessment and review of global bio-energy potentials to 2050. *Prog. Energ. Combust. Sci.* **33**, 56–106. http://doi:10.1016/j.pecs.2006.08.001
The Research Council of Norway (2011, February 9). Jatropha: Green biodiesel from African tree. Science Daily. Retrieved March 21, 2020 from www.sciencedaily.com/releases/2011/02/110208091656.htm
Torrey, M. 2010. Update on Jatropha. Accessed online on 20 March 2020 at https://www.aocs.org/stay-informed/inform-magazine/featured-articles/update-on-jatropha-january-2010
UNU-IAS. 2012. Biofuels in Africa: Impacts on Ecosystem Services, Biodiversity and Human Wellbeing. A UNU-IAS Policy Report access online on 12 March 2020 at https://collections.unu.edu/eserv/UNU:2902/Biofuels_in_Africa1.pdf
van Zyl, W.H., Chimphango, A.F.A., den Haan, R., Görgens, J.F. & Chirwa, P.W.C. 2011. Next-generation cellulosic ethanol technologies and their contribution to a sustainable Africa. *Interface Focus* (2011) **1**, 196–211. doi:10.1098/rsfs.2010.0017
Verma, M., Godbout, S., Brar, S.K., Solomatnikova, O., Lemay, S.P. & Larouche, J.P. 2012. Biofuels Production from Biomass by Thermochemical Conversion Technologies. International *Journal of Chemical Engineering,* 1–18. https://doi.org/10.1155/2012/542426
von Maltitz, G., Haywood, L., Mapako, M. & Brent, A. 2009. Analysis of opportunities for biofuel production in sub-Saharan Africa. Accessed online on 2 February 2020 at http://researchspace.csir.co.za/dspace/bitstream/handle/10204/3740/Von%20Maltitz_d1_2009.pdf?sequence=1
WBA (World Bioenergy Association) 2019. Global Bioenergy Statistics 2019. Accessed online on 18 March 2020 at https://worldbioenergy.org/uploads/191129%20WBA%20GBS%202019_LQ.pdf
Yılmaz, S. & Selim, H. 2013. A review on the methods for biomass to energy conversion systems design. *Renewable and Sustainable Energy Reviews* **25**, 420–430.
Yombouno, A. 2014. Oil palm production in West and Central Africa. Accessed online on 20 March 2020 at https://www.grain.org/article/entries/5034-oil-palm-production-in-west-and-central-africa
Zhang, L., Xu, C. & Champagne, P. 2010. Overview of recent advances in thermo-chemical conversion of biomass. *Energy Conversion and Management* **51**, 969–982.

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Atmospheric attenuation of the Ku band along the space-earth path due to clouds and rain

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Abstract. The weather conditions formed in troposphere causes the greatest signal attenuation in satellite communication systems especially at frequencies above 10 GHz. This paper describes possible signal attenuations on the satellite-earth path due to rain and clouds. It was measured whether it is advantageous to use the Ku band for data transmission over other bands. The measurement was carried out in the Czech Republic using a beacon signal from Eutelsat 12W satellite at 12.5 GHz. Clouds and the rainfall rate at the measured location were obtained from the CHMI portal. The measurements show that the clouds cause negligible attenuation. Significant attenuation was caused by rainfall. The measured values of slant path rain show a significant decrease in signal strength even in light rain. The measured cumulative rainfall rate was found to be close to the ITU-R model. The measurements show that the Ku band is advantageous for data transmission in rain poorer regions including Central Europe.

Key words: signal, attenuation, weather, satellite, rain, clouds.

INTRODUCTION

Telecommunications satellites are designed for data transmission. Signal transmission is ensured by a ground station, which sends a signal to the satellite, transposes it to another frequency, amplifies and sends it back to Earth (Elbert, 2004).

Radio signal transmission is very important for modern technology. Accurate transmission of information is very important in many specializations, including agriculture. Communication of ground units with satellites can gather information that can be used in the future to improve and refine agricultural practices. The radio spectrum is not unlimited, so it is necessary to allocate bands to certain applications with caution. It is also important to develop new principles for increasingly better and more secure information transfer. Without the possibility of transmitting information using satellites, it would not be possible to use the conventional satellite applications. The measured transmission of information in the Central European region mainly uses the Ku-band and the signal is transmitted through satellites located in space. These satellites are located in a geostationary orbit, which is about 37,000 kilometres from Earth, and therefore, for various reasons, attenuation occurs. On the Earth-satellite path, electromagnetic waves pass through different environments and each environment has a different effect on signal attenuation (Lee et al., 2011).

The literature shows that the greatest attenuations occur in the troposphere, where the weather is forming, other attenuations on the Earth-satellite route are negligible in practical use (Yeo et al., 2014).

Several models for rain attenuation have been developed based on empirical measurements. Best accuracy models provide in regions with mild to moderate rainfall rate. These models as SC EXCELL, BRYANT or ITU-R method don't work very well in tropical areas with heavy rainfall rate (Crane, 1982); Bryant et al., 2001; Capsoni et al., 2009).

The attenuation in the Ku band can be calculated with great accuracy, knowing certain parameters, so in practice it should not happen that newly installed terrestrial antennas will not be able to receive a signal with sufficient C/N reserve. In this paper, the use of the Ku band for the Central European region, namely in the Czech Republic, was measured and calculated.

The model ITU-R618 was chosen for the measurement. It was developed using geophysical observations of point rain speed statistics, horizontal structure and vertical temperature structure of the atmosphere, based on measured data from countries with mild climate. This model seems to be ideal for the Central European region (Crane, 1980).

On the contrary, it would not be very suitable for tropical regions as it could underestimate the attenuation in these regions. Other models have been designed specifically for regions with higher precipitation (Boonchuk et al., 2005; Lakanchanh et al., 2006).

MATERIALS AND METHODS

The measurements took place from 10.7.2018 to 6.3.2019. For the measurements was used fixed antenna Prodelin 1.8m directed to the satellite Eutelsat 12W B. The measurement was performed at the beacon frequency of this satellite. The received signal was converted by the LNB convertor to a frequency that can be recorded by a spectrum analyser and sent via coaxial and fiber optic cables to the Vista link LNB matrix from which the signal was connected to the Rhode & Schwarz FSC3 spectrum analyser. The correctness of the received signal is verified on the Ericsson RX 8200 SN 28097 receiver. For each measurement, data on the intensity of precipitation or cloudiness was assigned from the radar maps.

Attenuation due to clouds and fog calculation

Clouds or fog consist of tiny particles, which are usually smaller than 0.1 mm. The ITU Recommendation provides methods to predict signal attenuation due to cloud and fog on the Earth's satellite path. The methods described apply to frequencies less than 200 GHz. However, for correct prediction it is necessary to follow several principles. Terrestrial telecommunications systems shall use frequencies higher than 10 GHz. It is also necessary to know the total column water content contained in clouds for the attenuation calculation. (ITU-R, 2019)

The temperature T = 0 °C is used to calculate the probability of attenuation due to cloud cover. Furthermore, it is necessary to know the elevation and specific coefficient K_i and the value of the column water content, which can be read from the maps. The values read from the attached documents are approximately the following (ITU-R, 2019):

- 20% per year – 0.1 (kg m^{-2})
- 10% per year – 0.2 (kg m^{-2})
- 5% per year – 0.3 (kg m^{-2})
- 1% per year – 0.5 (kg m^{-2})

Specific attenuation by clouds and fog is expressed by:

$$\gamma_c(f,T) = Kl(f,T)M \quad [\text{dB km}^{-1}] \tag{1}$$

γ_c – specific attenuation [dB km^{-1}]; K_l – specific attenuation coefficient [(dB km^{-1})/(g m^3)]; [dB km^{-1}]; M – liquid concentration in cloud or fog [g m^{-3}]; f – frequency [GHz]; T – cloud liquid water temperature [K].

Liquid water density in fog:

- 0.05 g m^{-3} fog visibility about 300 m
- 0.5 g m^{-3} fog visibility about 50 m

Specific attenuation coefficient K_1:

$$K_1(f,T) = \frac{0.819f}{\varepsilon''(1+\eta^2)} \quad [(\frac{dB}{km})/(\frac{g}{m^3})] \tag{2}$$

$$\eta = \frac{2+\varepsilon'}{\varepsilon''} \tag{3}$$

The complex permittivity of water:

$$\varepsilon'(f) = \frac{f(\varepsilon_0-\varepsilon_1)}{f_p[1+(\frac{f}{f_p})^2]} + \frac{f(\varepsilon_1-\varepsilon_2)}{f_p[1+(\frac{f}{f_s})^2]} \tag{4}$$

$$\varepsilon'(f) = \frac{\varepsilon_0-\varepsilon_1}{[1+(\frac{f}{f_p})^2]} + \frac{\varepsilon_1-\varepsilon_2}{[1+(\frac{f}{f_s})^2]} + \varepsilon_2 \tag{5}$$

where:

$$\varepsilon = 77.66 + 103.3\,(\theta - 1) \tag{6}$$

$$\varepsilon 1 = 0.0671\varepsilon_0 \tag{7}$$

$$\varepsilon 2 = 3.52 \tag{8}$$

$$\theta = 300 / T \tag{9}$$

The principal relaxation frequency and secondary relaxation frequency:

$$fp = 20.2 - 146\,(\theta - 1) + 316\,(\theta - 1)2 \quad [\text{GHz}] \tag{10}$$

$$fs = 39.8fp \quad [\text{GHz}] \tag{11}$$

Slant path cloud attenuation is given by:

$$A = \frac{L_{red} K_l(f, 273.15)}{\sin \varphi} \ [db]\ pro\ 90° \geq \varphi \geq 5° \quad (12)$$

φ – elevation angle; L_{red} – total columnar content of liquid water reduced to a temperature of 273.15K at kg m^{-2}.

Total columnar content of liquid water at measured region L_{red} = 0.5 [kg m^{-2}].

Calculated attenuation due clouds and fog with probability of 1% per year for Eutelsat 12W B satellite for variable elevations at Table 1.

Table 1. Attenuation due to clouds and fog [dB] at frequencies [GHz]

Elev. [˚]	10.8	11.0	11.2	11.4	11.6	11.8	12.0	12.2	12.4	12.6	12.8	13.0
20.00	0.16	0.16	0.17	0.18	0.18	0.19	0.19	0.20	0.21	0.21	0.22	0.23
22.00	0.14	0.15	0.15	0.16	0.17	0.17	0.18	0.18	0.19	0.19	0.20	0.21
24.00	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.17	0.17	0.18	0.19	0.19
26.00	0.12	0.13	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.17	0.17	0.18
27.20	0.12	0.12	0.13	0.13	0.14	0.14	0.15	0.15	0.15	0.16	0.16	0.17
28.00	0.11	0.12	0.12	0.13	0.13	0.14	0.14	0.15	0.15	0.16	0.16	0.17
30.00	0.11	0.11	0.12	0.12	0.12	0.13	0.13	0.14	0.14	0.15	0.15	0.16
32.00	0.10	0.11	0.11	0.11	0.12	0.12	0.13	0.13	0.13	0.14	0.14	0.15

Calculation of long-term rain attenuation statistics

An important factor affecting the Earth-satellite link is rain. According to ITU-R P.618, rain attenuation can be calculated based on precipitation totals. The procedure provides estimates of long-term rainfall statistics with slope attenuation at frequencies below 55 GHz. (ITU-R, 2015)

To calculate the signal attenuation due to rain, it is necessary to know the model of the attenuation of rain for the measured location. Data for the final value are obtained from monthly precipitation totals and monthly measured temperatures. In tropical regions where rain attenuation is a major problem, many rainfall patterns are developed for specific regions, and models are tested using beacon frequencies on both uplink and downlink (Yeo et al., 2014).

The following procedure is described in ITU-R Recommendation P.618 and further refers to other recommendations. The resulting expression was calculated for attenuation in 0.01% of the average year. Subsequently, the attenuation was calculated in other percentages of the average year in the range of 0.001–5% based on the attenuation for 0.01%.

Rain height h_r is a key parameter for both direct and statistical models. The actual height and width of the melting layer can be estimated using weather radar data. According to ITU-R P.839, the annual average rainfall, higher than the mean sea level hmR, is obtained from an average annual isotherm height of 0 °C.

$$h_r = h_0 + 0.36\ [km] \quad (13)$$

h_0 was found from the document ITU-R 639 (ITU-R, 2013b)

In the second step the length of the signal path through the rain was calculated. The following formula was used for this particular case since the elevation angle was greater than 5°. If the elevation angle $\theta < 5$ °, it is necessary to use a different formula.

$$L_s = \frac{(h_r - h_s)}{sin\theta}\ [km] \tag{14}$$

h_s – ground station altitude; θ – elevation angle.

In next step is calculated Horizontal projection L_s, of the slant-path length:

$$L_G = L_s \cos\theta\ [\text{km}] \tag{15}$$

In the next step it is necessary to obtain a precipitation rate $R_{0.01}$ of the average year with an integration time of 1 minute. If the aggregate rate cannot be obtained from long-term statistics of the area, it can be estimated from the precipitation maps described in ITU-R Recommendation P.837. In next step was obtained the specific attenuation:

$$\gamma_R = k(R_{0.01})^{\alpha}\ [\text{dB km}^{-1}] \tag{16}$$

$R_{0.01}$ – point rainfall rate for the location for 0.01% of an average year (mm h^{-1}).

Now it is necessary to calculate the coefficients k and α, which depend on the angle of elevation of the antenna and the angle of polarization. The coefficients are also frequency dependent. The ITU-R P.838 recommendation for selected frequencies can be used to calculate the coefficients kH, kV, αH and αV.

$$k = \frac{[k_H + k_V + (k_H + k_v)cos^2\theta cos2\tau]}{2} \tag{17}$$

The next step is to calculate the horizontal decrease factor $r_{0,01}$

$$\alpha = \frac{[k_H + \alpha_H + k_V\alpha_V + (k_H\alpha_H + k_V\alpha_V)cos^2\theta cos2\tau]}{2k} \tag{18}$$

for 0.01% of the total time:

$$r_{0.01} = \frac{1}{1 + 0.78\sqrt{\frac{L_G\gamma_R}{f}} - 0.38(1 - e^{-2L_G})} \tag{19}$$

L_R and χ is calculated as:

$$\zeta = \tan^{-1}\left(\frac{h_R - h_s}{L_G\ r_{0.01}}\right)\ [°] \tag{20}$$

If ζ > θ:

$$L_R = \frac{L_G\ r_{0.01}}{cos\ \theta}\ [\text{km}] \tag{21}$$

Otherwise:

$$L_R = \frac{(h_R - h_s)}{sin\ \theta}\ [km] \tag{22}$$

$$\chi = 36 - |\varphi|\ [°] \tag{23}$$

For latitude of the earth station φ > 36°applies χ = 0, which corresponds to the latitude of the Central European region.

The next step is to calculate the vertical adjustment factor $v_{0.01}$. The L_R must be obtained according to ITU.R P.618(ITU-R, 2015)

$$v_{0.01} = \frac{1}{1 + \sqrt{sin\theta}(31\left(1 - e^{-\left(\frac{\theta}{1+\chi}\right)}\right)\frac{\sqrt{L_R\gamma_R}}{f^2} - 0.45)} \tag{24}$$

In the next step, the effective path length is calculated:

$$L_E = L_R\, v_{0.01}\ \text{[km]} \tag{25}$$

The next step is to calculate the predicted attenuation for 0.01% of the current year:

$$A_{0.01} = \gamma_R L_E\ \text{[dB]} \tag{26}$$

From the $A_{0.01}$ attenuation it is possible to determine attenuation for other percentages of the year in the range of 0.001–5%

$$A_p = A_{0.01}\left(\frac{p}{0.01}\right)^{-(0.655+0.33\ln(p)-0.045ln(A_{0,01})-\beta(1-p)\sin\theta}\ [dB] \tag{27}$$

Where p is the desired percentage and β is determined as follows:

$$\text{If } p \geq \text{ or } |\varphi| \geq 36°: \qquad \beta = 0 \tag{28}$$

$$p < 1\%\ \ |\varphi| < 36° \text{ a } \theta \geq 25°: \beta = -0.005(|\varphi| - 36) \tag{29}$$

Otherwise:

$$\beta = -0.005(|\ \varphi\ | - 36) + 1.8 - 4.25 \sin\theta \tag{30}$$

RESULTS AND DISCUSSION

Table 2 shows attenuations with a probability of 0.01% to 5% of time per year. The values are primarily calculated for a probability of 0.01% for the Earth-satellite link, where the location of the ground station is at 50.0310° N and 14.3913° E at an altitude of 310 m above sea level, for Eutelsat 12W B at 12.5°W. The calculations were carried out for the frequency of 12.5005 GHz, on which the empirical measurement was also performed.

Table 2. Attenuation due to rain [dB] for the satellite Eutelsat 12W in frequencies [GHz]

Probability [%]	11.2	11.4	11.6	11.8	12	12.2	12.4	12.5	12.6	12.8
0.01	8.42	8.59	8.75	8.91	9.07	9.22	9.38	9.45	9.53	9.68
0.1	2.26	2.31	2.36	2.41	2.46	2.5	2.55	2.57	2.59	2.64
0.5	0.85	0.87	0.89	0.91	0.93	0.95	0.97	0.98	0.99	1.01
1	0.64	0.66	0.67	0.69	0.7	0.72	0.73	0.74	0.74	0.76
5	0.19	0.19	0.2	0.2	0.21	0.21	0.22	0.22	0.22	0.22

From the calculated Table 3 for a certain frequency it is also possible to calculate the table of the frequency dependence on the attenuation due to precipitation.

Table 3. Long-term rain attenuation [dB] at the intensity of precipitation [mm h^{-1}]

Frequency [GHz]	1	1.5	2	4	10	20	30	40
11	0.44	0.65	0.86	1.67	3.43	5.69	7.48	8.98
11.5	0.45	0.67	0.88	1.71	3.55	5.94	7.84	9.44
12	0.45	0.68	0.89	1.74	3.60	6.18	8.19	9.89
12.5	0.46	0.68	0.91	1.77	3.77	6.41	8.52	10.32
13	0.46	0.69	0.92	1.79	3.88	6.63	8.84	10.74

Measured values

The difference between the measured average clear and cloudy attenuation is 0.235 dB. Graph 1 compares clear weather with cloud cover. Signal attenuation caused the signal to decrease by 2.73%. Rain attenuation was measured at certain rainfall rates. For precipitation up to 1 mm h^{-1}, the measured attenuation is 0.425 dB. Rain up to 2 mm h^{-1} caused a loss of 1.014 dB. Rain up to 4 mm h^{-1} caused a loss of 1.709 dB. All measured attenuation values due to rain are recorded in Table 2. Values with a total rainfall above 4 mm h^{-1} are not compared, as insufficient number of values was measured for statistical analysis.

Diagram 1. Measured data clear sky vs cloudy

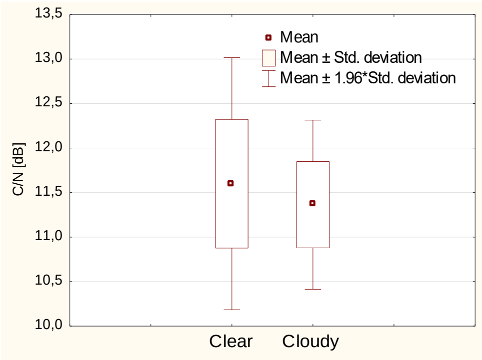

Diagram 2. Measured data clear sky vs rain

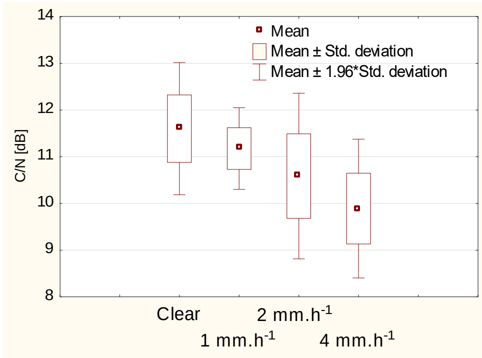

Table 4 shows statistical calculations. A two-sample t-test was used for evaluation and a significance level of $\alpha = 0.05$ was used. For clear vs clouds and clear vs 1 mm h^{-1}, this significance level cannot be rejected. For values of clear vs 2 mm h^{-1} and 4 mm h^{-1}, this significance level is rejected. All statistical calculations were performed in StatSoft, Inc. software. (2013). STATISTICA, version 12.0.1133.15.

Table 4. Statistical data processing

	Mean clear	Mean variable	t	E	p
clear vs cloudy	11.6	11.36471	1.015006	25	0.319826
clear vs 1 mm h^{-1}	11.6	11.175	1.692038	20	0.106161
clear vs 2 mm h^{-1}	11.6	10.58571	2.933984	22	0.007681
clear vs 4 mm h^{-1}	11.6	9.890909	5.274598	19	0.000043

In general, signal attenuation due to rain occurs at 10 GHz or more as the wavelength of the signal decreases and approaches the size of a raindrop. It is also true that the higher the rate of rainfall in a given area, the larger the droplets and the closer they are to the size of the carrier wave in the Ku-band. A prerequisite for the correct calculation of the total annual signal attenuation is preceded by the correct selection of the rain model for the given region. In the Central European region, annual precipitation is much lower than in the tropics. For this reason, the ITU-R model is used. In tropical areas, the choice of rain pattern may vary. G.N. Ezeh et al. compared the three rain models, the ITU-R model, the Ajayi method and the Alnutt model. The simulated results in the Matlab program are based on the Ajai method as the most accurate method in the tropical region. Different models are developed in each region. There are more of these models in the tropical regions, as there is an effort for the most accurate result that can be used in antenna design (Ezeh et al., 2014).

If the reception of the antenna signal is not continuous, it is economically advantageous to choose the smallest available antenna in the Central European region. Khairi Abdul Rahim et al. compares 0.6m and 13.2m antennas. The article compares an antenna for home use and a professional antenna. The measured distance from C / N noise was measured in clear weather. C / N values differ between antennas by 9.37dB. This difference is really striking and indicates how important the size of the antenna is in receiving the signal. As the measurement took place in Malaysia, which is located in a tropical area and the intensity of precipitation is several times higher than in the Central European region, the usability of the antenna with a diameter of 0.6 m is very limited. An antenna with a diameter of 13.2 m is more suitable for tropical areas (Rahim et al., 2009).

CONCLUSION

The attenuations were measured at the beacon frequency of the satellite, which serves, among other things, to set the satellite, so it is necessary that this frequency is permanently accessible. Therefore, its value reached 11.6 dB carrier to noise ratio in clear weather. This is a very high value for Earth-satellite data transmission. The measured values do not fully correspond to the calculated values in the cloud attenuation, which may be caused by the use of other measuring instruments than used in the processing of the prediction method or attenuation in optical cables on the LNB - spectrum analyzer route. Nevertheless, the trend of slight signal attenuation due to cloudiness is perceptible. Clouds in the Czech Republic vary between 20–40%, but the measurements show that the signal attenuation on the link has a very small contribution, in this particular case the attenuation due to cloudiness was 2.73% compared to clear weather.

It can be seen that light rain will cause more attenuation than clouds. Values for precipitation up to 1 mm h^{-1} and 4 mm h^{-1} correlate with calculated values. The measured attenuation at precipitation up to 2 mm h^{-1} was slightly higher than predicted by the ITU model. This inaccuracy may be caused by the same problem that occurred when measuring attenuation due to cloudiness.

With increasing frequency, the signal attenuation increases as well. Also, the higher the rainfall rate in a given area, the larger are the drops and the closer they are to the size of the carrier wave. The attenuation from the average year was calculated. In the measured case, it would be 81.47% attenuation for 52 minutes of the average year, without total signal loss. According to the measurement results, the Ku-band band is suitable for signal transmission on the Earth - satellite route in the Czech Republic. The measurements show that the ITU model for rain attenuation was chosen correctly and is suitable for the less wealthy region of Central Europe. Because the signal loss in the Czech Republic is at a low level, the Ku band should also be used for data transmission in agriculture.

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REFERENCES

Boonchuk, T., Hemmakorn, N., Supnithi, P., Iida, M., Tanaka, K., Igarashi, K. & Moriya, Y. 2005. Rain attenuation of satellite link in Ku-band at Bangkok. *2005 Fifth International Conference on Information, Communications and Signal Processing, 2005,* 1093–1096. https://doi.org/10.1109/icics.2005.1689222

Bryant, G.H., Adimula, I., Riva, C. & Brussaard, G. 2001. Rain attenuation statistics from rain cell diameters and heights. *International Journal of Satellite Communications* **19**(3), 263–283. https://doi.org/10.1002/sat.673

Capsoni, C., Luini, L., Paraboni, A., Riva, C. & Martellucci, A. 2009. A new prediction model of rain attenuation that separately accounts for stratiform and convective rain. *IEEE Transactions on Antennas and Propagation* **57**(1), 196–204. https://doi.org/10.1109/TAP.2008.2009698

Crane, R.K. 1980. Prediction of Attenuation by Rain. *IEEE Transactions on Communications*, **28**(9), 1717–1733. https://doi.org/10.1109/TCOM.1980.1094844

Crane, R.K. 1982. A two-component rain model for the prediction of attenuation statistics. *Radio Science* **17**(6), 1371–1387. https://doi.org/10.1029/RS017i006p01371

Elbert, BR. 2001. *The Satellite Communication Ground Segment and Earth Station Handbook*.

Ezeh, G.N., Chukwuneke, N.S., Ogujiofor, N.C. & Diala, U.H. 2014. EFFECTS OF RAIN ATTENUATION ON SATELLITE COMMUNICATION LINK. *Advances in Science and Technology Research Journal* **8**(22), 1–11. https://doi.org/10.12913/22998624.1105138

ITU-R. 2013a. Rain Height Model for Prediction Methods. *Rec. ITU-R P.839-4, 4,* 1–3. http://www.itu.int/ITU-R/go/patents/en

ITU-R. 2013b. Rain Height Model for Prediction Methods. *Rec. ITU-R P.839-4, 4,* 1–3. http://www.itu.int/ITU-R/go/patents/en

ITU-R. 2015. Propagation data and prediction methods required for the design of Earth-space telecommunication systems. *Recommendation ITU-R P.618-8*, **12**, 1–24. http://www.itu.int/ITU-R/go/patents/en

ITU-R. (2019). *ITU-R P.840-5 Attenuation due to clouds and fog*. *5*. https://www.itu.int/rec/R-REC-P.840-8-201908-I/en

Lakanchanh, D., Datsong, A., Leelaruji, N. & Hemmakorn, N. 2006. Rainfall rate and rain attenuation in Ku-Band satellite signal in Thailand and Laos. *2006 SICE-ICASE International Joint Conference*, 3928–3932. https://doi.org/10.1109/SICE.2006.314906

Lee, Yee Hui, jun xiang yeo, jin teong ong. 2011. *Rain Attenuation on Satellite to Ground Link for Beacon Signal Request PDF*. https://www.researchgate.net/publication/255413427_Rain_Attenuation_on_Satellite_to_Ground_Link_for_Beacon_Signal

Rahim, K.A., Ismail, M. & Abdullah, M. 2009. Satellite link margin prediction and performance of ASTRO Malaysia. *2009 International Conference on Space Science and Communication, IconSpace - Proceedings,* pp. 78–82. https://doi.org/10.1109/ICONSPACE.2009.5352666

Yeo, J.X., Lee, Y.H. & Ong, J.T. 2014. Rain attenuation prediction model for satellite communications in tropical regions. *IEEE Transactions on Antennas and Propagation*, **62**(11), 5775–5781. https://doi.org/10.1109/TAP.2014.2356208

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Assessment of spatial variability of environmental variables of a typical house of laying hens in Colombia: Antioquia state Case

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Abstract. This paper aimed to analyze the magnitude and spatial variability of environmental variables: Temperature and Relative Humidity Index (THI), Radiant Thermal Load (RTL), Globe Temperature and Relative Humidity Index (BGTH) and Enthalpy (H), inside a house for laying hens, in the state of Antioquia (Colombia) during the month of August. A traditional Colombian poultry house with natural ventilation was used. All variables were manually measured at equally spaced 1.0×1.0 m points, totaling 99 data collection points inside the poultry house. Geostatistical techniques were used through semivariogram analysis, and isochore maps were generated through data interpolation by kriging. The semivariogram was fitted by the restricted maximum likelihood method. The used mathematical model was the spherical one. After adjusting the semivariograms, the data were interpolated by ordinary kriging. The semivariograms and the isochore maps allowed identifying the non-uniformity of the spatial distribution of all evaluated variables throughout the poultry house. The results show that THI, RTL, BGTH and, H presented values above the comfort limits in the most significant part of the poultry house during the observed period. It is possible to concluded that the use of natural ventilation alone was not sufficient to guarantee the homeothermy conditions for the layers. Thus, it is suggested that in addition to natural ventilation, secondary modifications should be used to improve farm productivity.

Key words: animals production, animal welfare, natural ventilation.

INTRODUCTION

The environment in which birds are raised comprises all the physical, chemical, biological, social and climatic elements that influence their development and growth. Among those, environmental conditions, composites of air temperature, relative humidity, airspeed, and radiation, it has generated direct and immediate action on the behavioral, productive and reproductive responses of birds (Baêta & Souza, 2010).

Environmental conditions different from the thermoneutral zone could cause effects on the performance of the laying hens. When the birds are submitted to thermal stress conditions, it can compromise the most important vital functions of those animals, their homeothermy (Vale et al., 2016). Thermal stress may affect the animal welfare, and it also could result in economic losses for the industry, and it can not be ignored (Lee et al., 2015).

Generally, the hens that are exposed to environmental conditions different of their thermal comfort could suffer a reduction in their food consumption, which could be a probable cause of the decline in their productivity. In particular, thermal stress depresses egg production (Silva et al., 2015; Sousa et al., 2018), in addition to the deterioration in the quality of the eggs (Lemos et al., 2014; Lana, et al., 2017). Therefore, according to Freitas et al. (2017) and Lee et al. (2015), the understanding of the environmental conditions in which the birds are submitted is crucial for the laying hens rise with good productivity and adequate animal welfare.

It is expected that in a commercial production system, the environmental variables inside the facility will be homogeneous. The spatial distribution of these thermal variables could be assessed using spatialization and geostatic tools (Massari et al., 2016; Ribeiro et al., 2016). Geostatistics is a tool that allows having more knowledge of these factors that affect the environment where the animals are raised. This tool gives more precision and accuracy in the systems of exploration (Carvalho et al., 2012; Massari et al., 2016).

In tropical countries like Colombia, there are few studies to evaluate the thermal comfort of poultry production facilities, mainly for the egg production. In the most part of the year, these types of facilities have operated with natural ventilation, and they are located at altitudes above 1,800 meters. That means that these houses are located in cold temperatures for most of the year, with average air temperatures around 15 °C and relative humidity of 70%.

Therefore, the objective of this work was to analyze the magnitude and spatial variability of environmental variables: Temperature and Relative Humidity Index (THI), Radiation Thermal Load (RTL), Black Globe Temperature and Humidity (BGTH) and Enthalpy (H), inside a typical house for laying hen using natural ventilation without thermal insulation, located in the state of Antioquia (Colombia).

MATERIALS AND METHODS

The experiment was carried out in an experimental house for laying hens at the National University of Colombia Campus Medellín. The farm is located at the San Pablo Experimental Agrarian Station, in the eastern sector of the department of Antioquia, municipality of Rionegro, during August of 2017. August is known to be the mouth with the highest record of high temperatures of the year. The region is characterized by the most significant egg production in the department. Besides, it presents during the summer seasons, high temperatures and during the winter seasons high precipitation and hight thermal amplitude. These conditions generate a lot of problems for the environmental control of the different climatic variables, and they can cause a reduction in egg production in the state of Antioquia.

The farm is located at an altitude of 2,100 meters with an average annual air temperature between 12 and 23º C. Annual precipitation regime is around 2,280 mm and relative humidity of 75.5% that is considered according to Holdriged classification as a bmh - MB in the tropic (Espinal, 1992).

The outside of the facility used were 34.0 m long, 11.0 m wide. The laying hen house was built of reinforced concrete, and solid bricks, asbestos-cement roofing, concrete floor, the lateral side openings of mesh, having surrounding grass around the installation and surrounding vegetation that work as windbreaks on the south side of the plant. The longer axis facility is located East-West, which makes possible the use of natural ventilation in the summer and reduces the radiation (Fig. 1).

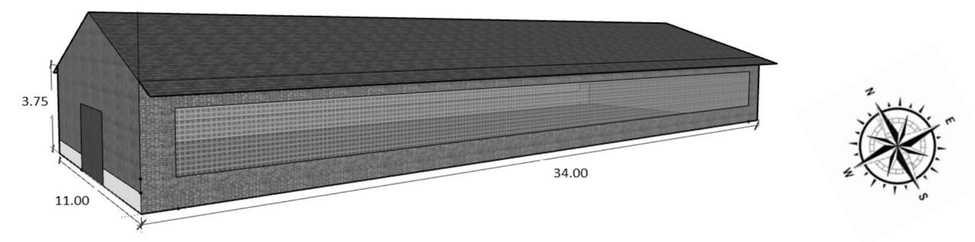

Figure 1. Scheme of the experimental facility.

The facility had three rows of cages arranged in a two-store vertical structure. Each cage has 2.90 meters long, 1.00 meters wide, and 1.90 meters high, with 4 chickens Lohmann Brown® per cages with a total of 1,200 birds. Water and feed for the birds were available *ad libitum* throughout the experimental period. The diets provided to the animals were formulated to meet the nutrient requirements for the age group.

To evaluate the thermal comfort measurement of dry bulb temperature (t_{db}, °C), black globe temperature (t_{bg}, °C), relative humidity (RH, %), air velocity (V_{air}, m s^{-1}) were made in the geometric center of each cage.

T_{db} and RH were measured using a hygrometer (Extech Instruments®, mod. RHT20, USA, the precision of ± 1%). The V_{air} was measured using one anemometer of hot wire (Extech Instruments®, mod. AN100, USA, precision de ± 3%). T_{gn} was measured using a BGT DELTA OHM HD 32.2 Thermal Stress, Italy, with a precision of ± 0.15 °C.

All variables were measured in 99 different points located at the same distance in one mesh of 1.0×1.0 m, inside of the facility. The data were collected in four moments of the day at 9:00 am, 12:00 pm, 15:00 pm, and 18:00 pm.

According to Behura et al. (2016), based on the collected thermal variables, some thermal indices were calculated. Temperature and Humidity Index (THI) that represents the combination of the effect of the air temperature, and humidity associated with the animal thermal stress level was proposed by Zulovich & Deshazer (1990), according to the Eq. (1):

$$THI = 0.6\, t_{db} + 0.4\, t_{wb} \quad (1)$$

where t_{db} – dry bulb temperature (°C); t_{wb} – wet bulb temperature (°C).

Radiation Thermal Load (RTL) was calculated with the Eq. (2), according to Esmay (1969), in W m^{-2}, and the Stefan-Boltzman ($\sigma = 5.67 \cdot 10^{-8}$ W $m^{-2} \cdot K^{-4}$).

$$RTL = \sigma \cdot (\mathrm{RTA})^4 \quad (2)$$

Radiant Temperature Average in K (RTA) was obtained by Eq. 3, the air velocity in m s^{-1} and t_{db} and t_{bg} in K:

$$\mathrm{RTA} = 100 \cdot \left[2.51 \cdot V_{air}{}^{0.5} \cdot (t_{bg} - t_{db}) + \left(\frac{t_{\mathrm{bg}}}{100}\right)^4\right]^{\frac{1}{4}} \quad (3)$$

Using the t_{bg}, and the THI, was calculated the Black Globe Temperature and Humidity (BGTH) according to Eq. 4. Values above 75 could generate low thermal comfort in the hens with age above 15 days of life (Rocha et al., 2010):

$$BGTH = t_{bg} + 0.36 t_{dp} + 41.5 \quad (4)$$

where t_{bg} – black globe temperature in °C; t_{dp} – dew point temperature in °C.

The enthalpy H was calculated using Eq. 5, according to Albright (1990), to the characterization of the thermal environment inside of the facilities.

$$H = 1.006 \cdot t_{db} + W \cdot (2.501 + 1.805 t_{db}) \quad (5)$$

where H – is the Enthalpy, in $kJ \cdot kg^{-1}_{\mathrm{dry\ air}}$; W in kJ·kg^{-1}; t_{db} – dry bulb temperature, in °C.

The spatial dependence of the environmental variables (THI, RTL, BGTH, and H) were analyzed using semivariogram adjustments, classic and ordinary Kriging interpolation. The classic semivariogram was estimated by Eq. 6, described by Bachmaier & Backes (2008):

$$\hat{\gamma}(\mathrm{h}) = \frac{1}{2\mathrm{N(h)}} \sum_{\mathrm{i=1}}^{\mathrm{N(h)}} [\mathrm{Z(x_i)} - \mathrm{Z(x_i + h]^2} \quad (6)$$

where N (h) – is the number of experimental pairs of observations Z (X_i); $Z_{(xi+h)}$ separate by a distance h.

The semivariogram is represented by the graph $\hat{\gamma}$(h)versus h. From the adjustment of a mathematical model, the calculated values of $\hat{\gamma}$(h), are estimated the coefficients of the theoretical model for the semivariogram called pepita effect, C0; contribution, C1; threshold, C0 + C1; and the reach described by Bachmaier & Backes (2008).

The method of ordinary least squares (OLS) or the method of maximum restricted likelihood (REML) was used to make the adjustment of a mathematical model. According to Mello et al. (2005), the ordinary least squares method consists consist to obtain of the values of the parameters of a model to minimize the sum of the difference square between the observed and estimated values.

The principle of REML is to estimate the parameters of the semivariogram by the maximum likelihood applied to the data using a linear transformation in order to maximize the probability of the profile of the semivariogram parameters is based on the transformation of the variables (Diggle & Ribeiro Jr. 2007). According to the same authors, REML is considered the estimator less suitable for variance parameters in small samples.

The spherical mathematical model for semivariograms was chosen for all environmental variables evaluated in this study. This model has widely used in geostatistical work for animal environments (Ferraz et al., 2016; Oliveira et al., 2016; Ribeiro et al., 2016), and its use could be explained due to its relatively easy ability to adjust to any point cloud. The spherical model is given as follows by Eq. 7:

$$\gamma(h) = \begin{cases} 0, & \text{if } h = 0 \\ C_O + C_1\left[\frac{3}{2}\left(\frac{h}{a}\right) - \frac{1}{2}\left(\frac{h}{a}\right)^3\right], & \text{if } 0 < h \leq \\ C_O + C_1 & \text{if } h > a \end{cases} \tag{7}$$

After adjusting the semivariograms, the interpolation of the data was done by ordinary kriging to allow visualization of the spatial distribution patterns of the evaluated variables.

The validation method was used to assess the quality of the adjust of theoretical spatial models. This method was also used by Faraco et al. (2008), Johann et al. (2010), and Ferraz et al. (2012).

According to Isaaks & Srivastava (1989), validation is the technique to evaluate the estimation of the errors that allow comparing predicted values with those sampled in the experimental process. The sample value, at a specific location $\hat{Z}\ (s_{(i)})$, is temporarily discarded of the data set, and then a prediction is made by kriging at the location using the remaining samples. In this way, it is possible to remove some values that would be very useful for choosing the method, such as the Mean Error (ME), the Standard Deviation of the Mean Error (DP_{EM}), the Reduced Mean Error (ER) and the Standard Deviation of the Reduced Mean Error (S_{ER}). Thus, the Mean Error by crossing validation (EM) is obtained by the following expression 8:

$$EM = \frac{1}{n}\sum_{i=1}^{1}\left(Z(s_i) - \hat{Z}(s_{(i)})\right) \tag{8}$$

where n – is the number of data; $Z(s_i)$, value observed at point s_i; $\hat{Z}(s_{(i)})$ is the value predicted by ordinary kriging at the point s_i, without considering the observation $Z(s_i)$ (Faraco et al., 2008).

According to Cressie (1993), the reduced mean error (ER), the standard deviation of mean errors (DP_{EM}), and the standard deviation of the reduced mean errors (SER) can be used to evaluate the models. The reduced mean error (ER) is defined by Eq. 9:

$$ER = \frac{1}{n}\sum_{i=1}^{n}\frac{Z(s_i) - \hat{Z}(s_{(i)})}{\sigma\left(\hat{Z}(s_{(i)})\right)} \tag{9}$$

where $\sigma\left(\hat{Z}(s_{(i)})\right)$ – is the kriging standard deviation at the s_i point without considering the $Z(s_i)$ observation.

The standard deviation of the reduced mean errors (S_{ER}) is obtained from Eq. 10:

$$S_{ER} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left\{\frac{Z(s_i) - \hat{Z}(s_{(i)})}{\sigma\left(\hat{Z}(s_{(i)})\right)}\right\}^2} \tag{10}$$

The average difference between the values will be closer to zero when the estimative will better. The selection criteria based on validation must find the EM and ER values closes to zero. The DP_{EM} value must be the lowest possible, and the S_{ER} value must be the closest to one.

Kriging is the method of Geostatistics interpolation, which uses the spatial dependence expressed in the semivariogram between samples closer to estimated values in any position within the field, without trend and with minimum variance. These features become the kriging an optimal interpolator. The condition of no tendency means that, on the average, the difference between the estimated and measured values is null, and the minimum variance condition means that, even though there may be different points between the predicted and the measured values, these differences are minimal (Burgess & Webster, 1980).

According to Vieira (2000), for the application of kriging, it is assumed that is important to know the realizations $z(x_1)$, $z(x_2)$..., $z(x_n)$ of the spatial random variable $Z(x)$, at locations $x_1, x_2,...,x_n$, (sample); and the semivariogram of the variable has already been determined; and that the interest is to estimate a value $\hat{z}$ at position x. The estimator $\hat{Z}(x)$ of $Z(x)$ is given by Eq. 11:

$$\hat{Z}(x) = \sum_{i=1}^{n} \lambda_i Z(x_i) \qquad (11)$$

where n –is the number of neighbors; $Z(x_i)$ – that involved the estimated value; λ_i – are the weights associated with each measured value.

To understand the conditions of optimal interpolator (kriging), Eqs 12 and 13 were used:

$$E\left\{\hat{Z}(x_0) - Z(x)\right\} = 0 \qquad (12)$$

$$Var\left\{\hat{Z}(x) - Z(x)\right\} = E\left\{\left[\hat{Z}(x) - Z(x)\right]^2\right\} = minimum \qquad (13)$$

In order to $\hat{Z}(x)$ be a non-biased estimator of $Z(x)$, the sum of the sample weights must be equal one (Eq. 14).

$$\sum_{i=1}^{N} \lambda_i = 1 \qquad (14)$$

The Lagrange multiplier (μ) was introduced to obtain the minimum variance. The result of the kriging system is deduced by Eq. 11. The solution of this system of simultaneous equations gives the kriging weights λi (Eq. 15).

$$\sum_{i=1}^{N} \lambda_i \gamma\left(x_{i,} x_j\right) + \mu = \gamma\left(x_{i,} x\right); \text{ i= 1 a N} \qquad (15)$$

To the geostatistical analysis and for making kriging maps, a computational system R Development Core Team (2019) was used, through the geoR library (Ribeiro Junior; Diggle, 2001).

RESULTS AND DISCUSSION

Based on the geostatistical analysis methodology, it was possible to quantify the magnitude and spatial dependence of THI, RTL, BGTH, and H. Through the validation that is shown in Table 1, it is observed that the adjustments of the semivariograms for the variables under study were well performed. Besides, it was observed that the criteria for a better fit were based on validation: the values of Average Error (EM) and Reduced Mean Error (ER) should be the closest to zero. The value of the Deviation Average Error

Standard (DP_{EM}) should be as small as possible, and the Reduced Average Error (S_{ER}) Standard Deviation value should be closer to 1.0.

The pepita effect (C_0) is an important parameter of the semivariogram, and this indicates unexplained variability, considering the sampling distance used. As it is impossible to quantify the individual contribution of these errors, the pepita effect can be expressed as a percentage of the threshold, thus facilitating the comparison of the degree of spatial dependence (GDE) of the variables under study (Trangmar, Yost & Uehara, 1985) (Table 1). According to Cambardella et al. (1994), the studied variables presented moderate GDE; only RTL presented strong GDE.

Table 1. Methods, models and estimated parameters of the experimental semivariograms for the variables: Temperature and Relative Humidity Index (THI), Radiation Thermal Load (RTL), Black Globe Temperature and Relative Humidity Index (BGTH) and Enthalpy (H, kJ $kg^{-1}_{dry\ air}$)

	C_0	C_1	$C_0 + C_1$	a	GDE	EM	DP_{EM}	ER	S_{ER}
THI	0.57	0.56	1.12	3.13	50.45	-0.001	1.21	-0.001	1.18
RTL	16.97	300.69	317.66	2.12	5.34	-0.238	-0.01	20.323	1.17
BGTH	3.05	0.62	3.68	3.49	83.04	-0.001	2.12	0.000	1.11
H	17.69	19.18	36.87	2.77	47.98	-0.010	6.87	-0.001	1.16

C_0 – pepita Effect; C_1 – Spatially dependent component; C_0 + C_1 – Sill; A – Range; SDD – Degree of Spatial Dependence; ME – Mean error; SDME – Standard deviation of mean error; RE – Reduced mean error; SDRE – Standard deviation of reduced mean error.

According to Cressie (1993), the range determines the space under which the variable is correlated. The longest range found was 3.49 m for BGTH, and the shortest range found among the variables under study was 2.12 m for RTL.

Once was made the semivariogram adjustments (Table 1) for the variables under study, the values of these variables were estimated using ordinary kriging. Therefore, it was possible to build spatial distribution maps (isolines) for all of them (Fig. 2), which allowed viewing the spatial variability of the temperature and relative humidity index (THI), the Radiation Termal Load (RTL), temperature index of the globe and relative humidity (BGTH) and enthalpy (H).

The distribution of THI (Fig. 2, A) indicates that most of the area is between 27.2 and 28.2 °C, mainly on the south side of the facility. On the north side, it presents values between 28.2 and 30.2 °C, which is the area that receives the most radiation during the day between 9:00 am and 6:00 pm as shown in Fig. 2. Most of the facility area presented values above 28.0° C, which is considered by Gates et al. (1995) as the upper limit allowed in the production of laying hens.

In the most part of the facility, the RTL (Fig. 2, B) has values below 470 W m^{-2}, on the north side, it has values between 450 and 470 W m^{-2}, which shows that direct solar radiation on the north side of the building causes that RTL presents values higher than 450 W m^{-2}, between 12:00 pm and 4:00 pm, which is the threshold recommended by Esmay (1969) and Rocha et al. (2010).

The BGTH (Fig. 2, C) and H (Fig. 2, D), showed similar behaviour to THI and RTL. Where on the north side of the facility, they present the worst indexes, and the highest amount of energy in the air, with BGTH, has values greater than 75, which could cause stress in the birds, according to Rocha et al. (2010). The H is increased in the north side and in places close to the walls. Also, it is where the airspeed is low since the prevailing winds enter from east to west with an inclination of 30° concerning the horizontal plane of the wall located in the East (Fig. 1). This situation does not allow a uniform distribution of air inside the building, generating a greater thermal load between 12:00 pm and 4:00 pm.

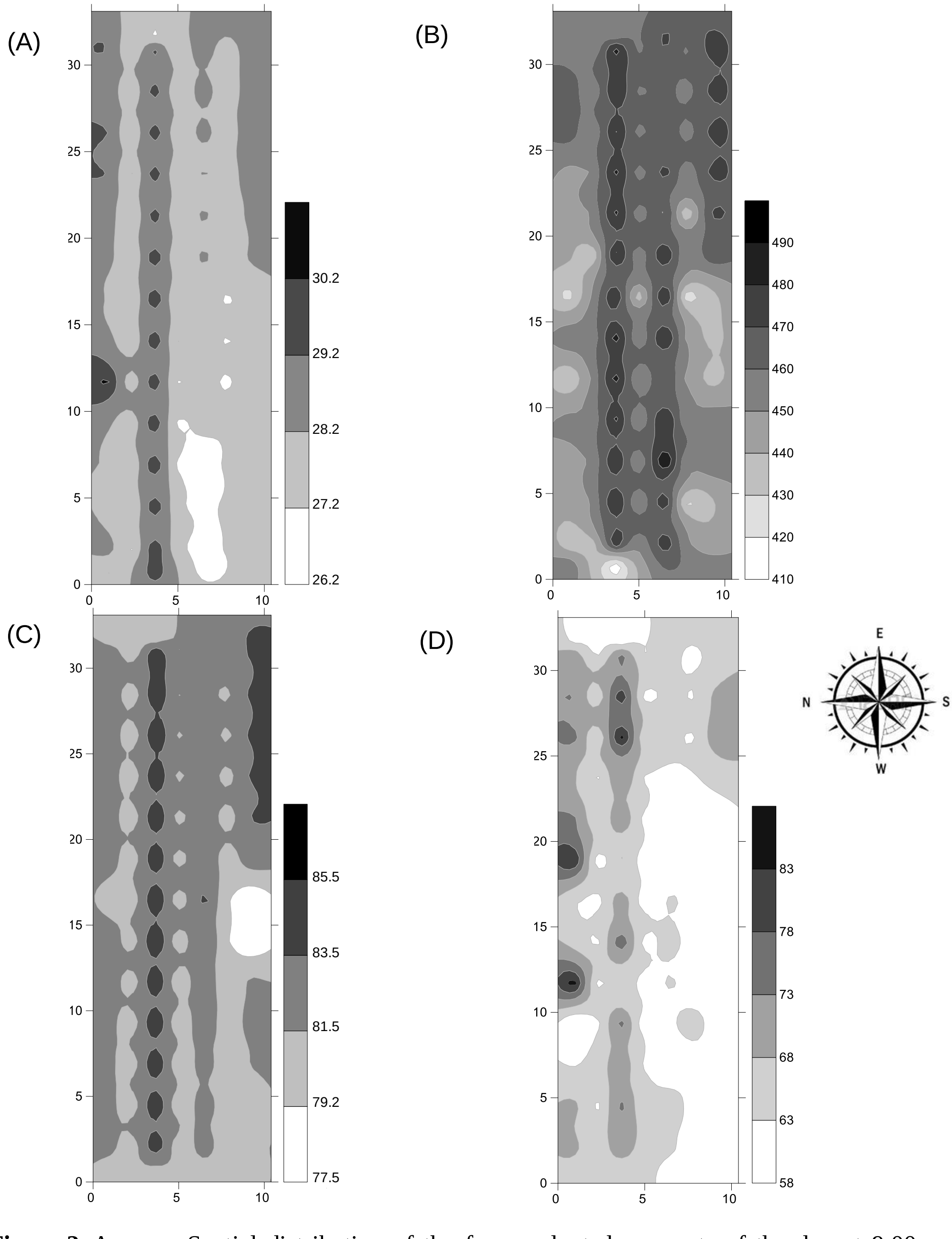

Figure 2. Average Spatial distribution of the four evaluated moments of the day at 9:00 am, 12:00 pm, 15:00 pm, and 18:00 pm of Temperature and Humidity Index (THI, adimensional) (A), Radiation Thermal Load (RTL, $W\ m^{-2}$) (B), Temperature and Humidity Index (BGTH, adimensional) (C), Enthalpy (H, $kJ.kg_{\text{dry air}}^{-1}$) (D).

The facility for laying hens studied in this paper is an ordinary Colombia system to egg production. The RTL, THI, BGTH, and H values were above the comfort limits to the hens. The results showed that the system is not efficient in keeping the animals under the thermal comfort, and it would be necessary to study different models of ventilation systems to be more efficient in the hottest months of the year. Besides the use of natural ventilation, other alternatives to improve the thermal environment, such as forced lateral ventilation or designing new models for Colombian poultry farming, could be adopted to improve the thermal environment. These alternatives can make the system more efficient and more competitive with the global agribusiness in this area of egg production.

CONCLUSIONS

The semivariograms allowed the characterization of the magnitude of spatial variability of thermal indices (RTL, THI, BGTH, and H) inside the studied hen facility.

It was possible to make isoline maps that allowed the observation of spatial variability, from the interpolation by kriging.

It was also possible to identify the heterogeneity of the spatial distribution of these parameters in the hen facility throughout the evaluated period.

The maps also allowed observing the existence of failures in the natural ventilation system in some regions of the hen facility. These failures can result in thermal condition above of the comfort in the most of the day. This condition out of the thermal zone may cause discomfort to the animals and productive and economic losses.

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REFERENCES

Albright, L.D. 1990. *Environment control for animals and plants*. American Society of Agricultural Engineers. 453 pp.

Bachmaier, M. & Backers, M. 2008. Variogram or semivariogram? understanding the variances in a variogram. *Precision Agriculture* **9**(1), 173–175.

Baêta, F.C. & Souza, C.F. 2010. Ambiência em edificações rurais, conforto animal. *Viçosa, MG: UFV*, 269 pp.

Behura, N.C., Kumar, F., Samal, L., Sethy, K., Behera, K. & Nayak, G.D. 2016. Use of Temperature-Humidity Index (THI) in energy modeling for broiler breeder pullets in hot and humid climatic conditions. *J. Livestock Sci* **7**, 75–83.

Burgess, T.M. & Webster, R. 1980. Optimal interpolation and isarithmic mapping of soil properties: II., block kriging. *Journal of Soil Science* **31**(2), 333–341.

Cambardella, C.A., Moorman, T.B., Novak, J.M., Parkin, T.B., Karlen, D.L., Turco, R.F. & Konopka, A.E. 1994. Field-scale variability of soil properties in central Iowa soils. *Soil science society of America journal* **58**(5), 1501–1511.

Carvalho, T.M.R., Moura, D.J., Souza, Z.M., Soura, G.S., Bueno, L.G.B., Lima, K.A.O. 2012. Use of geostatistics on broiler production for evaluation of different minimum ventilation systems during brooding phase. *Revista Brasileira Zootecnia* **41**(1), 194–202.

Cressie, N. 1993. Statistics for spatial data. *New York: J. Wiley*. 900 pp.

Rocha, H.P.D., Furtado, D.A., do Nascimento, J.W. & Silva, J.H. 2010. Bioclimatic and production parameters in different poultry houses in the semiarid region of Paraiba State. Revista Brasileira de *Engenharia Agrícola e Ambiental* **14**(12), 1330–1336 (in Portuguese).

Diggle, P.J. & Ribeiro Jr, P.J. 2007. Model based geostatistics. *New York: Springer*, 230 pp.

Esmay, M.L. 1969. Principles of animal environment. *2.ed. West Port: AVI*, 325 pp.

Espinal, L.S. 1992. Antioquia ecological geography. Life zones. Medellín: *Universidad Nacional de Colombia. Facultad de Ciencias Agropecuarias,* 146 pp. (in Spanish).

Faraco, M.A., Uribe-Opazo, M.A., Silva, A.A., Johann, J.A., Borssoi, J.A. 2008. Selection criteria of spatial variability models used in thematical maps of soil physical attributes and soybean yield. *Revista Brasileira de Ciência do Solo* **32**(2), 463–476 (in Portuguese).

Ferraz, G.A.S., Silva, F.M. Carvalho, L.C.C., Alves, M.C., Franco, B.C. 2012. Spatial and temporal variability of phosphorus, potassium and of the yield of a coffee field. *Engenharia Agrícola* **32**(1), 140–150 (in Portuguese).

Ferraz, P.F.P., Yanagi Junior, T., Ferraz, G.A.eS., Schiassi, L. & Campos, A.T. 2016. Spatial variability of enthalpy in broiler house during the heating phase. *Rev. bras. eng. agríc. Ambient.* **20**(6), 570–575.

Freitas, L.C.S.R., Tinôco, I.F.F., Baêta, F.C., Barbari, M., Conti, L., Teles Júnior, C.G.S., Cândido, M.G.L., Morais, C.V. & Souza, F.C. 2017. Correlation between egg quality parameters, housing thermal conditions and age of laying hens. *Agronomy Research* **15**(3), 687–693.

Gates, R.S., Zhang, H., CoUiver, D.G. & Overhults, D.G. 1995. Regional variation in temperature humidity index for poultry housing. *Transaction of the ASAE* **38**(1), 197–205.

Isaaks, E.H. & Srivastava, R.M. 1989. An introduction to applied geostatistics. *New York: Oxford University*, 561 pp.

Johann, J.A., Silva, M.C.A., Uribe-Opazo, M.A. & Dalposso, G.H. 2010. Spatial variability of profitability, harvest losses and productivity of beans. *Engenharia Agrícola* **30**(4), 700–714.

Lana, S.R.V., Lana, G.R.Q., Salvador, E.D.L., Lana, Â.M.Q., Cunha, F.S.A. & Marinho, A.L. 2017. Quality of commercial laying eggs stored at different temperatures and storage periods. *Rev. bras. saúde prod. anim*. **18**(1), 140–151 (in Portuguese).

Lee, J., Byeongjoon, N., Jang, S., Park, D., Chung, Y. & Chang, A. H. 2015. Stress detection and classification of laying hens by sound analysis. *Asian-Australas J. Anim. Sci*. **28**, 592–598.

Lemos, M. J., Calixto, L. F.L., Reis, T. L. & Rêgo, R. S. 2014. Quality of semi-heavy laying eggs of different ages stored at different temperatures. *Rev. Acad., Ciênc. Agrár. Ambient*. **12**(2), 133–140 (in Portuguese).

Massari, J.M., Moura, D.J., Curi, T.M.R.C., Vercellino, R.A. & Medeiros, B.B.L. 2016. Zoning of environmental conditions inside a wean-to-finish pig facility. *Engenharia Agrícola, Jaboticabal* **36**(5), pp. 739–748.

Mello, J.M., Batista, J.L.F., Junior, P.J.R. & de Oliveira, M.S. 2005. Adjustment and selection of spatial semivariogram models for the volumetric estimation of Eucalyptus grandis. *Scientia Forestalis*, Piracicaba, **69**, 25–37 (in Portuguese).

Oliveira, C.E.A., Damasceno, F.A., Ferraz, G.AS., Nascimento, J.A.C., Silva, E. & Ferreira, M.R. 2016. Geostatistics applied to the spatial distribution of thermal and noise conditions in Compost Barn installations with different ventilation systems. *Ciência ET Praxis* **9**(18), 41–48 (in Portuguese).

R DEVELOPMENT CORE TEAM. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2011. *Disponível em: <http://www.R-project.org/>*. Acesso em: 13 mar. 2011.

Ribeiro Junior, P. J., Diggle, P.J. June 2001. GeoR: a package for geostatistical analysis. *R-News, New York*, **1**(2), pp. 14–18.

Ribeiro, P.A.P., Yanagi Junior, T., Oliveira, D.D., Ferraz, G.A.S. & Lourenconi, D. 2016. Geostatistical analysis of illuminances in poultry for laying hens equipped with compact fluorescent and led lamps. *Engenharia Agrícola* **36**(1), 11–21 (in Portuguese).

Silva, R.C., Rodrigues, L.R., Rodrigues, V.P., Arruda, A.S. & Souza, B.B. 2015. Analysis of the effect of heat stress on poultry production, physiology and diet. *Agropecuária Científica no Semiárido* **11**(2), 22–26 (in Portuguese).

Sousa, F.C., Barbari, M., Baptista, F., Souza, C.F., Saraz, A.O., Coelho, D.J.R. & Silva, A.L. 2018. Diagnosis of air quality in broilers production facilities in hot climates. *Agronomy Research* **16**(2), 582–592.

Trangmar, B.B., Yost, R.S. & Uehara, G. 1985. Application of geostatistics to spatial studies of soil. *Advances in agronomy* **38**, 45–94.

Vale, M.M., Moura, D.J., Nääs, I.A., Curi, T.M.R.C. & Lima, K.A.O. 2016. Effect of a simulated heat wave in thermal and aerial environment broiler-rearing environment. *Engenharia Agrícola* **36**(2), 271–280.

Vieira, S. R. Geostatistics in studies of soil spatial variability. In: NOVAIS, R. F. de; Alvarez, V. H., Schaefer, C.E.G.R. (Ed.). 2000. *Tópicos em ciência do solo*. *Sociedade Brasileira de Ciência do Solo*, **1**, pp. 1–54.

Zulovich, J.M. & DeShazer, J.A. 1990. Estimating egg production declines at high environmental temperatures and humidity. *ASAE Paper No. 90-4021*, St. Joseph, MI.

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Understanding animal welfare by students and graduates of different studies

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Abstract. Animal welfare is one of the key elements of contemporary approach to animal production. Social consciousness of animal welfare concerns first of all persons responsible for practical implementation of individual solutions for animal welfare improvement. But what about other group of peoples and their relation to animal welfare? The aim of the paper was to analyze some aspects of animal welfare assessment including opinions given by young Polish citizens. The idea of the paper was to investigate, how kind of higher education represented by citizens show effect on understanding of animal welfare problems. The scope of the paper included survey, where 165 graduates of different studies (humanities, polytechnic, medical, economic, art and life sciences studies) had the possibility to present and assess their knowledge on animal welfare. In one of the questions, interpreting an ideal farm with animal production, most respondents, regardless of the field of study, pointed to the key role of maintaining the highest standards determining the welfare and comfort of livestock.

Key words: assessment, cattle, graduate, respondent, survey, welfare.

INTRODUCTION

The pursuit of profit maximization did not bypass farms associated with the production of livestock. The term maximizing profits for many people has negative associations, such as animal suffering, ruthless exploitation and poor living conditions. To measure the quality of livestock life, the term 'animal welfare' was introduced. According to the OIE (World Organization for Animal Health) definition given in introduction to the recommendations for animal welfare, animal welfare means the physical and mental state of an animal in relation to the conditions in which it lives and dies (Terrestrial Animal Health Code, 2010). Animal welfare includes a number of requirements that breeders should meet. Maintaining welfare at a high level translates into measurable benefits in the form of larger and more qualitative production as well as ensuring peace and safety in the herd.

Contemporary trends in scientific research in the area of animal production are moving in many directions, among which a special place is occupied by the pursuit of an increasingly better understanding of animal welfare problems, including livestock. Animal welfare thus becomes a key element in the sustainable development of agricultural production and its individual sectors (von Keyserlingk et al., 2009). In the sustainable development of dairy farms, along with animal health, welfare will be the main factor in increasing cow productivity and milk production efficiency over the next few decades (Britt et al., 2018).

In the context of the quality of the broadly understood food production chain, the issue of animal welfare generates an approach considered from various points of view, including the level of farm production as well as consumers (Blokhuis et al., 2003). Each approach to the concept of animal welfare is distinguished by specific features, among which a special place is occupied by the environment and the surrounding conditions included in many definitions regarding animal welfare.

According to Hughes (1976), welfare is defined as a state of physical and mental health in which animals are in full harmony with the environment in which they live and at the same time develop. The considerations made by Broom (1993) confirming the importance of linking animals with the surrounding environment have indicated the interdisciplinary nature of research in the field of welfare, taking into account animal sciences and ethics in farm production. Jensen & Sandøe (1997) emphasized that welfare can also refer to satisfying animal preferences with the increasing level of animal welfare.

In practice, animal welfare is considered at farm level. And it is fully justified. On the farm, the conditions that determine the comfort and well-being of animals are shaped. However, consumers' awareness of animal welfare also seems important. Consumers are recipients of agricultural products, hence they can decide on shaping the food market through their conscious preferences resulting indirectly from knowledge about animal maintenance and welfare (Gołębiewska et al., 2018).

The aim of the investigation was to compare the knowledge of students or graduates representing various kind of study in the field of animal welfare and dairy production. The undertaken aim of the study can be considered in line with the trend of linking education and educational programs with the implementation of broadly understood progress in dairy production (Chase et al., 2006).

MATERIALS AND METHODS

Conducting detailed research in the field of animal welfare knowledge required the appropriate design of research activities. In general, research design takes into account the selection of a specific approach from qualitative, quantitative and mixed methods (Creswell, 2013), which facilitates later interpretation of the results, and in the case of own research considered – results obtained on the basis of a survey. Consumer surveys are among the more and more common methods of assessing the links between the agricultural production area and the recipients on the market of plant and animal products.

Detailed information on the methodical approach to the undertaken research is as follows:

- An electronic survey was used. The respondent completed the questionnaire on his/her own after entering the appropriate link to the website in the advertisement.
- The survey was anonymous and contained 20 questions.
- 165 students or university graduates took part in the survey. Most of them, i.e. 140 respondents, were young people (18–25 years old).

• Users posted on social networks voluntarily responded, provided that only university students or graduates respond.

• Data taken into account in the study were collected from 12/04/2019 to 11/05/2019.

Group of 165 people (most were women) took part in the survey. Among the respondents, six groups representing the following fields of study were distinguished:

• Humanities studies – 16 people (9.70%),
• Polytechnic studies – 18 people (10.91%),
• Medical studies – 15 people (9.09%),
• Economic studies – 22 people (13.33%),
• Art studies – 5 people (3.03%),
• Life sciences studies – 89 people (53.94%).

The most popular field of study among the respondents were life sciences studies with the following specializations: Agronomy and Agribusiness, Animal Behavior, Food Safety, Animal Bioengineering, Biology, Biotechnology, Biotechnology in breeding and animal health protection, Agricultural chemistry, Ecology, Breeding and protection of accompanying and wild animals, Ecological Engineering, Production Engineering, Forestry, Microbiology, Environmental Protection, Horticulture, Agriculture, Food Technology, Technologies in Environmental Protection, Commodity Science in the Bioeconomy, Veterinary Medicine, Zootechnics, Human Nutrition and Food Evaluation.

The age diversity of the respondents was as follows:

• 18–25 years – 140 people (84.85%),
• 26–35 years – 25 people (15.15%).

The two most numerous (out of four distinguished) groups of people participating in the survey lived in the countryside and in cities of over 100,000 residents.

Participants were recruited online from Poland. The survey was conducted on a group of young people living in the Masovian region. Data were collected via an online platform. Participants, before answering the study question, were first asked several multiple-choice demographic questions. The survey was completely anonymous.

The survey completed by respondents included in total 20 questions directly or indirectly related to the welfare of livestock, mainly dairy cattle. For individual questions, the survey included response options to choose from, in order to facilitate not only answering, but also in the next stage to develop research results. Depending on the detailed scope of the questions formulated, several groups of answers were distinguished. One group included 'yes', 'no' and 'I have no opinion / I don't know' answers. Another group of answers included the following gradation of 'yes', 'rather yes', 'rather not' and 'no'. For some questions, the answers proposed included more specific response options, as appropriate. Of the total of 20 questions, only a part of the questions and the results – the answers are presented in this article.

The condition of proceeding to complete the survey was confirmation by the potential respondent that he / she had or has contact with a farm, in particular keeping dairy cattle. This information was taken into account at the preparation stage for completing the survey. People who had not previously visited the farm or farms did not participate in the survey.

The proposed approach to surveys was an alternative solution to the research presented in the literature within the research group. In studies conducted by Cardoso

(2016), people who had never been associated with the dairy industry before were selected for the survey on some aspects of dairy production.

Survey results have been developed using descriptive statistics. The differences between the research results for the considered groups of students / graduates for individual questions were analyzed by ANOVA. Results – answers within individual questions have been properly prepared for ANOVA analysis. The Statistica v.13 software (StatSoft Polska, Cracow, Poland) was used for the analysis. Significance was declared at $\alpha = 0.05$.

RESULTS AND DISCUSSION

The first question concerned general knowledge about welfare was worded as follows: 'What do you think animal welfare is about?' For this question, four answer options were considered: 'On the satisfaction of the biological needs of animals', 'On the satisfaction of the emotional needs of animals', 'On the satisfaction of the biological and emotional needs of animals' and 'I do not know'. Only one answer could be chosen. Only one person (representing polytechnic studies) replied that he did not know what animal welfare is. Most, i.e. 92.7% of respondents answered that animal welfare is about meeting the biological and emotional needs of animals. No respondent indicated that animal welfare is about meeting their emotional needs. Comparison of the number of responses indicated by the respondents in the case of the four considered answer options showed their significant statistical differentiation (at $F = 4.3795$ the p value was 0.0159).

In a more detailed question, respondents were asked to assess their own knowledge of animal welfare, taking into account four response options, ranging from specialist knowledge to the statement 'I have never heard of animal welfare'. Among graduates or people studying at life sciences universities, answers confirming possession of specialist and good knowledge about animal welfare dominated (66.3%). In the group of people from other types of universities, the majority of respondents (from 60 to 94% for individual types of universities) indicated an average level of their own knowledge about the welfare of farm animals. The results of this survey show the current state of knowledge about animal welfare. The results of the one-way analysis of variance did not show a significant differentiation (with $F = 1.8749$, the p-value was 0.1664) between the response options regarding the assessment of the respondents' own knowledge about livestock welfare. Even more interesting, however, is how this knowledge changes. In the studies of Ventura et al. (2016) an approach consisting in assessing the change in respondents' knowledge about dairy production before and after the visit to the dairy farm was presented. This knowledge was assessed on the basis of answers to questions about various activities undertaken in the barn.

Then it was asked whether, in the respondents' opinion, the required level of animal welfare in Poland was maintained. In the groups of respondents from polytechnic and life sciences universities, the distribution of answers was similar. Most people (around 90%) were not fully resolved and answered 'rather yes' and 'rather not' as to whether the required level of animal welfare in Poland was maintained. More pessimistic opinions on the presented issue were expressed by students and graduates of humanities and medical universities. The number of 'rather not' and 'no' answers covered 63–73% of all answers from these universities. On the other hand, people associated with economic and artistic studies spoke most negatively about maintaining the required level of animal welfare in Poland. In this case, the answers 'rather not' and 'no' constituted 90% and 100% respectively. Only 5 respondents from art universities took part in the study, therefore the obtained test result (100% negative answers) would require verification on a larger population of people with artistic education. The results of one-way analysis of variance did not show a significant differentiation (with F = 2.2124, the p-value was 0.1182) between response options regarding the assessment - in the respondents' opinion - of maintaining the required level of animal welfare in Poland.

In the next question, respondents generally agreed that the law should extend strict regulations on the welfare of livestock. The results of the survey presented in Fig. 1 indicate a rigorous approach of the respondents to the systematic extension of regulations regarding the welfare of livestock. In the case of each group of students / graduates of the included fields of study, the acceptance for extending the assessed regulations was about 80%, and in the case of artistic studies even 100%.

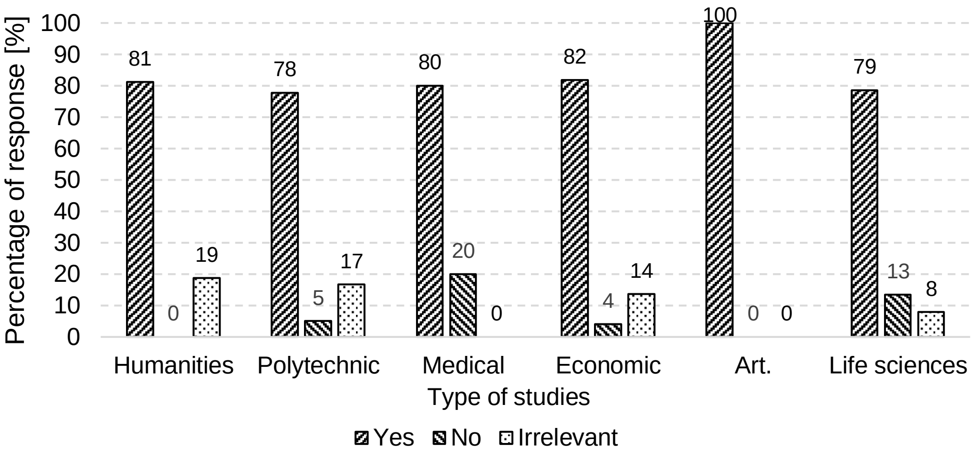

Figure 1. Percentage distribution of answers to the question: Should the law extend strict regulations regarding the welfare of livestock?

The questionnaire also asked respondents the following question: What in your opinion can contribute to improving the welfare of farm animals? This question included the possibility of providing several answers (maximum three answers) from the following suggestions:

- higher qualifications / training of owners of farms with animal production,
- additional subsidies from the European Union,
- introduce stricter animal protection rules,
- increased controls on state inspections and animal recipients.

The distribution of answers to the question 'What in your opinion can contribute to improving the welfare of farm animals?' is presented in Table 1.

The calculated average values of the percentage share of the considered response options (Table 1) indicated that respondents assessed the significance of three factors that could improve the welfare of livestock animals to a similar percentage. In the respondents' opinion, additional subsidies from the European Union may have the least impact on the improvement of animal welfare. On the other hand, analysis of variance did not show a significant differentiation ($p > 0.05$) between the number of responses given by individual respondents to the given issues.

Table 1. Percentage share of each option to answer the question: What in your opinion can contribute to improving the welfare of farm animals?

Answer option	Studies						Av
	Humanities	Polytechnic	Medical	Economic	Art	Life sciences	
Higher qualifications / training of owners of farms with animal production	20.9	32.6	23.3	28.3	25.0	30.6	26.8
Additional subsidies from the European Union	18.6	14.0	14.0	20.8	18.8	16.4	17.1
Introduce stricter animal protection rules	27.9	23.2	30.1	26.4	31.2	24.6	27.2
Increased controls on state inspections and animal recipients	32.6	30.2	32.6	24.5	25.0	28.4	28.9

Abbreviation: Av – Average value.

Taking into account the consumer aspect in the survey, respondents were asked whether, in their opinion, the state of health and comfort of cattle have an impact on the quality of animal products, including milk and meat. In all fields of study, answers dominating that the state of health and comfort of cattle are of key importance for the quality of animal products. The average percentage share of answers confirming the assessed issue for the full population of respondents was 71%, the most in the group of people from artistic studies (80%) and the least from polytechnic and medical studies (67%). Only three persons out of 165 persons participating in the study stated that the health and comfort of cattle are not important for the quality of milk, meat and other animal products. The one-way analysis of variance did not show a significant differentiation (for F = 2.9392 the p-value was 0.0837) between the number of responses 'are of key importance', 'have some impact' and 'it does not matter'.

One of the questions concerned the issue of modernity in animal production and animal welfare. It was asked whether, according to respondents, the use of modern technology on a farm may affect the welfare of livestock. A significant proportion of respondents (on average 83% of the entire respondent population) confirmed that the level of mechanization in animal production can affect animal welfare. On average, 5% of respondents said that mechanization is not important when assessing animal welfare. The remaining respondents, most in the group of people associated with humanities and medical studies, had no opinion on the issue under consideration. A one-way analysis of variance did not show a significant differentiation (for F = 3.2372, p value was 0.0678) between the number of responses confirming and denying the impact of mechanization on animal welfare. Modernization of agriculture and the associated introduction of increasingly higher mechanization of technological processes, including those related to dairy production is a response to the processes of urbanization and globalization (Britt et al., 2018). It therefore seems justified to raise the problem of how the introduction of more modern animal production technologies and their automation affect animal welfare. This is even more important because at the same time the importance of technical and technological modernization of dairy farms and its impact on the possible reduction of costs of obtaining dairy products for the needs of consumers in urban areas is emphasized (Nicholson et al., 2011).

The purpose of one of the questions in the survey was to assess the sensitivity of respondents to the problems of animals kept on the farm. The following question was raised: In your opinion, is it acceptable for cows or calves to suffer prolonged pain and suffering in the milk production process on the farm? The percentage distribution of answers to this question is presented in Fig. 2.

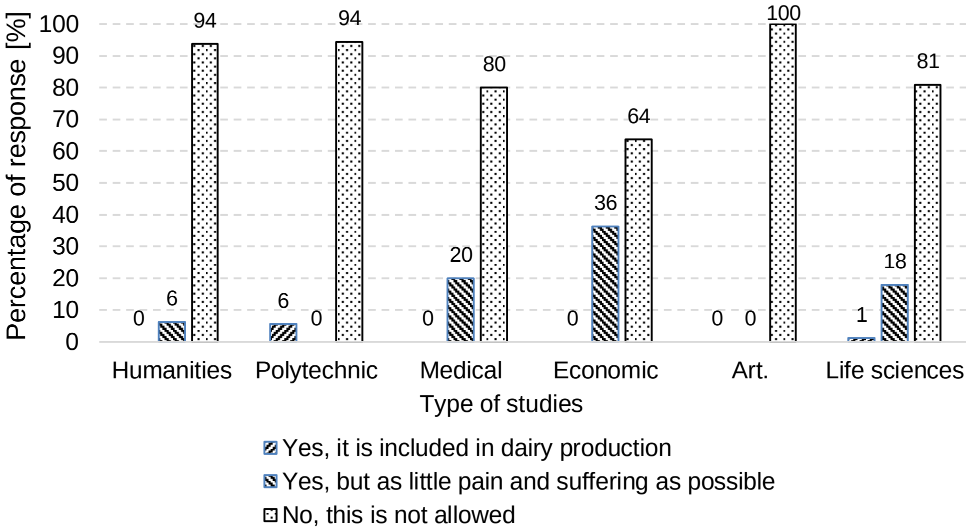

Figure 2. Percentage distribution of answers to the question: In your opinion, is it acceptable for cows or calves to suffer prolonged pain and suffering in the milk production process on the farm?

The percentage distribution of responses shows (Fig. 2) that among respondents representing individual fields of study, opinions prevailed that animals were not allowed to experience pain and suffering. Depending on the field of study, between 64 and 100% of respondents showed sensitivity to the pain and suffering of livestock, considering them unacceptable. The most sensitive to the pain and suffering of farm animals were those associated with artistic studies. The survey was completed by only 5 people from artistic studies, which is why this part of observation would certainly require confirmation in additional research. At the same time, it is worth paying attention to a similar distribution of answers in the case of respondents from medical and life sciences. Definitely the most opinions (36%) authorizing even minimal pain and suffering of cattle were expressed by people associated with economic studies (Fig. 2). One-way analysis of variance showed a significant differentiation (for F = 3.8505 p value was 0.0447) between the number of responses to the highlighted issues related to the nature of animal pain and suffering in dairy production. Questions about the pain and suffering of livestock are often associated with stress, which is one of the determinants of welfare assessment (von Keyserlingk & Weary, 2017). Therefore, in future surveys, it will be worthwhile to address the issues of stress and factors affecting animal stress.

The next question in the survey referred to the previously discussed issue of animal pain and suffering. The question addressed to the respondents was: Do you think that interference with the animal's body (e.g. putting on an ear tag, chip under the skin, etc.) reduces its welfare? In the case of such a question, the following answer options were considered: 'Yes, always', 'Yes, but not always, depends on the nature of the interference', 'Does not reduce', 'It's difficult to say'.

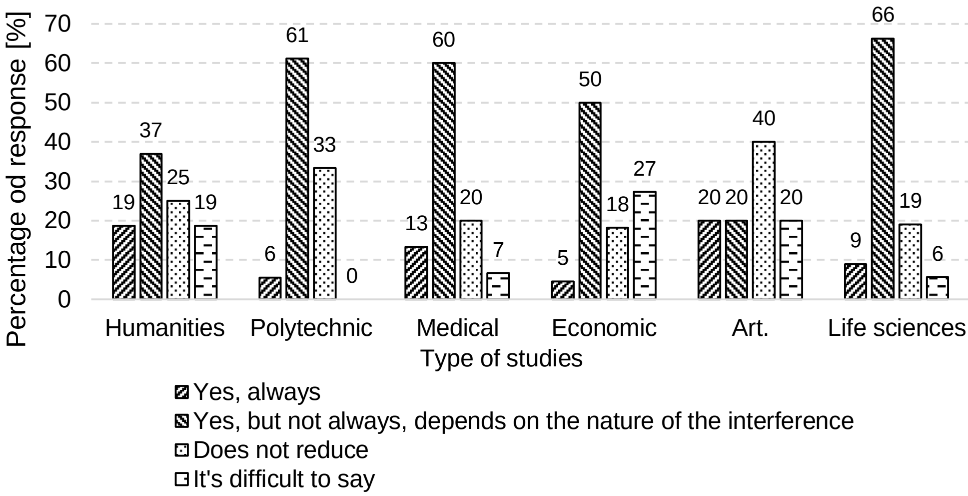

Figure 3. Percentage distribution of answers to the question: Do you think that interference with the animal's body (e.g. putting on an ear tag, chip under the skin, etc.) reduces its welfare?

Comparison of the percentage distribution of answers presented in Fig. 3 shows that the respondents are dominated by the opinion (on average 49% for the total population) that it is not always possible, but according to the specificity of production needs, to interfere with the animal's body. One-way analysis of variance showed no significant differentiation (for F = 1.9654 the p-value was 0.1517) between the number of responses to individual issues – opinions on interference with the animal's body. Questions about pain, suffering and interference in the animal's body become particularly important in the situation of improving dairy cattle production technology, especially when the animals are exposed to many different sources of stress (Herbut et al., 2019). In many discussions, animal pain and suffering are confronted with solutions that can benefit the farmer. Exemplary studies in large herds of dairy cattle did not give an unequivocal answer as to whether docking tails guarantees the expected benefits, i.e. greater cleanliness of cows and less udder health problems (Schreiner & Ruegg, 2002). On the other hand, the issue of the impact of tail docking on the welfare of dairy cattle is raised (von Keyserlingk et al., 2009), and in particular the possibility of chronic pain in animals undergoing tail docking (Eicher et al., 2006). The problem of pain and suffering is also raised in the case of dehorning of calves and related methods of dealing with varying degrees of pain perception by young animals (Stafford & Mellor, 2005). Examples of activities that lead dairy cattle to animal pain and suffering point to the need to develop a public debate on animal welfare. The conclusions of this debate, taking into account the results of surveys and consumer opinions, could contribute to a balanced approach to improving animal production technology.

The interference in the animal body raises issues of ethics in animal production. This problem was addressed in the next question in the survey, which was worded as follows: Does ethics apply to dairy production and keeping dairy cows on the farm?

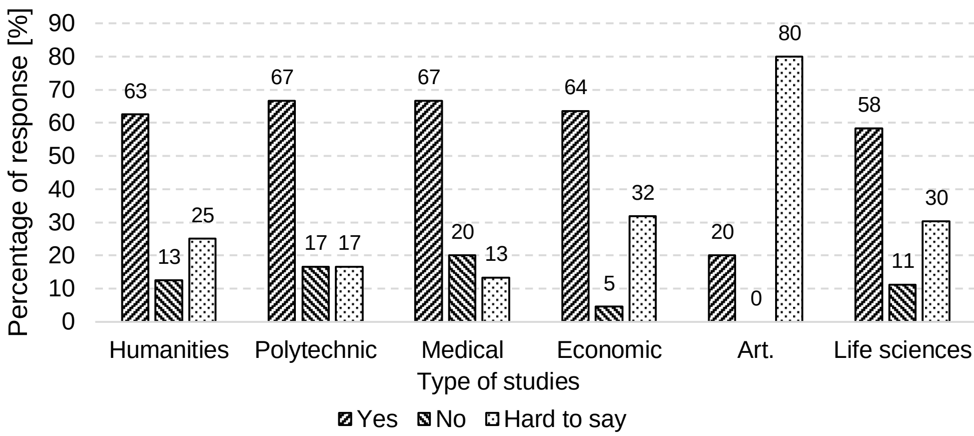

Figure 4. Percentage distribution of answers to the question: Does ethics apply to dairy production and keeping dairy cows on the farm?

Students and graduates of almost all fields of study predominantly indicated that the principles of ethics apply to dairy production on the farm. Only respondents representing art studies did not have a clear opinion on the issue in question (Fig. 4). In studies carried out by Cardoso et al. (2016), respondents, considering the importance of ethical aspects related to animal handling, indicated that acceptance of milk production in ethical terms is possible when animals are properly treated. According to Gaworski (2006), ethics refers to the entire food chain and its transformations, hence the survey questions on ethics can be extended to other stages related to food production.

The last of the presented issues was formulated in the survey as follows: 'An ideal farm with animal production' is in your opinion a farm that puts priority as following ... The possible answers suggested in this case included such options as:

- maximizing production income,
- minimizing financial outlays and labour outlays on production,
- maintaining the highest standards of comfort and welfare of livestock,
- protection of the natural environment related to the use of animal wastes.

The results of the survey covering the opinion of respondents on the ideal farm with animal production indicate the key importance attached to maintaining the highest standards of comfort and animal welfare. On average, in the entire population of people completing the survey, 82% of respondents indicated this approach (Table 2), taking into account the range from 67% (people associated with polytechnic studies) to 100% (people associated with medical studies). Other options for answering the image and interpretation of an ideal farm with animal production have received relatively little confirmation of significance among the respondents participating in the survey. One-way analysis of variance showed a significant differentiation (for F = 4.1862 p value was 0.0188) between the number of responses under individual options related to the interpretation of the concept of an ideal farm with animal production.

Table 2. Percentage share of each option to answer the problem: 'An ideal farm with animal production' is in your opinion a farm that puts priority as following ...

Answer Option	Studies						Av
	Humanities	Polytechnic	Medical	Economic	Art	Life sciences	
Maximizing production income	12.5	11.1	0.0	0.0	0.0	3.4	4.6
Minimizing financial outlays and labour outlays on production	0.0	5.5	0.0	9.1	20.0	3.4	6.3
Maintaining the highest standards of comfort and welfare of livestock	87.5	66.7	100.0	81.8	80.0	78.6	82.4
Protection of the natural environment related to the use of animal waste	0.0	16.7	0.0	9.1	0.0	14.6	6.7

Abbreviation: Av – Average value.

In the survey conducted by Cardoso et al. (2016), the respondents were asked to freely express their opinion on the ideal farm, which allowed to obtain descriptive information subject to discussion and comparison with the opinions presented in other papers. However, our approach to interpreting the concept of 'ideal farm' was slightly different from the approach presented in the literature. First of all, the term 'ideal farm' has been narrowed down to the term 'ideal farm with animal production'. This, in our opinion, facilitated the approach to gathering the respondents' opinions based on the selection from the suggested answers.

The method and scope of formulated response options constitute an individual proposal within the given research. However, it seems to be more important that respondents' preferences for specific responses can be compared numerically or by percentage. Such a numerical (percentage) comparison of the results of own research among the available response options showed the leading importance of maintaining the highest standards of comfort and animal welfare in the context of the interpretation of 'ideal farm with animal production'. Such indications of respondents are consistent with the results of surveys obtained by Cardoso et al. (2016), in which the majority of respondents (90%) pointed to animals and their needs as the dominant elements in the interpretation of the concept of an ideal dairy farm. Quality of treatment given to animals was a priority concern for people completing the survey. The role of sustainable development and the needs of animals in shaping the future model of farms was also indicated by the respondents in the survey conducted by Boogaard et al. (2008).

The question about 'an ideal farm with animal production' is a premise to develop a wide spectrum of research. The term 'ideal' can be applied to many farm activities. For example, on a dairy farm it would be a precise selection of a milking installation for the size of a dairy cow herd (Gaworski et al., 2018), creation of ideal working conditions in a milking parlour (Papez & Kic, 2015), selection of ideal bedding materials in the lying area of the barn (Leso et al., 2019) as well as use of litter that has always played a fundamental role on the physical wellbeing of the animals (Bambi et al. 2018). Opinions on the ideal dairy farm can be gathered both among external respondents and farmers themselves. A comparison of these opinions could be a valuable contribution to the discussion on the farm development vision.

CONCLUSIONS

The survey showed that the majority of young respondents, regardless of the type of study, interpret animal welfare as meeting their biological and emotional needs. In this way, respondents demonstrated a mature approach to assessing the needs of livestock, which is not only about providing feed needs, but also about the ability to express natural behaviour and comfort.

Answering the question about the factors that could increase the welfare of livestock, young respondents primarily pointed to the knowledge, training and qualifications of farm owners, as well as the control of animal herds by the relevant inspection services. Thus, students and graduates of universities confirmed the importance of acquiring knowledge and its practical application, as well as the role of an efficient system for controlling agricultural activity, which translates into food production safety.

Students and graduates of all fields of study unanimously confirm the opinion regarding the interpretation of an ideal farm with animal production. They pointed to the key role of maintaining the comfort and welfare of animals, which in their opinion are more important than economic and labour-related aspects.

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REFERENCES

Bambi, G., Rossi, G. & Barbari, M., 2018. Comparison between different types of bedding materials for horses. *Agronomy Research* **16**(3), 646–655. doi: 10.15159/AR.18.124

Blokhuis, H.J., Jones, R.B., Geers, R., Miele, M. & Veissier, I. 2003. Measuring and monitoring animal welfare: Transparency in the food product quality chain. *Animal Welfare* **12**, 445–455.

Boogaard, B.K., Oosting, S.J. & Bock, B.B. 2008. Defining sustainability as a socio-cultural concept: Citizen panels visiting dairy farms in the Netherlands. *Livestock Science* **117**(1), 24–33. doi: 10.1016/j.livsci.2007.11.004

Britt, J.H., Cushman, R.A., Dechow, C.D., Dobson, H., Humblot, P., Hutjens, M.F., Jones, G.A., Ruegg, P.S., Sheldon, I.M. & Stevenson, J.S. 2018. Learning from the future – A vision for dairy farms and cows in 2067. *Journal of Dairy Science* **101**(5), 3722–3741. doi: 10.3168/jds.2017-14025

Broom, D.M. 1993. Assessing the welfare of modified or treated animals. *Livestock Production Science* **36**(1), 39–54. doi: 10.1016/0301-6226(93)90136-6

Cardoso, C.S., Hötzel, M.J., Weary, D.M., Robbins, J.A. & von Keyserlingk, M.A.G. 2016. Imagining the ideal dairy farm. *Journal of Dairy Science* **99**(2), 1663–1671. doi: 10.3168/jds.2015-9925

Chase, L.E., Ely, L.O. & Hutjens, M.F. 2006. Major advances in extension education programs in dairy production. *Journal of Dairy Science* **89**(4), 1147–1154. doi: 10.3168/jds.S0022-0302(06)72183-X

Creswell, J.W. 2013. Designing of scientific research. Qualitative, quantitative and mixed methods / Projektowanie badań naukowych. Metody jakościowe, ilościowe i mieszane. *Wydawnictwo Uniwersytetu Jagiellońskiego*, Kraków, pp. 272. (in Polish).

Eicher, S.D., Cheng, H.W., Sorrells, A.D. & Schutz, M.M. 2006. Behavioral and physiological indicators of sensitivity or chronic pain following tail docking. *Journal of Dairy Science* **89**(8), 3047–3051. doi: 10.3168/jds.S0022-0302(06)72578-4

Gaworski, M. 2006. Ethics and transformation of Polish food chain. In: *6th Congress of the EurSafe on Ethics and the politics of food*. Oslo, Norway, pp. 270–273.

Gaworski, M., Leola, A., Kiiman, H., Sada, O., Kic, P. & Priekulis, J. 2018. Assessment of dairy cow herd indices associated with different milking systems. *Agronomy Research* **16**(1), 83–93. doi: 10.15159/AR.17.075

Gołębiewska, B., Gębska, M. & Stefańczyk, J. 2018. Animal welfare as one of the criterion determining Polish consumers' decisions regarding their purchase of meat. *Acta Scientiarum Polonorum* **17**(3), 17–21. doi: 10.22630/ASPE.2018.17.3.33

Herbut, P., Angrecka, S., Godyń, D. & Hoffmann, G. 2019. The physiological and productivity effects of heat stress in cattle – a review. *Annals of Animal Science* **19**(3), 579–593. doi: 10.2478/aoas-2019-0011

Hughes, B.O. 1976. Behaviour as an index of welfare. In: *Proceedings 5th European Poultry Conference*, Malta, 1005–1012.

Jensen, K.K. & Sandøe, P. 1997. Animal welfare: Relative or absolute? *Applied Animal Behaviour Science* **54**(1), 33–37. doi: 10.1016/S0168-1591(96)01203-8

Leso, L., Pellegrini, P. & Barbari, M. 2019. Effect of two housing systems on performance and longevity of dairy cows in Northern Italy. *Agronomy Research* **17**(2), 574–581. doi: 10.15159/AR.19.107

Nicholson, C.F., Gomez, M.I. & Gao, O.H. 2011. The costs of increased localization for a multiple-product food supply chain: Dairy in the United States. *Food Policy* **36**(2), 300–310. doi: 10.1016/j.foodpol.2010.11.028

Papez, J. & Kic, P. 2015. Heating and ventilation in milking parlours. *Agronomy Research* **13**(1), 245–252.

Schreiner, D.A. & Ruegg, P.L. 2002. Effects of tail docking on milk quality and cow cleanliness. *Journal of Dairy Science* **85**(10), 2503–2511. doi: 10.3168/jds.S0022-0302(02)74333-6

Stafford, K.J. & Mellor, D.J. 2005. Dehorning and disbudding distress and its alleviation in calves. *The Veterinary Journal* **169**(3), 337–349. doi: 10.1016/j.tvjl.2004.02.005

Terrestrial Animal Health Code, 2010. The World Organization for Animal Health – OIE, Paris, France.

Ventura, B.A., von Keyserlingk, M.A.G., Wittman, H. & Weary, D.M. 2016. What difference does a visit make? Changes in animal welfare perceptions after interested citizens tour a dairy farm. *PLoS ONE* **11**(5), e0154733. doi: 10.1371/journal.pone.0154733

von Keyserlingk, M.A.G., Rushen, J., de Passillè, A.M.B. & Weary, D.M. 2009. The welfare of dairy cattle – Key concepts and the role of science. *Journal of Dairy Science* **92**(9), 4101–4111. doi: 10.3168/jds.2009-2326

von Keyserlingk, M.A.G. & Weary, D.M. 2017. A 100-Year Review: Animal welfare in the Journal of Dairy Science – The first 100 years. *Journal of Dairy Science* **100**(12), 10432–10444. doi: 10.3168/jds.2017-13298

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https://doi.org/10.15159/AR.20.073

Comparison of two perennial energy crops for biomass production at the end of their life cycle

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Abstract. Nowadays fossil fuels are decreasing, causing the world's interest in renewable energy sources to rapidly grow. One of the most interesting renewable and ecologically pure fuels is biomass, which is considered to be carbon neutral. Biomass is a promising source of energy, as it can be used directly as an energy resource. Its quality characteristics such as gross calorific value and ash content are of paramount importance so as to improve the combustion process. Furthermore, during the last three decades, there has been an increasing interest in the production of biomass pellets for domestic and industrial use. Alternative feed stocks will need to be sourced to meet the demand for biomass pellets. Investigation for new energy crops that produce high amounts of biomass under low inputs and of high energy efficiency are the main tasks of this field. Therefore, the aim of this study is to assess the biomass yield and the quality characteristics (gross calorific value and ash content) of two perennial energy crops (*Cynara cardunculus* L. and *Panicum virgatum* L.) growing in a typical soil (*Fluventic Xerochrept*) of the main agricultural land of central Greece. The comparison for both cultivated crops was made in order to show the results during their 8th growing year. The examined factors were the irrigation (two levels: irrigated and rainfed) and the nitrogen fertilization (two levels: 0 and 80 kg N ha^{-1}) as well as their effect on the dry biomass yield and the gross calorific value. It was found that higher dry biomass yield was produced from cardoon (21.3 vs. 14.23 t ha^{-1}), while the higher average gross calorific value was observed for switchgrass biomass (17.31 vs. 15.65 Mj kg^{-1}). Finally, multiplying the dry biomass yield (t ha^{-1}) with the gross calorific value (Mj kg^{-1}) it was found that 334 and 245 Gj ha^{-1} from a cardoon and a switchgrass cultivation could be produced, respectively. Cardoon has better results than switchgrass probably due to the fact that switchgrass is growing from March till October; while cardoon's growing period is from October to June and in such areas precipitation is in shortage during summer months. Both crops could achieve high amounts of energy per hectare and thus their introduction in future land use systems, for an environmentally friendly energy production should be seriously taken into consideration.

Key words: cardoon, switchgrass, yield, gross calorific value, biomass.

INTRODUCTION

Nowadays fossil fuels which are used by people on a daily basis are decreasing and therefore the world's interest in renewable energy sources is rapidly growing. One of the most interesting renewable and ecologically pure fuels is biomass (Li et al., 2012; Kosov et al., 2014; Nishiguchi & Tabata, 2016). The use of biomass for energy generation is considered to be carbon neutral, since its harvest is carried out from a source achieved in a sustainable way (Acda & Devera, 2014).

Biomass is an organic product generated by a natural process (Kunecova & Hlavač, 2019) and constitutes a promising source of energy, as it can be used directly as an energy resource and will help to reduce greenhouse gases (Pizzi et al., 2018). Biomass quality characteristics such as moisture content, gross calorific value, volatile matter, fixed carbon, and ash content are of paramount importance. Such knowledge is quite important so as to improve the combustion process (Torgrip et al., 2017). In particular, calorific value is an essential parameter in the specification of biomass quality to set the price of the product. It has been referred that moisture content affects biomass gross calorific value negatively (Preece et al., 2013; Posom et al., 2016), while increases the final costs due to increased transportation and drying costs (Everard et al., 2012; García et al., 2014). Ash content affects combustion efficiency (García et al., 2014; Toscano et al., 2016), its knowledge is applied for the operating combustion systems (Liu et al., 2014) and finally, the remaining amount must become known so that it could be stored for further management (e.g. use in compost manufacturers).

Furthermore, during the last three decades, there has been an increasing interest in the production of biomass pellets for both domestic and industrial use (Gil et al., 2010; Toscano et al., 2014). In the beginning, pellet manufacturers were using sawdust and wood shavings as a feedstock (Arshadi et al., 2008; Carroll et al., 2012). The rapid increase of pellet use for heating in many countries raised the issue of consuming sawdust but in some countries firewood as well (Carroll et al., 2012). Therefore, alternative feedstocks will need to be sourced to meet the demand for biomass pellets.

Investigation for new energy crops that produce high amounts of biomass under low inputs and of high energy efficiency are the main tasks of this field. There is a considerable number of energy sources from biomass for biofuel production. These sources are mainly plant remains of agricultural crops, waste of woodworking industry and energy crops such as miscanthus, giant reed etc., (Christou et al., 2001; Da Borso et al, 2018).

There are different divisions that someone could make for energy crops. The most common one is in annual and perennial, according to their nature. Perennial energy crops are well acclimatized to the certain conditions and form high yield of biomass grown on non efficient soils, indicating their low input requirements. Such crops are cardoon, silver grass, switchgrass, miscanthus and others.

Switchgrass (*Panicum virgatum* L.), a warm-season (C4) perennial grass with a deep-root system, requires limited use of fertilizers (Giannoulis & Danalatos 2014). Switchgrass has been investigated as a biofuel co-fired with coal for power generation (McLaughlin et al., 2005; Qin et al., 2006). Switchgrass produces its biomass mostly in dry Mediterranean areas during spring and summer, where extended and extreme summer drought occurs frequently, which can be a major factor in the reduced yields and survival of plants (Clifton-Brown et al., 2001; Vamvuka et al., 2010). In literature a remarkable decrease is mentioned in both the leaf area index (Barney et al., 2009) and biomass productivity (Petrini et al., 1996; Vamvuka et al., 2010; Cosentino et al., 2014; Giannoulis & Danalatos, 2014), which was the result of prolonged severe drought. Therefore, irrigation support in such areas as the Mediterranean basin is needed in order to ensure high biomass production, where crop growth is restricted by low water availability and high evapotranspiration rates during the summer (Vamvuka et al., 2010; Zema et al., 2012).

On the other hand, cardoon (*Cynara cardunculus* L.) is a perennial C3 crop, sown early in autumn (Giannoulis et al., 2011). Cardoon is characterized as a crop of its deep-root system which forms a leaf rosette during winter, while flowering stem elongates in spring, and the flowering heads appear in June. Cardoon produces its high biomass during spring and summer (Danalatos et al., 2006), and re-grows after the first rains in autumn. The dry biomass is harvested during July (with a plant moisture content of 15%). The crop produces annual biomass up to 10 years (Fernadez et al., 2006).

The increasing interest for new crops with modest inputs and cultivation requirements, as well as the increasing interest for alternative renewable environmental friendly energy has led the scientific community to the investigation of the yield of energy crops. These crops are divided into two large categories: i) the annual and the perennial. In the case of the perennial crops, most of the studies refer to the biomass production till the fourth year of their growth (Christou et al., 2001; Danalatos et al., 2006; Fernadez et al., 2006; Giannoulis & Danalatos, 2014; Giannoulis et al., 2016). There is none or limit of literature about the yield and the gross calorific value of the biomass produced at the end of the productive life cycle of such perennial crops like cardoon and switchgrass (8–10 and ≥ 10 productive years for cardoon and switchgrass, respectively; Fernández et al., 2005; Angelini et al., 2009; Amarasekara, 2014). Therefore, the aim of the current study was the investigation of the yield of these perennial plants (cardoon and switchgrass) towards the end of their production cycle (8^{th} growing year) and the energy efficiency of the above-mentioned biomass yield under moderate low inputs.

MATERIALS AND METHODS

Two field experiments were established in 2008–2009 in a typical soil-climatic Mediterranean environment in Thessaly plain, central Greece (East Thessaly; Velestino area; Magnesia) to evaluate the effect of moderate irrigation and distinct nitrogen dressings on cardoon and swithcgrass (Alamo variety) yield. The results of the present study refer actually to the experimentation year 2017–2018, where the cultivations were in their 8^{th} growing year. The experimental site is located at 39° 02´ N and 22° 45´ E (Velestino area; Magnesia). Velestino soil was classified as Calcixerollic Xerochrept, according to USDA (1975). Soil characteristics are presented in Table 1.

Table 1. Soil characteristics in depth 0–40 cm

	pH	Composition			Organic	Total N,	Available P	Available K
		Sand, %	Silt, %	Clay, %	Matter, %	%	(mg kg^{-1})	(mg kg^{-1})
0–10 cm	8.3	21	41	38	2.7	0.47	8	254
10–40 cm	8.1	19	39	42	2.3	0.1	4	178

A 2×2 factorial split-plot design was used (during all the cultivating years) with four replications (blocks) and four plots per replication (4×4 = 16 plots per crop). Irrigation comprised the main factor (two levels: moderate irrigation-150 mm and rainfed) and the nitrogen fertilization as the sub-factor (two levels: 0 and 80 kg N ha^{-1}) with four replications. Plot size was 33 m^2 (11 m width ×3 m length). The choice of moderate irrigation was made because there is an increased cultivation cost in the wider area due to the high cost of pumping water from the deep underground aquifer (average pumping depth 170 m). In the last fifteen years there has been an effort to find crops of satisfactory yield using low inputs. It has to be mentioned that irrigation water was supplied by 5 equal doses (30 mm each) during March - May in cardoon case and during May - August in the case of switchgrass cultivation.

No pesticides were used because the crops were in their 8th growing year and were more competitive, but even during the establishment year all plots were hand weeded and no pesticides were used.

Biomass yield and calorific value measured during final harvest (end of June and end of September for cardoon and switchgrass Alamo variety, respectively). The plants were cut 8–10 cm above ground and in order to avoid any border effect, 1 m^2 in the inner plot was harvested. The samples were weighed in the field and then a sub-sample was taken for further laboratory measurements and air drying. Even in cardoon case where biomass was almost dried the sub-samples where air-dried so as to have the same moisture content (about 8%) in all studied samples.

Thereafter, the dry samples were chopped and grounded. After grinding, samples were placed in a stack of sieves, so as to obtain the geometric mean diameter of the sample and geometric standard deviation of particle diameter according to ASAE standard S319.3 (2001). Thereafter, an oxygen bomb calorimeter (Model C5000 Adiabatic Calorimeter, 2004) was used to determine the calorific value of each grind sample.

The weather data was recorded by an automated meteorological station on a daily basis, which was installed next to the experimental field.

Finally, the statistical package GenStat (7th Edition) was used for the analysis of variance (ANOVA) within sample timings for all measured and derived data. The $LSD_{0.05}$ was used as the test criterion for assessing differences between means (Steel & Torrie, 1982) of the main and/or interaction effects.

RESULTS AND DISCUSSION

Weather condition

The experimental area is characterized by a typical Mediterranean climate with cool humid winters and hot and dry summers. Fig. 1 illustrates measured weather data during the cultivating period September 2017 (reemergence of cardoon) till September 2018 (switchgrass harvest). Actually, as depicted from Fig. 1, average temperatures in January and February were closed to 7 °C, showing a mild winter with no frosts that could offset the cultivation of cardoon which was at the rosette stage. Then, air temperature fluctuated around 12–13 °C in March and the first half of April and increased up to 20 °C in May. Finally, during the summer months the average air temperature fluctuated between 21.3 to 26.1 °C.

During cardoon growing period (September to half of June) about 320 mm of rain were recorded while only the 90 mm in spring and in June till harvest date (moderate irrigation period as shown above). On the other hand, during switchgrass growing period (reemergence in March and final harvest at the end of September) about 290 mm were recorded (Fig. 1). During May and the first ten days of June only 34 mm of precipitation were recorded, followed by a period of 20 days with high recorded rainfall (120 mm) and then (July till the first ten days of September) the observed rainfall remained low (90mm; moderate irrigation period, see above).

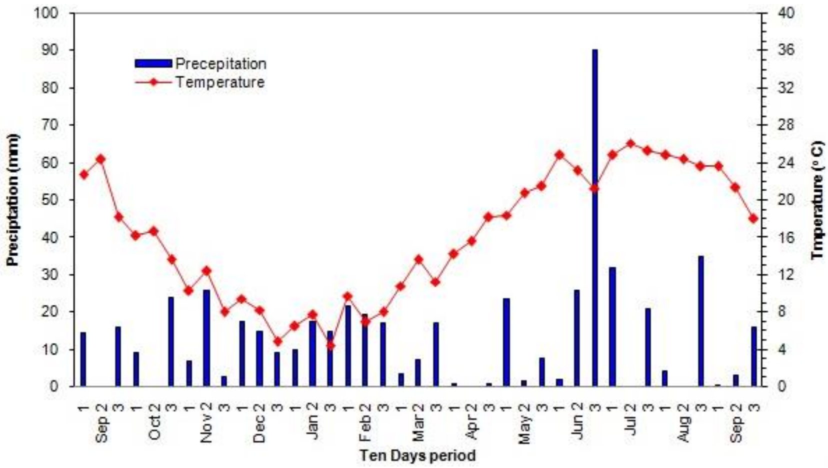

Figure 1. Mean 10-day air temperature and precipitation, recorded in the study area during the growing period of 2017–2018.

Yield

Switchgrass dry biomass yield was significantly affected ($P > 0.05$) by irrigation while nitrogen fertilization had no effect (Fig. 2). Dry yield was fluctuated between 9.34 to 14.23 t ha^{-1}, with the higher dry yield corresponds to the irrigated treatment without fertilization. This yield is lower than the reported yield of literature (Kimura et al., 2015; Collins et al., 2010; McLaughlin & Kszos, 2005) and almost the same (decreased by 2–2.5 t ha^{-1}) with the reported yield by Kimura et al. (2018), Nazli et al. (2018) and Boyer et al. (2013), although the switchgrass was in its eighth growing year. This fact proves that this crop retains its productivity even at an age that tends to embody its productive life cycle (≥ 10 growing years). However, due to the absence of groundwater table in such soils like the soil of the current site, biomass yields may remain at low levels even under (moderate) irrigation. As in these soils the effect of capillary rise is absent, 150 mm of irrigation water plus approximately 290 mm of rain (Fig. 1) comprise only 55% of the potential evapotranspiration.

In cardoon case, dry biomass yield was significantly affected only by nitrogen fertilization. The higher yield (21.31 t ha^{-1}) was found at rainfed treatments fertilized with 80 kg N ha^{-1}, while the lower (19.10 t ha^{-1}) for the irrigated unfertilized treatments.

The current results are generally in agreement with the available scientific literature from other studies in Mediterranean basin. Foti et al. (1999) reported a yield of about 18.8 t ha^{-1} in wild cardoon under low irrigation input (100 mm), while lower yields have been reported under rainfed conditions (Piscioneri et al., 2000; 10–15 t ha^{-1}). The dry biomass yields were equal with a 3–4 year average yield, reported by Mauromicale & Ierna (2004) and Raccuia & Melilli (2007) under low irrigation input (50 mm). Finally, the measured yield is in agreement with the dry biomass produced under supplementary irrigation (90 mm) that was reported by Vasilakoglou & Dhima (2014) in Central Greece, a site close to the study area. The difference with the previous reported study is that the cultivation was at the zenith of its productive life cycle whereas in the case of the present study cardoon was in the nadir, indicating that this crop retains its productivity even at an age close to the end of its productive life cycle (8–10 growing years).

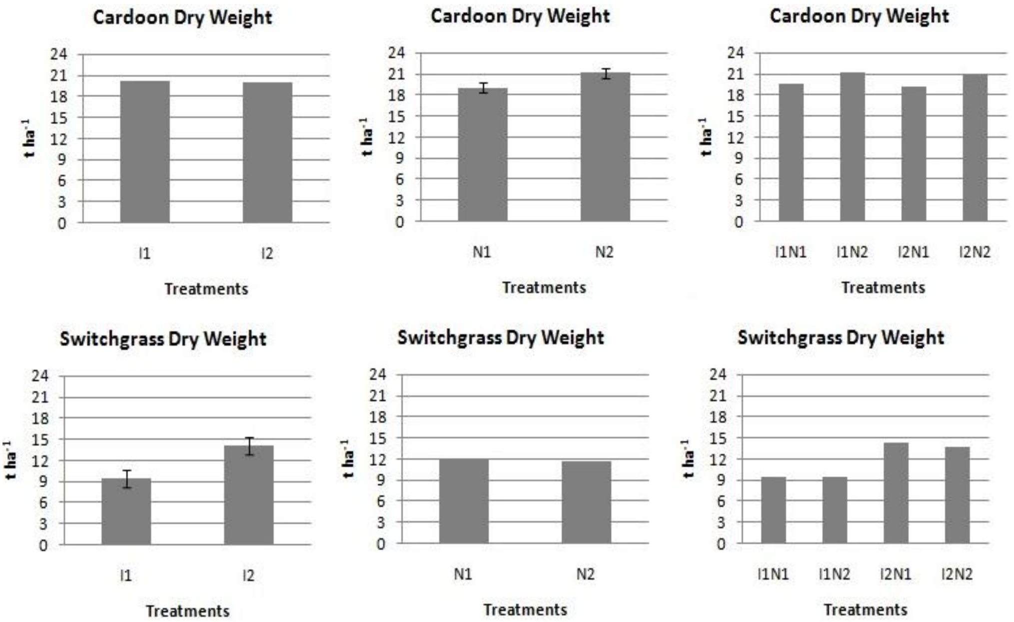

Figure 2. Effects of different irrigation (irrigated-150 mm and rainfed) and N-fertilization levels (0 and 80 kg ha^{-1}) on dry yield of the crops (cardoon upper graphs, switchgrass bottom graphs). $LSD_{0.05}$ is illustrated with (I) were it was found.

Calorific value

The energy content of biomass is determined by its calorific value. The calorific value is influenced by biomass elemental composition. Fig. 3 shows average GCV (MJ kg^{-1}) for all the samples of the studied treatments.

Only in irrigation case statistical significant differences of cardoon calorific values were found. The average values were 15.57 and 15.24 MJ kg^{-1} with the rainfed treatments having higher calorific values. In the case of nitrogen fertilization no significant differences were found. The above values are between the reported values for the cardoon pellets (14.8 MJ kg^{-1}, González et al., 2004; 17.3 MJ kg^{-1}, Christodoulou et al. 2014), the calorific value of cardoon biomass (without seeds) is 13.7 MJ kg^{-1} and the case that seeds are included in biomass 17.1–17.3 MJ kg^{-1} (Fernández et al., 2006; Grammelis et al., 2008).

On the other hand, in switchgrass case no significant differences were found for the tested irrigation and N-fertilization levels to the found biomass calorific values. The average calorific values were 17.29 and 17.26 MJ kg^{-1} for I1 and I2, respectively, while in the case of N-fertilization the average calorific values were 17.25 and 17.29 MJ kg^{-1} for N1 and N2, respectively, (Fig. 3). The above values are lower according to previous studies, where the average calorific value for switchgrass is reported to be 18.7 MJ kg^{-1} (Kludze et al., 2013) while it is the same with sorghum dry biomass calorific value (17.17 MJ kg^{-1}) that is reported by Pannacci & Bartolini (2018). The above found calorific value is a little bit lower compared to the calorific value of the most common hardwood species for energy production in Brazil (Chiteculo et al., 2018). It is reported that the calorific value of maize straw pellets ranged from 16.63 to 17.80 MJ kg^{-1} (Krizan et al., 2018) values similar with the switchgrass calorific value of the current study.

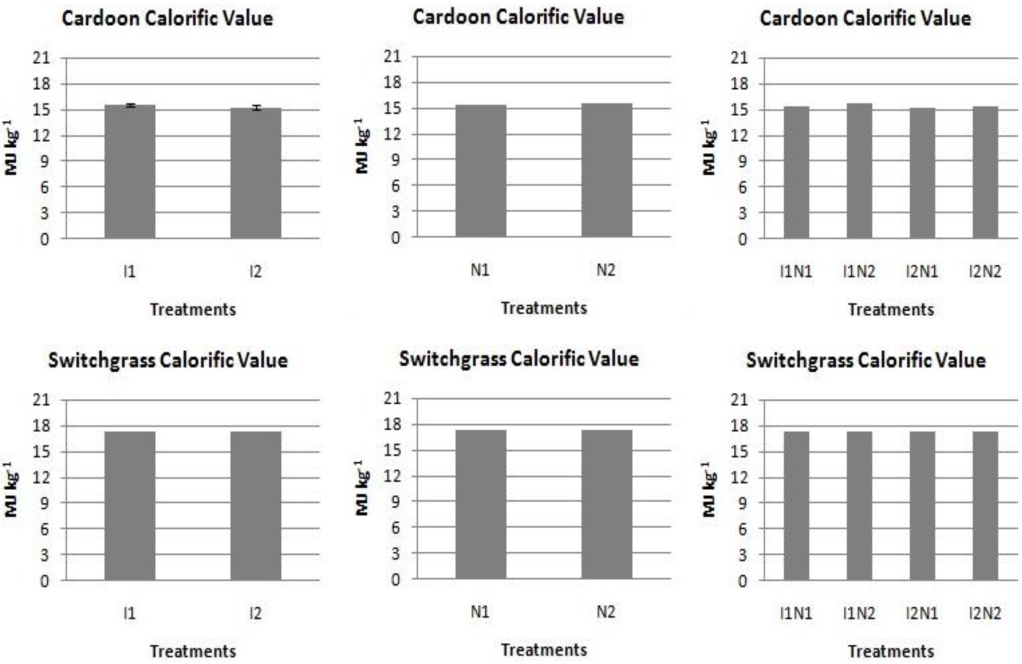

Figure 3. Effects of different irrigation (irrigated-150 mm and rainfed) and N-fertilization levels (0 and 80 kg ha^{-1}) on calorific value of the crops (cardoon upper graphs, switchgrass bottom graphs). $LSD_{0.05}$ is illustrated with (I) were it was found.

Multiplying the heating value by the dry yield of biomass produced in each treatment reflects the production of energy per hectare. As it is illustrated in Fig. 4, statistical significant differences were found for the nitrogen fertilization in case of cardoon (291 and 328 GJ ha^{-1} for the N1 and N2, respectively) and for the irrigation in switchgrass case (162 and 241 GJ ha^{-1} for the I1 and I2, respectively).

Furthermore, as it is illustrated in Fig. 4, it was found that cardoon in every case could produce higher energy per hectare (average 300 GJ ha^{-1}) than switchgrass (200 GJ ha^{-1}).

It has been reported that the annual energy produced by hectare is depending on the biomass type. There are many types of biomass that have been tested for their energy production per hectare, like wheat straw which can produce 123 GJ ha^{-1}, poplar (173–259 GJ ha^{-1}), SRC willow (187–280 GJ ha^{-1}) and coppiced willow chips (108 GJ ha^{-1}), miscanthus (222–555 GJ ha^{-1}), switchgrass which is reported to produce 139–150 GJ ha^{-1} and cardoon producing energy equal to 171–346 GJ ha^{-1} (Duffy & Nanhoue, 2002; McKendry P. 2002; Tharakan et al., 2005; Fernández et al., 2006; Grammelis et al., 2008). Therefore, it seems like both tested cultivation, cardoon and switchgrass, even at the end of their perennial life cycle they have the ability to produce large amounts of energy per hectare and makes them interesting solutions of low-input energy crops.

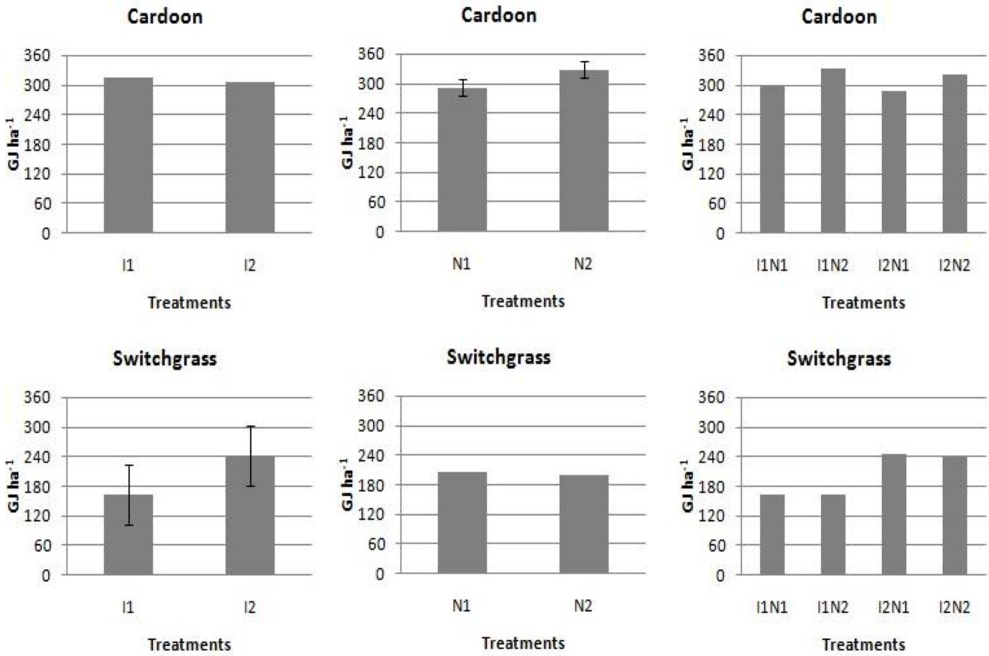

Figure 4. Effects of different irrigation (irrigated-150 mm and rainfed) and N-fertilization levels (0 and 80 kg ha^{-1}) on energy produced per hectare by the crops (cardoon upper graphs, switchgrass bottom graphs). $LSD_{0.05}$ is illustrated with (I) were it was found.

CONCLUSIONS

The main conclusion that may be drawn from this study is that even in their 8th growing year, cardoon and switchgrass can achieve remarkable dry biomass yield characterized of a high calorific value ranging from 15.4–17.3 MJ kg^{-1}. The energy produced by hectare is higher for cardoon cultivation than in switchgrass.

Furthermore, it was found that irrigation affected switchgrass dry biomass while in the case of cardoon dry biomass the higher nitrogen fertilization produced higher yield. Both cultivations can produce high amounts of dry biomass which can lead to high energy production. The differences to the calorific value are mainly due to different carbon content (main energy source) or the experimental site.

Therefore, depending on the experimental site (if there is or not the possibility to irrigate the cultivation) and the climatic conditions of the area, cardoon and switchgrass could achieve high amounts of energy per hectare, forming a promising solution for biomass production and their use in future cultivating scenarios for an environmentally friendly energy production should be seriously taken into consideration.

REFERENCES

Acda, M. & Devera, E. 2014. Note physico-chemical properties of wood pellets from forest residues, *J. Trop. For. Sci.* **26**(4), 589–595.

Amarasekara, A.S. 2014. *Handbook of Cellulosic Ethanol*. Department of Chemistry, Prairie View A&M University, Texas, Usa. Co-published by John Wiley & Sons, Inc Hoboken, New Jersey, and Scrivener Publishing LLC, Salem, Massachusetts. ISBN 978-1-118-23300-9.

Angelini, L.G., Ceccarini, L., Nassi o Di Nasso, N. & Bonari, E. 2009. Long-term evaluation of biomass production and quality of two cardoon (*Cynara cardunculus* L.) cultivars for energy use. *Biomass Bioenergy* **33**, 810–816.

Arshadi, M., Gref, R., Geladi, P., Dahlqvist, S. & Lestander, T. 2008. The influence of raw material characteristics on the industrial pelletizing process and pellet quality. *Fuel Process Technol.* **89**(12), 1442–1447.

ASAE Standards S319.3, 2001. Method of determining and expressing fineness of feed materials by sieving. St. Joseph, MI: American Society of Agricultural Engineers 573–576.

Barney, J.N., Mann, J.J., Kyser, G.B., Blumwald, E., Deynze, A.V. & DiTomaso, J.M., 2009. Tolerance of switchgrass to extreme soil moisture stress: ecological implications. *Plant Sci.* **177**, 724–732.

Boyer, C.N., Roberts, R.K., English, B.C., Tyler, D.D., Larson, J. & Mooney, D.F. 2013. Effects of soil type and landscape on yield and profit maximizing nitrogen rates for switchgrass production. *Biomass Bioenergy*, **48**, 33–42.

Carroll, J.P. & Finnan, J. 2012. Physical and chemical properties of pellets from energy crops and cereal straws. *Biosyst Eng.* **112**(2), 151–159.

Chiteculo, V., Brunerova, A., Surovy, P. & Brozek, M., 2018. Management of Brazilian hardwood species (Jatoba and Garapa) wood waste biomass utilization for energy production purposes. *Agronomy Research* **16**(2), 365–376.

Christodoulou, C., Tsekos, C., Tsalidis, G., Fantini, M., Panopoulos, K.D., de Jong, W. & Kakaras, E. 2014. Attempts on cardoon gasification in two different circulating fluidized beds, *Case Stud. Ther. Eng.* **4**, 42–52.

Christou, M., Mardikis, M. & Alexopoulou, E. 2001. Research on the Effect of Irrigation and Nitrogen upon Growth and Yields of Arundo donax L. in Greece. *Asp. Appl. Biol.* **65**, 47–55.

Clifton-Brown, J.C., Lewandowski, I., Andersson, B., Basch, G., Christian, D.G., Kjeldsen, J.B., Jorgensen, U., Mortensen, J.V., Riche, A.B., Schwarz, K.U., Tayebi, K. & Teixeira, F. 2001. Performance of 15 Miscanthus genotypes at five sites in Europe. *Agron. J.* **93**, 1013–1019.

Collins, H.P., Smith, J.L., Fransen, S., Alva, A.K., Kruger, C.E. & Granatstein, D.M. 2010. Carbon sequestration under irrigated switchgrass (*Panicum virgatum* L.) production, *Soil Sci. Soc. Am. J.* **74**, 2049–2058.

Cosentino, S.L., Scordia, D., Sanzone, E., Testa, G. & Copani, V. 2014. Response of giant reed (*Arundo donax* L.) to nitrogen fertilization and soil water availability in semiarid Mediterranean environment. *Eur. J. Agron.* **60**, 22–32.

Da Borso, F., Di Marco, C., Zuliani, F., Danuso, F. & Baldini M., 2018. Harvest time and ensilage suitability of giant reed and miscanthus for bio-methane production and characterization of digestate for agronomic use. *Agronomy Research* **16**(1), 22–40.

Danalatos, N.G, Archontoulis, S.V., Giannoulis, K. & Rosakis, S. 2006. Miscanthus and Cardoon as Alternative Energy Crops for Solid Fuel production in Greece, HAICTA 2006, International Conference on: *Information Systems in Sustainable Agriculture, Agroenvironment and Food Technology*, Volos, Greece, 20 – 23 September 2006, pp. 387−397.

Duffy, M.D. & Nanhoue, V.Y. 2002. Cost of Producing Switchgrass for Biomass in Southern Iowa. Trends in new crops and new uses. J. Janick and A. Whipkey. Alexandria, VA, ASHS Press.

Everard, C.D., McDonnell, K.P. & Fagan, C.C. 2012. Prediction of biomass gross calorific values using visible and near infrared spectroscopy, *Biomass Bioenergy* **45**, 203–211.

Fernandez, J. & Aguado, PL. 2006. Industrial applications of *Cynara cardunculus* L. for energy and other uses. *Industrial Crops and Products* **24**, 222–229.

Fernández, J., Curt, M.D. & Aguado, P.L. 2006. Industrial applications of *Cynara cardunculus* L. for energy and other uses. *Ind. Crop. Prod.* **24,** 222–229.

Fernández, J., Hidalgo, M., Del Monte, J.P. & Curt, M. 2005. *Cynara cardunculus* L. as a perennial crop for non-irrigated lands: yields and applications. *Acta Hortic.* **681**, 109–116.
Foti, S., Mauromicale, G., Raccuia, S.A., Fallico, B., Fanella, F. & Maccarone, E. 1999. Possible alternative utilization of Cynara spp. Part I. Biomass, grain yield and chemical composition of grain. *Ind. Crops Prod.* **10**, 219–228.
García, R., Pizarro, C., Lavín, A.G. & Bueno, J.L. 2104. Spanish biofuels heating value estimation. Part II: proximate analysis Data, *Fuel* **117**, 1139–1147.
Giannoulis, K.D. & Danalatos, N.G. 2014. Switchgrass (*Panicum virgatum* L.) nutrients use efficiency and uptake characteristics, and biomass yield for solid biofuel production under Mediterranean conditions. *Biomass Bioenergy* **68**, 24–31.
Giannoulis, K.D., Danalatos, N.G. & Sakellariou, M. 2011. Switchgrass, Cardoon and Miscanthus, perennial crops as Alternatives for solid biofuel production in central Greece. 19th European Biomass Conference & Exhibition**,** Berlin, Germany, June 2011, pp. 692–696.
Giannoulis, K.D., Karyotis, T., Sakellariou-Makrantonaki, M., Bastiaans, L., Struik, P.C. & Danalatos, N.G. 2016. Switchgrass biomass partitioning and growth characteristics under different management practices. *Wageningen Journal of Life Sciences* **78**, 61–67.
Gil, M.V., Oulego, P., Casal, M.D., Pevida, C., Pis, J.J. & Rubiera, F. 2010. Mechanical durability and combustion characteristics of pellets from biomass blends. *Bioresour Technol.* **101**(22), 8859–8867.
González, J.F., González-Garcı́a, C.M., Ramiro, A., González, J., Sabio, E., Gañán, J. & Rodrı́guez, M. 2004. Combustion optimisation of biomass residue pellets for domestic heating with a mural boiler, *Biomass Bioenergy* **27**, 145–154.
Grammelis, P., Malliopoulou, A., Basinas, P. & Danalatos, N.G. 2008. Cultivation and characterization of *Cynara cardunculus* for solid biofuels production in the Mediterranean region. *Int. J. Mol. Sci* .**9**, 1241–1258.
IKA®-WERKE C 5000 control/duo control, 2004. Operating instructions. Ver. 09 02.04
Kimura, E., Collins, H.P. & Fransen, S. 2015. Biomass production and nutrient removal by switchgrass under irrigation, *Agron. J.* **107**, 204–210.
Kimura, E., Fransen, S.C., Collins, H.P., Stantond, B.J., Himes, A., Smith, J., Guyg, S.O. & Johnston, W.J. 2018. Effect of intercropping hybrid poplar and switchgrass on biomass yield, forage quality, and land use efficiency for bioenergy production. *Biomass and Bioenergy* **111**, 31–38.
Kludze, H., Deen, B. & Dutta, A. 2013. Impact of agronomic treatments on fuel characteristics of herbaceous biomass for combustion. *Fuel Processing Technology* **109**, 96–102.
Kosov, V.V., Sinelshchikov, V.A., Sytchev, G.A. & Zaichenko, V.M. 2014. Effect of torrefaction on properties of solid granulated fuel of different biomass types, *High Temp.* **52**, 907–912.
Krizan, M., Kristof, K., Angelovic, M., Jobbagy, J. & Urbanovicova, O. 2018. Energy potential of densified biomass from maize straw in form of pellets and briquettes. *Agronomy Research* **16**(2), 474–482.
Kunecova, D. & Hlavač, P. 2019. Determination of activation energy of the pellets and sawdust using thermal analysis. *Agronomy Research* **17**(6), 2306–2316.
Li, H., Chen, Q., Zhang, X., Finney, K.N., Sharifi, V.N. & Swithenbank, J. 2012. Evaluation of a biomass drying process using waste heat from process industries: a case study, *Appl. Therm. Eng.* **35**, 71–80.
Liu, Z., Fei, B., Jiang, Z. & Liu, X. 2014. Combustion characteristics of bamboo-biochars, *Bioresour. Technol.* **167**, 94–99.
Mauromicale, G. & Ierna, A. 2004. Biomass and grain yield in *Cynara cardunculus* L: genotypes grown in a permanent crop with low input. *Acta Hortic*. **660**, 593–597.
McKendry, P. 2002. Energy production from biomass (part 1): overview of biomass. *Bioresource Technology* **83**, 37–46.

McLaughlin, S.B. & Kszos, L.A. 2005. Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States, *Biomass Bioenergy* **28**, 515–535.
Nazli, R.I., Tansi, V., Öztürk, H.H. & Kusvuran, A. 2018. Miscanthus, switchgrass, giant reed, and bulbous canary grass as potential bioenergy crops in a semi-arid Mediterranean environment. *Industrial Crops & Products* **125**, 9–23.
Nishiguchi, S. & Tabata, T. 2016. Assessment of social, economic, and environmental aspects of woody biomass energy utilization: direct burning and wood pellets, *Renew. Sustain. Energy Rev*. **57**, 1279–1286.
Pannacci, E. & Bartolini, S. 2018. Effect of nitrogen fertilization on sorghum for biomass production. *Agronomy Research* **16**(5), 2146–2155.
Petrini, C., Bazzocchi, R., Bonari, E., Ercoli, L. & Masoni, A. 1996. Effect of irrigation and nitrogen supply on biomass production from Miscanthus in Northern-Central Italy. *Agricoltura Mediterranea* **126**, 275–284.
Piscioneri, I., Sharma, N., Baviello, G. & Orlandini, S. 2000. Promising industrial energy crop, *Cynara cardunculus*: a potential source for biomass productiona nd alternative energy. *Energy Convers. Manage.* **41**, 1091–1105.
Pizzi, A., Toscano, G., Pedretti, E.F., Duca, D., Rossini, G., Mengarelli, C., llari, A., Renzi, A. & Mancini, M. 2018. Energy characteristic assessment of olive pomace by means of FT-NIR spectroscopy, *Energy* **147**, 51–58.
Posom, J., Shrestha, A., Saechua, W. & Sirisomboon, P. 2016. Rapid non-destructive evaluation of moisture content and higher heating value of *Leucaena leucocephala* pellets using near infrared spectroscopy, *Energy* **107**, 464–472.
Preece, S.L.M., Auvermann, B.W., MacDonald, J.C. & Morgan, C.L.S. 2013. Predicting the heating value of solid manure with visible and near-infrared spectroscopy, *Fuel* **106**, 712–717.
Qin, X., Mohan, T., El-Halwagi, M., Cornforth, G. & McCarl, B.A. 2006. Switchgrass as an alternate feedstock for power generation: an integrated environmental, energy and economic life-cycle assessment, *Clean Technol. Environ. Policy* **8**, 233–249.
Raccuia, S.A. & Melilli, M.G. 2004. Cynara cardunculus L., a potential source of inulin in Mediterranean environment: screening of genetic variability. *Aust. J. Agric. Res.* **55**, 693–698.
Steel, R.G.D. & Torrie, J.H. 1982. Principles and Procedures of Statistics. A Biometrical Approach, 2nd ed., McGraw-Hill, Inc., 633.
Tharakan, P.J., Volk, T.A., Lindsey, C.A., Abrahamson, L.P. & White, E.H. 2005. Evaluating the impact of three incentive programs on the economics of cofiring willow biomass with coal in New York State. *Energy Policy* **33**, 337–347.
Torgrip, R.J.O. & Fernandez-Cano, V. 2017. Rapid X-ray based determination of moisture-, ash content and heating value of three biofuel assortments, *Biomass Bioenergy* **98**, 161–171.
Toscano, G., Duca, D., Amato, A. & Pizzi, A. 2014. Emission from realistic utilization of wood pellet stove. *Energy* **68**(0), 644–650.
Toscano, G., Duca, D., Foppa Pedretti, E., Pizzi, A., Rossini, G., Mengarelli, C. & Mancini, M. 2016. Investigation of woodchip quality: relationship between the most important chemical and physical parameters, *Energy* **106**, 38–44.
USDA (Soil Survey Staff), 1975. *Soil Taxonomy. Basic System of Soil Classification for Making and Interpreting Soil Surveys*. Agricultural Handbook, vol. 466, USDA, Washington, DC, 754 pp.
Vamvuka, D., Topouzi, V. & Sfakiotakis, S. 2010. Evaluation of production yield and thermal processing of switchgrass as a bioenergy crop for the Mediterranean region. *Fuel Proc. Technol.* **91**, 988–996.
Vasilakoglou, I. & Dhima, K. 2014. Potential of two cardoon varieties to producebiomass and oil under reduced irrigation and weed control inputs. *Biomass Bioenergy* **63**, 177–186.
Zema, D.A., Bombino, G., Andiloro, S. & Zimbone, S.M. 2012. Irrigation of energy crops with urban wastewater: effects on biomass yields, soils and heating values. *Agric. Water Manag.* **115**, 55–65.

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Comparative environmental analysis of soil sampling methods in precision agriculture for lime application in Paraná State, Brazil

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Abstract. Precision agriculture (PA) provides techniques that favour the localized application of inputs allowing their rational use. This makes the PA a potential indicator of reduced operational costs, input volume, and environmental impacts. The objective of this study was to evaluate and compare the environmental effects of three different sampling methods used in PA for the lime application. The first sampling method evaluated was the grid sampling (GS). It was performed at a density of one sample per hectare in a 100×100 m georeferenced grid. The second method was the directed sampling, that was performed after defining the management zones by soil apparent electrical conductivity (ECa) using a soil electrical conductivity sensor. The lest sampling method was the Altitude-based management zone (AMZ) sampling that was developed based on altitude maps of the field. These sampling methods were tested in three different areas in the south of Brazil. This study evaluated the spatial variability of the lime volume in the soil and compared quantitatively and spatially the recommended application volumes achieved by each sampling method. Results highlighted that the sensor-directed soil sampling method was the alternative that would generate the lowest environmental impact.

Key words: environmental impact, management zones, sample grids, soil apparent electrical conductivity.

INTRODUCTION

Liming is a common and well-established practise in many agricultural Brazilians regions where is generally made on a single composite sample unit representing supposedly homogeneous areas of ten or more hectares (Hurtado et al., 2009). Thus, uniform lime applications can result in areas with levels below or above what is necessary, damaging crop development and/or negatively impacting the environment.

Brazil is a tropical country that has typically acid soils and with high aluminium content which is toxic and influences directly on the nutrients available for crops (Rodriguero et al., 2015). One of the ways to correct this problem is to promote liming since it is a natural, low-cost and widely available input (Rodriguero et al., 2015; Melo et al., 2019).

Applying variable soil amendment rates based on precision agriculture (PA) is an alternative for enhancing production and minimizing the impact of agricultural activity on the environment (Oliveira et al., 2008; Temizel, 2016). For this purpose, the spatial variabilities of soil attributes must be characterized by different soil sampling strategies that can represent such variations (Bottega et al., 2013).

The most commonly used soil sampling methods to identify soil properties in PA include grid sampling (GS) and management zone sampling (Ragagnin et al., 2010). In GS sampling method, the area is divided into square or rectangular sections of equal or reduced size so farmers can sample each section (Morgan & Ess, 1997; Zinkevičius, 2008). In management zone sampling, an area is divided into subareas or homogeneous areas to apply uniform input doses (Prado et al., 2015). These management zones can be obtained via relief, productivity, and soil attribute maps (Alves et al., 2013; Bernardi et al., 2015).

Several studies have compared the quality and optimal size of different sampling grids and management zones (Peralta et al., 2015; Tripathi et al., 2015; Ferraz et al., 2017; Ferraz et al., 2018). However, according to Keskin & Sekerli (2016), soil sampling methods are an important part of the PA it is, sometimes, not well known and corrected used. So, it is interesting to carry out studies to understand better the soil sampling methods.

Lombardo et al. (2018) and Bambi et al. (2019) stated that rural areas are facing different challenges, and according to Barbari et al. (2014a, 2014b) and Conti et al. (2017) it is needed to use technologies with less environmental impact and more sustainable. According to Kanal (2004), agronomic practices need to be better understand to be more environmentally friendly. The soil sampling methods based on PA technologies are used to determine the amount of product to be applied to the soil. The incorrect application rate of products can result in the increased cost of fertilizers and correctives, reduction of crop growth and also negative environmental effects (Šima et al., 2013; Ferraz et al., 2019). So, regarding environmental analysis, gaps in knowledge must be filled to demonstrate and consolidate the positive environmental impacts of PA soil sampling technologies.

Therefore, this study evaluated and compared the environmental effects of three sampling methods used to apply lime in PA.

MATERIALS AND METHODS

Study site

The present study was conducted at Cupim Farm (25°32’66” South and 51°34’65” West) and Juquiá Farm (25°16’45” South and 52°6’01” West) in the municipalities of Guarapuava and Cantagalo, respectively, in the central-southern region of the state of Paraná, Brazil. The experiments were conducted in three distinct areas of the farms: A1

(Cupim Farm, 154.82 ha), A2 (Cupim Farm, Jordãozinho, 18.64 ha), and A3 (Juquiá Farm, 62.63 ha) (Fig. 1).

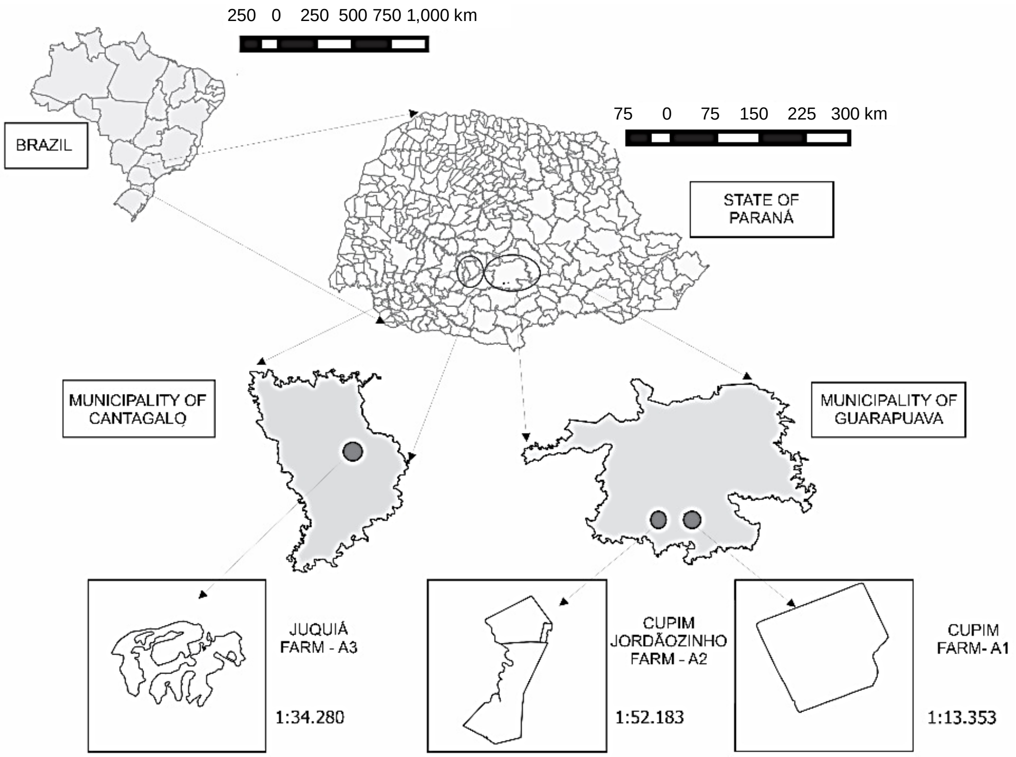

Figure 1. The geographical location of study areas.

The climate in Guarapuava and Cantagalo is Köppen classification type Cfa, characterized as subtropical humid, with a mean annual temperature of 18 °C, a maximum temperature of 36° C, and minimum temperature of 6.8 °C. The experimental area soils were classified as cambic aluminic dark latosol (per the Brazilian Soil Classification), with prominent A horizon, smooth undulating relief, and basalt substrate and a textural class varying from clayey to very clayey (Fontoura et al., 2015).

In these areas crop rotation has been the management method performed for at least 16 years, with rotational planting of soybeans, oats, corn, wheat, and barley. Seedings are performed twice yearly in June through November, with harvests in November, February, and March.

Grid sampling

In the GS method, the sampling grids were defined as 100×100 m, (1 point per hectare), using the sampling grid generation tool in the software, SMS Advanced (Advanced Spatial Management System), version 15.1 (AgLeader Technology, 2019). Thus, 158 georeferenced sampling points were obtained for area A1, 23 points for area A2, and 65 points for area A3 (Fig. 2).

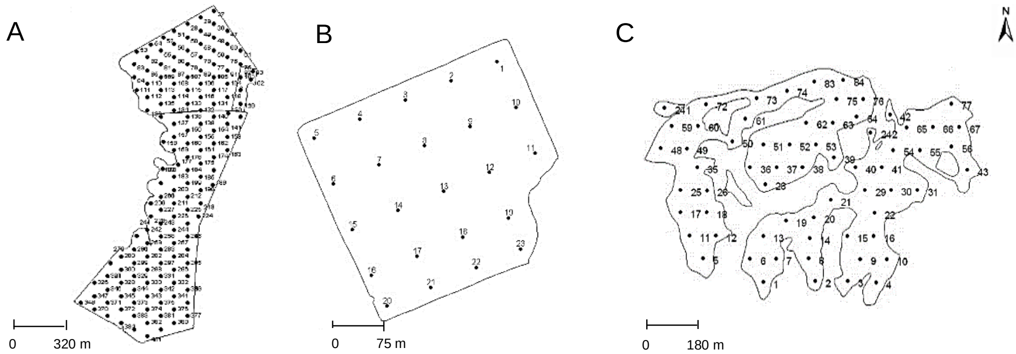

Figure 2. Representation of the areas A1 (A), A2 (B), and A3 (C) sampling grids.

After defining the sampling grids, from 11 to 15 subsamples were collected at each georeferenced point and duly homogenized, forming a composite sample representative of the sampling point.

Directed soil sampling (DS)

For the DS method, the apparent electrical conductivity (ECa) of the soil was obtained using a Veris PMC® electrical conductivity sensor coupled to an agricultural tractor. The ECa sampling density for each sampling area was 200 to 320 points per hectare. After collection, outliers were removed from the dataset using the standard deviation (SD) method, i.e. data with values above or below 2.5 SD were removed. Subsequently, the data were interpolated using the punctual kriging method in Vesper 1.6. Besides, experimental semivariograms were constructed for each point, which was automatically interpolated by the software to generate spatial variability maps of the ECa data obtained by the sensor to direct the soil sample collection (Fig. 3).

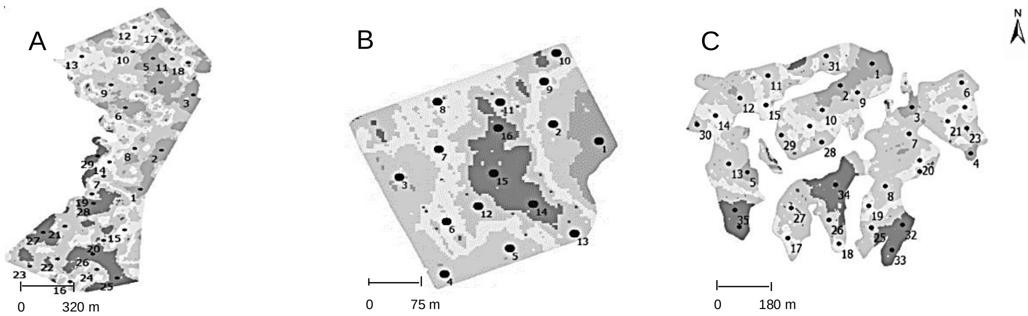

Figure 3. Directioning of collection points by management zone for areas A1 (A), A2 (B), and A3 (C).

Once the management zones were defined, samples were collected in a zigzag pattern in each zone (Silva et al., 2015). A variable number from 11 to 15 subsamples were collected and homogenized, yielding composite samples representing each zone.

Altitude-based management zone (AMZ) sampling

The AMZs for the areas were obtained by harvest maps and interpolated with the punctual kriging method using Vesper 1.6. After kriging, the maps were divided into three altitude classes high, medium and low respectively to define the collection classes. Thus, no fixed scale was defined, since each area was evaluated by its degree of inclination, and three zones were defined. Generating and interpreting these maps enabled identifying areas with the same altimetric profile to collect the samples (Fig. 4).

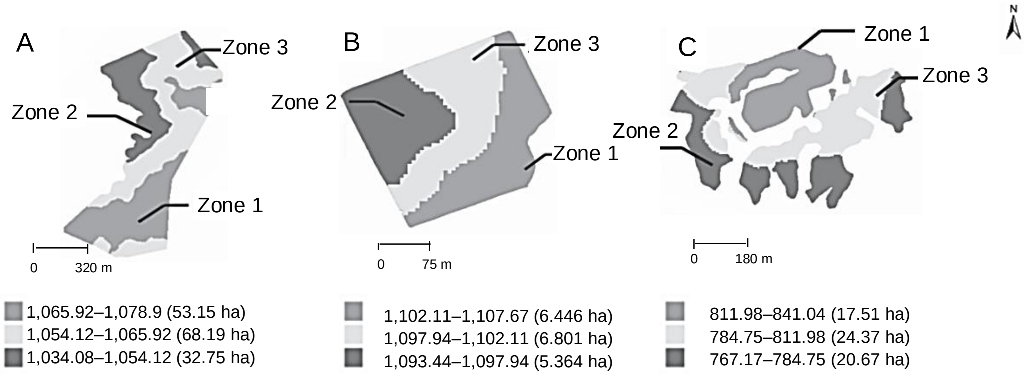

Figure 4. Representation of the altitude-based management zones of areas A1 (A), A2 (B), and A3 (C).

After creating the maps, the same procedure for the DS method was followed. Specifically, from11 to 15 subsamples were collected in a zigzag pattern in each zone (Silva et al., 2015), which were homogenized to form a composite sample representing the area being studied.

Chemical analyses and liming recommendations

Soil chemicals properties from layer ranging from 0.0–0.2 m deep were analyzed. The lime dose recommendations applied for each method followed the methodology proposed by EMBRAPA (1997). This procedure was used for all three sampling methods (AMZ, GS, and DS).

According to (Fontoura et al., 2015) the liming application needs to ensure a greater residual effect, which means, greater number of years in which the acidity levels of the soil will be above the values determined as critical (4,9 for pH-CaCl and 60% of V). It is indicated that the desired base saturation value in liming should be 70% (equivalent to pH CaCl of 5.2), according to Eq. 1:

$$N/C(\text{t ha}^{-1}) = [(V2 - V1) \text{ x } CTC_{ph7} \text{ x } f] / 100 \qquad (1)$$

where NC – liming requirement, in t ha^{-1}, for the soil layer 0–20 cm; V2 – value that you want to increase base saturation (in this case, 70%); V1 – base saturation value obtained by the soil analysis; f – correction factor based on limestone PRNT (f – 100/PRNT).

According to Fontoura et al. (2015), the application of lime, to be carried out in the doses calculated by the Eq. 1, must be carried out on the soil surface, without the incorporation of the lime by the ploughing and harrowing. The same authors infer that the effects of this lime application can be observed in the correction of pH and base saturation in the first year after liming and up to 60 cm in depth.

Recommendation maps

The chemical recommendation maps were generated based on the laboratory analysis results obtained from the sampling methods. This step provided the amount of lime associated with the levels. These results were input and compiled using SMS Advanced (Advanced Spatial Management System), versions 15.1 (AgLeader Technology, 2019), to generate the recommendation/application maps. Each map shows interpretation ranges on per cent scales of lime dosage distribution by colour. Thus, correlating the colours with the percentages enables spatially differentiating the dosages. The interpolator used for the GSs was kriging. The semivariograms were constructed using GS+ 7.0 software (Dalchiavon et al., 2012; Dalchiavon et al., 2013; Dalchiavon et al., 2015), and the interpolations were performed using SSToolBox 4.0 (SST Development Group, Stillwater, OK, USA) (Molin & Faulin, 2013; Sana et al., 2014).

RESULTS AND DISCUSSION

Analyzing the liming recommendation maps for area A1 (Fig. 5) obtained from the three sampling methods revealed low spatial variability for the lime doses in the AMZ and DS methods compared with the GS method. Larger lime doses were recommended for the AMZ and GS methods (Figs 5, A and 5, B), ranging from 981.63–1,392.04 kg ha^{-1} and 1,392.04–1,802.45 kg ha^{-1}, respectively, with a more uniform spatial distribution in these ranges in the AMZ method. For the DS method (Fig. 5, C), the most recommended lime doses ranged from 0–571.22 kg ha^{-1} and 571.22–981.63 kg ha^{-1}.

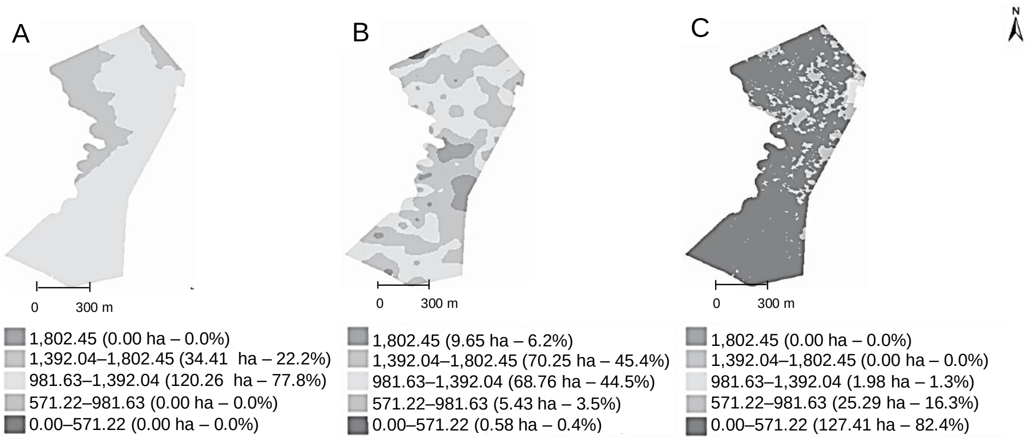

Figure 5. Liming recommendation maps of area A1 obtained by altitude-based management zone sampling (A), grid sampling (B), and directed sampling (C).

For the whole lime to be applied in area A1, the DS method presented the lowest value per specific area with 154 t. The AMZ and GS methods yielded similar values of 213 and 219 t, respectively. Therefore, environmentally evaluating the three methods showed that DS was a more viable alternative compared with the AMZ and GS sampling methods, which may permit excess limings of 59 t for AMZ and 65 t for GS.

Fig. 6 shows the liming recommendation maps for area A2. Similar to area A1, the lime doses presented low spatial variability for the AMZ and DS methods relative to the GS method.

Like in the area A1, the most recommended lime dosages produced by the AMZ and GS methods (Figs 6, A and 6, B) ranged from 981.63–1,392.04 kg ha^{-1} and 1,392.04–1,802.45 kg ha^{-1}, respectively, with a more uniform spatial distribution in these ranges for the AMZ method. In contrast, the opposite was observed for the DS method, with most liming recommendations in the range of 1802.45 kg ha^{-1} and no recommendations in the range of 0–571.22 kg ha^{-1} (Fig. 6, C).

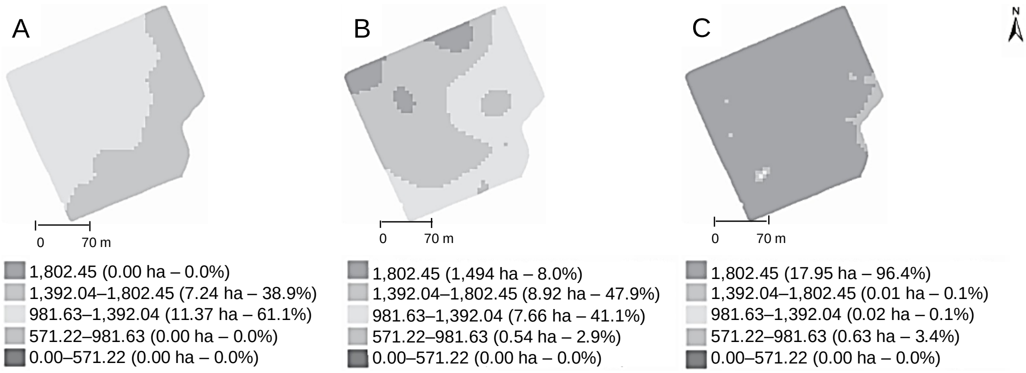

Figure 6. Liming recommendation maps of area A2 obtained via altitude-based management zone sampling (A), grid sampling (B), and directed sampling (C).

The total recommended a lime application for area A2 for both the GS and AMZ methods was 27 t area^{-1}. For the DS method, the total application was 40 t area^{-1}. Thus, based on a general comparison of the methods and considering the environmental impact, the DS method is a less viable alternative, since it indicated a smaller spatial distribution of lime in the area and recommended a lime volume of 13 t area^{-1} higher than that recommended by the AMZ and GS methods. In its turn, the GS method is the much more achievable viable environmental alternative because, although it recommended the same total lime application as the AMZ method, it indicated a more varied lime spatial distribution.

In contrast to areas A1 and A2, the recommendation maps for area A3 (Fig. 7) showed high spatial variability in the lime rates using the GS and DS sampling methods, with emphasis on the GS method (Fig. 7, B). In this method, similar proportions of recommendations were observed in the five dosage ranges. For the DS method, most recommendations ranged from 0–837.07 kg ha^{-1} and 1,952.06–2,733.98 kg ha^{-1} (Fig. 7, C). For the AMZ method, most recommendations ranged from 837.07–1,356.59 kg ha^{-1} and 1,356.59–1,952.01 kg ha^{-1} (Fig. 7, A).

For the total application of lime to area A3, the DS method showed a lower recommendation at 74 t area^{-1}, evidencing a possible excess recommendation of 34 t for the AMZ method and 40 t for the GS method. Besides, the DS method presented a better spatial distribution, thus being a more viable environmental alternative.

Per Ribeiro et al. (1999), the amount of lime to apply depends on the soil analysis, which may prevent overdosage. These authors indicate that excessive liming is as harmful as high acidity, which may make soil corrections more difficult by precipitating several soil nutrients, such as P, and inducing a higher predisposition to damage the physical properties of the soil. This finding highlights that aspects related to liming must be considered.

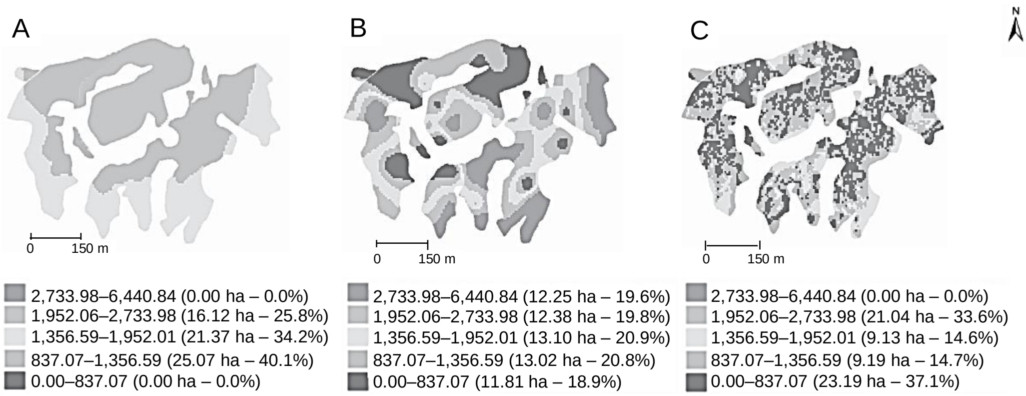

Figure 7. Liming recommendation maps of area A3 obtained via altitude-based management zone sampling (A), grid sampling (B), and directed sampling (C).

Moreover, although lime is not a fertilizer but rather functions to correct pH, its excess dosage may indirectly affect the environment, for example, by facilitating herbicide leaching. Refatti et al. (2014) assessed the effect of liming on leaching the herbicides, imazethapyr, and imazapyr and found that the soil is the final destination of most herbicides and that these herbicides increase groundwater contamination after a lime overdosage due to the increased pH.

In addition to the aspects mentioned above, lime applications using PA methods can bring economic benefits. Schadeck (2015), found that when fixed-rate and varied-rate lime applications were compared in soybean, oat, and wheat cultivation areas in the city of Santo Ângelo, Rio Grande do Sul, Brazil, there was a 43% reduction in the use of lime.

Thus, these previous findings on lime application corroborate this study, since, in addition to the possibility of reducing costs using localized dosages, soil contamination with other nutrients or chemical products that may act in association with the overliming conditions may be prevented.

CONCLUSIONS

The environmental effects of AMZ sampling, GS, and directed sampling by soil ECa methods were compared.

When comparing the recommendations by sampling method, differences were identified among the lime dosages that each method suggested, thus indicating the need to improve soil sampling techniques, because, regardless of the technology chosen, uncertainty will remain regarding deficits or overdosages in input applications.

When the environmental effects were evaluated, the ECa sensor-directed soil sampling method was found to be the alternative that would have fewer environmental impacts as a function of the applied lime volume.

REFERENCES

Ag Leader. 2019. Spatial Management System - SMS, version 15.1. Ag Leader: Ames, IA.

Alves, S.M.F., Alcantara, G.R., Reis, E.F., Queiroz, D.M. & Valente, D.S.M. 2013. Definition of management zones from maps of electrical conductivity and organic matter. *Bioscience Journal* **29**(1), 104–114 (in Portuguese).

Bambi, G., Iacobelli, S., Rossi, G., Pellegrini, P. & Barbari, M. 2019. Rural tourism to promote territories along the ancient roads of communication: case study of the rediscovery of the St. Francis's ways between Florence and La Verna. *European Countryside* **11**(3), 462–474.

Barbari, M., Monti, M., Rossi, G., Simonini, S. & Sorbetti Guerri, F. 2014b. Simple methods and tools to determine the mechanical strength of adobe in rural areas. *Journal of Food, Agriculture & Environment* **12**(2), 904–909.

Barbari, M., Monti, M., Rossi, G., Simonini, S. & Sorbetti-Guerri, F. 2014a. Proposal for a simple method of structural calculation for ordinary earthen buildings in rural areas. *Journal of Food, Agriculture & Environment* **12**(2), 897–903.

Bernardi, A.C.C., Bettiol, G.M., Andrade, R.G., Rabello, L.M. & Inamasu, R.Y. 2015. Precision agriculture tools as aid to soil fertility management. *Cadernos de Ciência & Tecnologia* **32**(1/2), 211–227 (in Portuguese).

Bottega, E.L., Queiroz, D.M., Pinto, F.A.C. & Souza, C.M.A. 2013. Spatial variability of soil attributes in no a no-tillage system with crop rotation in the brazilian savannah. *Revista Ciência Agronômica* **44**(1), 1–9 (in Portuguese).

Conti, L., Goli, G., Monti, M., Pellegrini, P., Rossi, G. & Barbari, M. 2017. Simplified method for the characterization of rectangular straw bales (RSB) thermal conductivity. *In IOP Conference Series: Materials Science and Engineering*. Prague, Czech Republic, pp. 052035.

Dalchiavon, F.C., Carvalho, M.P., Andreotti, M. & Montanari, R. 2012. Spatial variability of the fertility attributes of Dystropheric Red Latosol under a no-tillage system. *Revista Ciência Agronômica* **43**(3), 453–461 (in Portuguese).

Dalchiavon, F.C., Carvalho, M.P., Montanari, R., Andreotti, M. & Dal Bem, E.A. 2013. Sugarcane trash management assessed by the interaction of yield with soil properties. *Revista Brasileira de Ciência do Solo* **37**(6), 1709–1719.

Dalchiavon, F.C., Montanari, R., Andreotti, M., Dallacort, R. & Souza, M.F.P. 2015. Relationship between sunflower productivity and soil's chemical properties by geostatistical techniques. *African Journal of Agricultural Research* **10**(35), 3525–3532.

EMBRAPA - Empresa Brasileira De Pesquisa Agropecuária. 1997. *Soil analysis methods manual*. EMBRAPA, Rio de Janeiro, 212 pp. (in Portuguese).

Ferraz, G.A.S., Avelar, R.C., Bento, N.L., Souza, F.R., Ferraz, P.F.P., Damasceno, F.A. & Barbari, M. 2019. Spatial variability of soil fertility attributes and productivity in a coffee crop farm. *Agronomy Research* **17**(4), 1630–1638. https://doi.org/10.15159/AR.19.142

Ferraz, G.A.S., Oliveira, M.S., Silva, F.M., Avelar, R.C., Silva, F.C. & Ferraz, P.F.P. 2017. Methodology to determine the soil sampling grid for precision agriculture in a coffee field. *Dyna* **84**(200), 316–325.

Ferraz, G.A.S., Oliveira, M.S., Silva, F.M., Sales, R.S. & Carvalho, L.C.C 2018. Plant sampling grid determination in precision agriculture in coffee field. *Coffee Science* **13**(1), 112–121 (in Portuguese).

Fontoura, S.M.V., Vieira, R.C.B., Bayer, C., Viero, F., Anghinoni, I. & Moraes, R.P. 2015 *Soil fertility and its management in no-tillage system in South Center of Paraná*. Fundação Agrária de Pesquisa Agropecuária, Guarapuava, 146 pp. (in Portuguese).

Hurtado, S.M.C., Silva, C.A., Resende, Á.V.D., Von Pinho, R.G., Inácio, E.D.S.B. & Higashikawa, F.S. 2009. Spatial variability of soil acidity attributes and the spatialization of liming requirement for corn. *Ciência e Agrotecnologia* **33**(5), 1351–1359.

Kanal, A. 2004. Effects of fertilisation and edaphic properties on soil-associated Collembola in crop rotation. *Agronomy Research* **2**(2), 153–168.

Keskin, M. & Sekerli, Y.E. 2016. Awareness and adoption of precision agriculture in the Cukurova region of Turkey. *Agronomy Research* **14**, 1307–1320.

Lombardo, S., Sarri, D., Vieri, M. & Baracco, G. 2018. Proposal for spaces of agrotechnology co-generation in marginal areas. *Atti Della Societa Toscana Di Scienze Naturali, Memorie Serie B* **125**, 19–24.

Melo, R.M., Vieira, M.C., Carnevali, T.O, Gonçalves, W.V., Torales, E.P, Tolouei, S.E.L, Santos, C.C. 2019. Liming and substrate texture affect the development of Campomanesia adamantium (Cambess.) O. Berg. *Revista de Ciências Agrárias* 2019, **42**(1), 99–108 (in Portuguese).

Molin, J.P. & Faulin, G.D.C. 2013. Spatial and temporal variability of soil electrical conductivity related to soil moisture. *Scientia Agrícola* **70**(1), 1–5.

Morgan, M. & Ess, D. 1997. *The precision farming guide for agriculturists*. Moline, Deere, 117 pp.

Oliveira, R.B., Lima, J.S.S., Xavier, A.C., Passos, R.R., Silva, A.S. & Silva, A.F. 2008. Comparison between soil sampling methods for conilon coffee liming and fertilization recommendation. *Engenharia Agrícola* **28**(1), 176–186 (in Portuguese).

Peralta, N.R., Costa, J.L., Balzarini, M., Franco, M.C., Córdoba, M. & Bullock, D. 2015. Delineation of management zones to improve nitrogen management of wheat. *Computers and Electronics in Agriculture* **110**, 103–113.

Prado, E.V., Machado, T.A. & Prado, F.M.T. 2015. Management zone generation and correlation using spad sensor and soil-seed electrical conductivity for irrigated in coffee growing in the Zona da Mata Mineira. *Revista Científica Eletrônica de Agronomia*, **1**, 1–7 (in Portuguese).

Ragagnin, V.A., Júnior, D.G.S. & Silveira Neto, A.N. 2010. Recommendation of liming at variable rates under different sampling intensities. *Revista Brasileira de Engenharia Agrícola e Ambiental* **14**(6), 600–607.

Refatti, J.P., Avila, L.A., Agostinetto, D., Manica-Berto, R., Bundt, A.D.C. & Elgueira, D.B. 2014. Efeito da calagem na lixiviação de imazethapyr e imazapyr em solo de cultivo de arroz irrigado. *Ciência Rural* **44**(6), 1008–1014.

Ribeiro, A.C., Guimarães, P.T.G. & Alvarez, V.V.H. 1999. *5ª Approach – Recommmendations for the use of correctives and fertilizes in Minas Gerais*. Comissão de Fertilidade do Solo do Estado de Minas Gerais, Viçosa, 359 pp. (in Portuguese).

Rodriguero, M.B, Barth, G. & Caires, E.F. 2015. Surface application of lime with different Magnesium contents and particle sizes under a no-till system. *R. Bras. Ci. Solo*, **39**, 1723–1736, (in Portuguese).

Sana, R.S., Anghinoni, I., Brandão, Z.N. & Holzschuh, M.J. 2014. Spatial variability of soil physical-chemical attributes and their effects on cotton yield. *Revista Brasileira de Engenharia Agrícola e Ambiental* **18**(10), 994–1002.

Schadeck, F.A. 2015. *Soil fertility and technical feasibility – economic of precision agriculture in the region of Missions - RS*. Dissertation, Universidade Federal de Santa Maria, 48 pp. (in Portuguese).

Silva, S.F., Mendes, D.F., Quarto Junior, P., Lima, W.L., Rangel, O.J.P. & Ferrari, J.L. 2015. Spatial variability of soil chemical attributes under pasture. *Vértices* 17, 25–37 (in Portuguese).

Šima, T., Nozdrovický, L., Krištof, K., Dubeňová, M. & Krupička, J. 2013. Effect of the nitrogen fertiliser rate on the nitrous oxide flux from haplic luvisol soil in the laboratory experiment. *Agronomy Research* **11**(1), 97–102.

Temizel, K.E. 2016. Mapping of some soil properties due to precision irrigation in agriculture. *Agronomy Research* **14**(3), 959–966.

Tripathi, R., Nayak, A.K., Shahid, M., Lal, B., Gautam, P., Raja, R., Mohanty, S., Kumar, A., Panda, B.B. & Sahoo, R.N. 2015. Delineation of soil management zones for a rice cultivated area in eastern India using fuzzy clustering. *Catena* **133**, 128–136.

Zinkevičius, R. 2008. Influence of soil sampling for precision fertilizing. *Agronomy Research* **6**, 423–429.

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Improvement of the elk domestication technology at Sumarokovsky State Nature Reserve

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Abstract. The paper discusses the technology of elk domestication and measures for its improvement at Sumarokovsky state nature reserve, the largest world center for the domestication of elks, as well as an environmental, research, cultural and educational institution. The article contains the data on ethological observations, the evolution of elk behavior in the conditions of ecological tourism, on elk keeping and on the conditions of the food base, on technological processes and suggests possible ways to correct them. It has the description of the organizational structure of the reserve, of the forestlands around and of their forage capacity; gives information about the vegetation composition, the mode of keeping and feeding elks. The reserve, due to its presence in a specially protected area, experiences significant restrictions on forest use on its territory, which leads to a deterioration and decrease in the area and quality of forage land for domesticated elks. The article shows the necessity of improving the regulatory framework as well as of the development and implementation of biotechnological measures to preserve and increase the forage capacity of frestland. It also shows that the technological chain of domestication makes it possible to get a controlled, stress-resistant, calm, friendly and safe animal, to carry out the selection period for the formation of dairy herds earlier, to obtain unique milk with both high nutritional and medicinal properties. Considering the fact that the largest number of domesticated elks in the world is concentrated in the reserve, the technology there is unique. Because of the potential danger of an elk as a source of human infection with various pathogens, the veterinary service requires intensification. It includes monitoring, development of treatment methods, drug application, prevention and control measures for diseases, provision of normative documents, etc. A change in the elk domestication technology under the increasing role of ecological tourism has led to the formation of a new economical type of a reserve, which combines elements of a stationary-exit, multidisciplinary and enclosure types.

Key words: domestication, elk, elk farm.

INTRODUCTION

First attempts to domesticate elks date back to ancient times, as evidenced by cave paintings in the valleys of Siberian Rivers. The information about it appeared later in the Baltic countries, Scandinavia and other regions. However, they were random in nature. Purposeful work in this direction began in the last century in Russia. The first experiments on domestication of elks started in the Moscow Zoo by P.A. Manteifel, and later were continued in the Serpukhov experimental hunting farm. L.G. Kaplanov and S.N. Popov carried out a similar work in Western Siberia. The work of E.P. Knorre from 1937 to 1941 is also of great importance. In the reserve ‘Buzuluksky Bor’ he first introduced the system of keeping elks on free pasture

The biological and economic features of the European elk are unique. They allow considering this animal as promising for the livestock sector. The moose has a significant weight (up to 500 kg), early maturity, multiple fertility, unpretentiousness, accessibility to a wide range of rough branch feed and forest forbs. A variety of products can be obtained from elks - meat, skin, horns, antlers for the extraction of pantocrine (Baranov et al., 2010; Sokolov, 2012). At first glance, such products are more economically viable when hunting for wild elks, and can be obtained in larger volumes. Products from domesticated elks are very expensive, and their output is very small (Sokolov, 2012). This suggests the unprofitability of elk farming, economic insolvency, and the unjustified costs of domestication of the animal. However, elk domestication is determined by the uniqueness of the main product received from it - milk, which is now also applied in humane medicine. In addition, the elk itself is an object of scientific research and, largely, of meeting the needs of human communication with nature. It has led to the formation of a new direction - ecological tourism.

Elk breeding as an industry in Russia has two directions. The first is the organization of elk farms in the Gorky, Yaroslavl, Ivanovo and other regions according to the principle of traditional livestock farming with a concentration of livestock in limited areas. Limited areas help to reduce the cost of production. The second direction is the formation of elk farms as an integral part of the scientific units: Pechoro-Ilychsky reserve and the elk farm of the Kostroma state regional agricultural station (currently division of 'Sumarokovskiy' state nature reserve) (Smirnov, 2015).

Four types represent elk farms focused on industrial livestock breeding. The stationary multidisciplinary type exploits specific territories based on the long-term use of a compound feed base. The stationary exiting type has stationary objects, permanent fodder territories, where elk calves move from summer to winter camps. Forest-nomadic type is designed for a year-round use of the forage base, using portable hedges, and cutting down elks of all ages in groups. Enclosure type is a purely tourist option that does not have industrial significance due to the high cost of production (Baranov et al., 2010).

In all cases, elk breeding technology has a high concentration of animals in limited areas, which leads to depletion of the food supply, violation of the hygienic standards, and insufficient supply of natural feed. Farms also need additional supply with imported animals, which raises the cost of production. Among the difficulties, there is also intensification of the work of the veterinary service due to animals' metabolic disorders, surgical pathologies, and massive diseases of the newborn. Attempts to expand the territories of fodder resources by allowing free grazing outside or by creating giant pens will lead to a decrease in the efficiency of elk domestication. Animals prone to vagrancy may leave the farm and, as a result, the farm will have a loss of a domesticated livestock due to predators - wolves and poachers (Baranov, 2015).

E. Knorre laid the scientific basis for the implementation of elk domestication of the second direction in 1949 on the elk farm of Pechora-Ilychsky Reserve in the Komi Autonomous Soviet Socialist Republic. He showed the possibility of elk domestication in conditions created by human and developed methods for imprinting elk calves at the tutor and their further manual feeding. E. Knorre paid considerable attention to environmental issues, behavior, physiology, feeding, milk productivity and the quality of elk milk as well as to the development of methods for the prevention and treatment of diseases of various etiologies (Knorre, 1959, 1961; Kozhukhov, 1973). Further experiments on the elk domestication continued in the Sumarokovsky elk farm in the Kostroma region. The work of Mikhailov, Dzhurovich, Vitakova, Baranov, Sokolov and other employees made a significant contribution to the study of the elk biology: behavior, reproduction, morphology. They determined individual physiological and biological indicators, developed machine milking of elks, methods of preserving milk, etc. They developed and implemented in practice the technology of rearing the young animals and keeping the adult, including: imprinting the newborn calves to humans; overexposure of the newborn calves for 2–6 weeks in boxes and then up to 6 months of age in a summer camp, their further transfer to the winter camp when they get one-year-old; keeping in corrals grazing and milking. (Mikhailov, 1973; Dzhurovich et al., 1984; Smirnov, 2015).

In recent years, the elk domestication technology has changed again. The aim of this research is to analyze the new technology and to identify possible ways of its improvement in the reserve.

MATERIALS AND METHODS

The studies were carried out in 2017–2019 at Sumarokovsky nature reserve, which is an experienced elk farm, an elk sanctuary and a forest site. The object of the study is the European elk (Alces alces L).

The research consisted in observation of elks (over 1,200 hours). In total 146 animals took part in it: 57 elk cows; 63 calves (32 females, 21 males); 15 adolescents of first year (12 females, 3 males) and 11 adolescents of second year (8 females, 3 males). Studies included ethological observations, studying the evolution of the behavioral foundations of domesticated elks of various age groups, assessing the food supply, conditions, technological processes and possible ways to correct them.

Currently, the behavior of elks in the farm is accessed by visual observations, by the analysis of photo and video materials, taking into account aggressive and 'friendly' actions to a person. As the reserve is at the same time a tourist object, the main attention is paid to collecting materials on the behavior of animals in the presence of tourists. A calf gets excluded from the list of applicants to the milking herd if, when being visited by a group of tourists in the contact pen, it shows excessive aggressiveness (butting, attacking a person, etc.) or, conversely, avoided communication, is shy when crowding a group of people, runs away, etc. Credulity, curiosity, nonfearfulness, lack of aggression towards humans are regarded as positive criteria during the selection for the milking herd.

Elks on the farm are monitored by radio-tagging according to Minaev & Purikov (2015). Similarly to the IQRF-monitoring system of cattle (Hartova & Hart, 2018), the method allows to determine the location of animals with an accuracy of several meters. The methods are equal, but in first case the data transmission is carried out in a more economical mode and the equipment can operate in unlicensed frequency ranges.The paper has some archival materials of the reserve as well.

RESULTS AND DISCUSSION

The reserve is an environmental protection center, a unique research center and an educational institution, contributing to the formation of a new direction - ecological tourism. The boundary borders of the reserve include an area of 40,391 ha. The area of the reserve itself is 36,176 hectares, including forestry lands – 9,989.2 hectares and agricultural lands – 26,186.8 hectares. The lands located in the district borders of the reserve, but not included in it count 4,215 hectares. Large forests cover about 52% of the territory. The farmland is interspersed with coppices, clumps of shrubs with the dominance of willow, birch, and alder.

On the territory of the reserve there are 86 settlements (the village of Gridino, the village of Bogovarovo, the village of Sumarokovo, Kharitonovo, Khalipino, etc.), and the land near them is used for grazing. This makes the contact between elks and domestic livestock, and vice versa possible, which to a certain extent determines infectious diseases among them. Invasive diseases are of particular danger. Their hosts are lynxes, wolves, bears, and especially dogs that use slaughter and other wastes for food.

In spring (during calving), the number of elks varies between 40–50 heads, and by winter it decreases to 25–30 due to the sale of young animals. Over the observation period, the reserve obtained 63 calves and sold 71% of them to other reserves and zoos.

Elk farming can be successfully developed only in regions with a sufficient supply and diversity of fodder plants. As Baranov et al. (2015; 2010) say, the fodder land should be accessible to elks, protected from adverse climatic conditions, bloodsucking insects and predators. Remote inaccessible forest areas are the most consistent with these requirements. They are less susceptible to anthropogenic impact and have maximum protective properties. Sumarokovsky elk farm belongs to the group of so-called mosaic lands represented by forests around the regional center, which are protected with fields, meadows and other cultural areas. They are a subject to significant anthropogenic influences and require constant protection from poachers, as well as from packs of feral dogs. Because of this, the farm annually loses 2–3 elk individuals. The forestlands correspond to the III class of bonitet, with the number of about three elk individuals per 100 ha (IV class of bonitet has a critical density of elks of 7–10 animals per specified area) (Baranov et al., 2010 and Shabrov, 2015).

The vegetation composition in the area of the elk farm is represented by 231 species, incl. 89 species used by elks as feed. Animals prefer soft tree species, especially aspen according to Brough et al. (2017), as well as willow, birch, mountain ash, bird cherry, etc., less readily - spruce. The diets may also include aquatic and semi-aquatic plants – codfish, water lilies, horsetail growing on cutting areas – fireweed, sorrel, umbellate, and mushroom, berries, lingberry, and blueberry branches closer to autumn.

Although the elk is a food flexible animal, and with a lack of feed it can switch to food with relatively new plants, there is still a need to feed them. This question is especially acute when concentrating the entire herd in a winter camp, when elks can use only branches and aspen bark for food (independently, only during thaws). In this regard, young calves and milking queens are fed according to the season of the year during the daytime with green grass (fireweed, meadowsweet, clover, etc.), branch feed of aspen, willow, birch etc., freshly cut aspen bark, steamed oats, etc. In winter, the camp feeding grounds are equipped for these purposes with a cut material, which requires additional processing for sanding aspen logs. Largely it increases the cost of production.

Because of the long-term use of enclosures, elks do not leave the camp for a long time and the food supply is greatly depleted. Restriction of forest use on the territory of the reserve due to entering the recreation zone of Kostroma leads to a deterioration of forestland for elk domestication. The territories with overgrown plantings get bigger (Shabrov, 2015). In this regard, it is necessary to improve the regulatory work aimed at the development and implementation of biotechnological measures to preserve and increase the forage capacity of forestland, taking into account the interests of the forestry and the reserve. As one of the options, this could be the creation of large forage pens for the formation of a forage base for grazing and feeding elks with annual alternation. It is also possible to resolve the issue of engaging the forests of the reserve for harvesting wood in a planned manner, using felling material for elk feed, but followed by forest inventory (planting willow, aspen, shrubs and other fodder plants).

Taking into account elk groups, different modes of keeping and feeding animals were developed at the elk farm: a box-pavilion mode (for newborns where they are kept with the mothers for several hours and for calves up to 3–6 weeks of age), summer and winter camp-pasture modes, a free pasture mode and a corral mode. The analysis showed that the main technological technique is the organization of elk keeping in natural conditions.

Below there is a diagram of elk keeping depending on the age and calendar time with a more detailed description of the techniques used in this case.

Farm modes based on the age and calendar time:

1. Newborn elks (birth time is the end of April - beginning of May) - within 3–4 hours after birth with the mother, and then weaned.

2. After weaning from the mother until June 1 (up to 3–6 weeks of age) - a veranda with a canopy and a limited paddock for walking (‘nursery’ with the access only to service personnel in replaceable clothes).

3. From June 1 to mid-January (from 3–6 weeks to 8–9 months of age) - ‘kindergarten’ for young animals, from 10:00 to 15:00 - in the pen, from 15:00 to morning - grazing.

4. From mid-January to mid-April (from 8–9 - to 11–12 months of age) - in the winter camp.

5. From mid-April to mid-January (from 12 to 21 months of age) - adolescents (one year old) are kept in the pen.

6. From mid-January to mid-April (from 21 to 24 months of age) - teenagers (one and a half year old) are kept in a winter camp.

7. From mid-April to August- September (age from 24 to 29 months) - teenagers (two years old) are kept in the pen. Selective - free grazing.

8. From August - September to mid-April (elks and female teenagers 2.5 years of age) are in the summer-winter camp, free-range.

9. Pregnant animals (queens, two-year-old adolescents) two weeks before calving (mainly in mid-April) are in the maternity pad, after calving (three days after the birth) - from 8:00–10:00 to 18:00 in the milking pad (May-August), from 18:00 to 8:00–10:00

- free range. From September until the first half of January - free-range in the summer-autumn camp. From the second half of January up to mid-April - in the winter camp.

As can be seen from the scheme (item 9), mainly in mid-April, all adult and young elk queens that can potentially bring offspring (usually teenage females of sufficient fatness and development at the age of 2 years) are temporarily placed in a maternity shelter with an area of about 4 ha. Before giving birth (in a few hours, days) a pregnant elk cow usually begins to worry - it tries to find a way out of the corral in order to bring offspring away from everyone. Neither before nor after childbirth, she lets anyone in, protecting the future calf. Employees (milking technologists) are waiting for the onset of labor, during which the elk's attention is dulled and people can calmly approach her to attend the birth. This allows them to fall into the so-called 'inner circle'. Milkmaids take a calf and give it a suck on the udder, the first servings of colostrum. After 3–4 hours (leaving elk calves for a longer period with the mother is impractical for domestication) the calf is hidden from the mother and taken away from the paddock and transferred for further care to the caregiver (baby rearing technologist and research assistant) in the 'nursery'. The milkmaid immediately returns to the elk and continues to milk it and let it sniff and lick the traces of the afterbirth from her hands. According to these criteria (appearance at the time of birth, the presence of the smell of a newborn) the elk captures the person as cubs. Having accepted the milkmaid for a calf, an elk cow remembers her personal smell, voice, appearance and even gait and later will distinguish this employee from others.

The first stage of domestication is imprinting. It consists in developing a newborn calf after weaning the imprint of the educator instead of the elk cow (Sokolov, 2012). The foundations of the future attitude of the elk to humans start from the first feeding from the nipple drinkers and at least five times daily (up to 1.5–2 liters).

Weaned elk calves live in a separate box up to 6 days of age, and then move to an enclosure, where they are taught to eat green feed, red clay (a natural source of minerals). (Figs 2, 3).

Figure 1. Teaching calves to the nipple.

Figure 2. Teaching calves to green feed.

Figure 3. Teaching calves to additional mineral forage.

At the age of one month, when they begin to consume green food actively (branches of trees and shrubs, grass), table salt is introduced into the diet, and the assembled elk milk is replaced with a substitute for whole milk, which is drunk from a separate bowl.

On June, 1 elk calves move to a summer camp ('kindergarten') - a barn equipped with feeders and drinking bowls. During this period, combining paddock content with grazing, they develop the skills of submission to a person during grazing, learn to follow a person, get skills of behavior in a group (Figs 4, 5).

Figure 4. Calves in the summer camp.

Figure 5. Obedience to man (following the tutor).

This educational scheme consists in focusing elk calves on one person (as a disadvantage), so when they meet strangers this may lead to an inadequate response (from fear to aggression).

The involvement of ecotourism has led to a change in the technological process of raising elk calves (earlier contact with strangers and targeted selection of elk calves to form a dairy herd), as well as to a change in the behavior of domesticated young animals.

Guided tours in small groups started to visit the farm in 1997/98. Every year, the flow of tourists increased due to individual visitors. So in 2000, 750 people visited the reserve, in 2014 about 40 thousand people, and in 2019 more than 50 thousand people. At the same time, the form of their communication with elks has changed. At first, visitors were admitted to the elks without any hedge, accompanied by specialists. Subsequently, taking into account the safety requirements, a tourist zone was allocated where adult animals can be fed through the hedge (Elokhina & Elokhin, 2015).

Currently, tourists are allowed to communicate with elk cubs from 1.5 months of age (they can go into the pen, but it is forbidden to touch and feed the animals). From 2 months before moving to a winter camp tourists can touch, but cannot feed. (Fig. 6, 7)

Figure 6. Contact with tourists.

Figure 7. Calves in the winter camp.

This technique causes significant changes in the behavior of the young animals. Being in the tourist's attention zone, elk calves become less aggressive, less afraid of a large number of people, screams, camera flashes, noises and traffic (domesticated elks do not get into car accidents), i.e. they become more socialized. This allowed farm employees to evaluate the behavior of young animals in relation to humans, and to conduct an earlier selection for the formation of dairy herds. Observations established that already in the first six months of life people can determine the nature of the calf without economically expending it for growing up to 2–3 years (at this age, the elk gives its first offspring).

Along with this, elks become more stress-resistant. Before some of them reacted negatively to any stimulus by a decrease in milk yield, refused milking, and did not arrive to the farm. This showed the importance of making a change in the milking technology. Before the staff was not recommended to talk during milking, now it is desirable.

Currently, all elk farms are divided into four groups according to the type of technological processes: stationary multidisciplinary, stationary exiting, forest-nomadic and enclosure. Sumarokovsky elk farm previously related to the second type, but now does not fit into any of them. In this regard, we can talk about the formation of a new, fifth type, combining the characteristics of the second (the presence of the main stationary objects, a constant feeding area, the annual movement of elks in summer and winter camps) with the fourth (cage-driven). To a certain extent, this necessitated changes in the process. Earlier the transfer of animals to the winter camp was carried out in November, but now in connection with tourism, in the second half of January. To some extent, this affected the environmental situation in the pens, and increased the additional costs of procuring the imported feed.

Taking into consideration the positive impact of human communication with elks, some drawbacks should be noted in this case: isolated cases of hypertrophic attention to a person (increased interest, importunity). Some animals show anxiety and even loss of appetite, periodic overeating with unnatural feeds, in particular carrots, and, very significantly, there is an increased risk of the presence of pathogens of infectious diseases that are also dangerous for humans.

A variety of products can be obtained from elks, and the main one is milk. By its qualities, it significantly exceeds the cow milk as well as milk obtained from other types of domestic animals. Compared with cow milk it is by 6–7% higher in protein and fat (fluctuations in fat from 7.91% to 13.65%, protein - from 7.66% to 10.95%) and has a lesser extent of casein and globulin (Baranov et al., 2013). In the cow milk, these indicators range from 3.77% to 4.75% (casein) and from 3.14% to 3.75% (globulin), in goat milk - from 3.09% to 5.04% (casein) and 2.74% to 3.96% (globulin) (Michlová et al., 2016; Tatar et al., 2015). The highest level of fat in the milk during lactation for 2017/19 was in July, as evidenced by the data in the Table 1.

Table 1. Fat content in elk milk in 2017/2019 (%)

No.	Month	Fat content (%)			M ± m
		2017	2018	2019	
1	June	9.7	8.0	7.2	8.39 ± 0.74
2	July	13.3	12.2	9.2	11.57 ± 1.23
3	August	10.5	10.2	10.8	10.50 ± 0.17*

Note: * – $P < 0.05$ compared to June.

The use of natural feeds also contributes significantly to the increase of calcium, phosphorus, magnesium and some trace elements. P. Stypinski (2011) analyzed the data of many authors on the influence of forage pastures on the quality and quantity of milk. The data showed that the presence of grass with a large botanical diversity contributes to the production of milk with a higher content of fatty acids and antioxidants that are beneficial to human health. All this significantly increases its nutritional value, and in combination with a pronounced bacteriostatic and bactericidal action due to the high lysozyme content of 70–80,[3] (Savin & Sokolov, 2015), it opens the way for using it in human medicine in the treatment of radiation sickness, stomach ulcer and other gastrointestinal diseases. In practice, this is implemented in the health resort of Ivan Susanin in Kostroma region.

During the lactation period (from May to September) elk cows give from 134.3 to 302.5 liters of milk. Over the past 3 years the farm has received 7,382.8 liters of milk. Milking elks is carried out manually using the same technology as cows (Ugodskaya & Sokolov, 2015). The milking process itself has several features. It is carried out inside the milking hangar, located in the pen of the same name and divided into several sections. Availability of premises is necessary to separate elk cows from each other and to avoid conflicts between them for a milkmaid, who is simultaneously recognized as an "elk calf" for several elk cows. In one room, as a rule, they milk up to two elks at a time. The elk cows stand in a special machine, where they are also fed a treat. Due to the high growth of the animal and the small size of the udder (even during lactation the mammary gland along with the nipples protrudes only a few centimeters from the abdominal line) employees milk either standing up or slightly leaning on the longitudinal poles of the machines. Several technologists practice milking elk cows at the same time (no more than four). At this time, the entrance to the premises of other personnel is extremely undesirable. Since the elk cow considered the milkmaid to be her calf, she will not only give milk, but also protect her like a real elk calf.

A characteristic feature of technology in recent years is that between morning and evening milking, animals stay in the pen, and not released for grazing. This allowed to reduce the number of passes ('no shows') for milking in 2017/2019 by 27.2%; 9.8%; 12.7%, respectively, compared to 2014, and to increase the average annual milk yield from 180.9 liters in 2017 to 197.6 liters and 205.9 liters in subsequent years. Along with this, the new technology made it possible to normalize the work schedule of milkmaids. Before early morning milking was in the period from 7 to 12 o'clock in the morning, evening milking was from 18 o'clock until the last elk cow arrived. This necessitated the payment of overtime work, which reached 5–7 extra days a month. The regulation of evening milking from 6 p.m. to 9 p.m. avoided this, made it possible to reduce labor costs and the cost of milk. Thus, it can be assumed that the new milking technology influenced the production of milk according to Cielava L. et al. (2017) who argued that milk production directly depended on the system of keeping and feeding animals.

Work with adult males due to their absence on the farm for the past seven years was not carried out.

Considering the technology of elk domestication in the reserve at the present stage, we believe that the issue of imprinting should be considered somewhat more broadly. In addition to the effect of imprinting, 'self-studies' play a large role in the socialization of elk calves with an increasing flow of tourists. At the same time animals also 'analyzing' the situation, understand that the presence of a person next to them creates a favorable forage base (top dressing with branches of trees, shrubs, grass, steamed crushed oats). In addition, near people it is less likely to meet predators. As a result, this leads to a loss of migration and to the development of elk attachment to one territory. A similar point of view belongs to R. Found et al (2019).

Considering the above, it can be assumed that at the same time as the elk milkmaid is perceived as a calf, and a calf perceives a caregiver as a mother, animals also receive additional information about the favorable environment (good food base that a human creates, the absence of predators), which also contributes to that animals remain in the reserve.

One of the main problems in elk breeding are the diseases of various etiology. They appear because of the high concentration of elks in a limited area, because of the content of animals of different ages in the boxes, because of the inevitable undermining of the natural food supply when sanitary and hygiene rules are not followed, because of the contact with livestock, because of the uniformity of the imported feed when keeping animals in the pens, and because of apparent inactivity of elks. According to Baranov et al., (2015), metabolic disorders and the birth of non-viable offspring take place, and the incidence of newborn calves has reached up to 50%. Also 62% of the elk population is infected with helminthes (Okunev et al., 2012). The current research showed that elk calves up to 20 days of age had diseases, accompanied by diarrheal and respiratory syndromes. Injuries to organs and tissues of various origins were quite widespread in the reserve too. Diseases of the hooves (wounds of the sole, phlegmon of the corolla, deformation of the hoofed horn) were also a relatively common phenomenon. This issue requires further discussion. Because of the potential danger of moose as a possible source of human infection with various pathogens, it is necessary to improve the system of veterinary service for elk breeding (monitoring, development of treatment methods, prevention and control measures for diseases of various etiologies, drugs and their application schemes, ensuring events normative documents, etc.)

CONCLUSIONS

1. Sumarokovsky state nature reserve is the world's largest center for domestication of elks, an environmental research and education institution.

2. The widespread introduction of ecological tourism in the elk domestication technology has contributed to the formation of a new type of elk farming, combining the elements of a stationary-exiting and multidisciplinary.

3. The technological process of growing young animals in the conditions of ecological tourism contributed to:

- changes in the behavior of elk calves, which made it possible to get controlled, stress-resistant, calm animals with a positive reaction to strangers at an early age;
- the possibility of conducting targeted selection at the age of 4–5 months for the formation of dairy herds, which can help to avoid the cost of growing an unpromising breeding stock;
- an increase in the tourist season by three months (from June until first half of January instead of from June until first half of November) and in the number of visits (from 40000 visitors in 2014 to 56000/65000 visitors in 2018/2019, respectively).

4. The reserve is the only farm in the world that receives a unique product from elks - milk. Optimization of elk milking technology (their content in the pen between morning and evening milking, free grazing only at night) contributed to:

- a decrease in the number of ‘no shows’ for milking in 2017–2019 by 27.2%, 9.8% and 12.7%, respectively, compared with 2014;
- the positive dynamics of an increase in the average annual milk yield per head from 180.9 liters in 2017 to 197.6/205.9 liters in 2018/2019;
- rationing the working time of milkmaids and reducing the cost of paying overtime hours, which before reached 5–7 extra working days per month.

5. Restriction of forest use on the territory of the reserve leads to a deterioration, decrease in the area and quality of forage land for a herd of domesticated elks. In this regard, it is necessary to improve the regulatory framework aimed at the development and implementation of biotechnological measures taking into account the interests of the forestry and the reserve.

6. Because of the potential danger of elks as possible sources of human infection with various pathogens, it is necessary to develop new diagnostic methods as well as measures of their prevention and control.

REFERENCES

Baranov, A.V., Sokolov, N.V., Sokolov, A.N. & Sitnikova, O.N. 2015. Feed base for elks. *Salmon farming: problems, searches, solutions. Interregional scientific-practical conference.* Kostroma, pp. 42–47 (in Russian).

Baranov, A.V., Sokolov, N.V., Sokolov, A.N. & Ugodskaya, E.K. 2013. *Otsenka molochnoy productivnosti odomashnivaemih losih* [Milk Productivity Assessment of domesticated elks]. Kostroma, 26 pp. (in Russian).

Baranov, A.V., Sokolov, N.V., Dzhurovich, V.M., Kudryashov, D.I., Khramskaya, K.G. & Okunev, I.S. 2010. *Tehnologiya organizatsii losevedcheskih hosyaistv* [Technology for the organization of elk farms]. Kostroma, 46 pp. (in Russian).

Brough, A.M., DeRose, R.J., Conner, M.M. & Long, J.N. 2017. Summer-fall home-range fidelity of female elk in northwestern Colorado: Implications aspen management. *Forest Ecology and Management* **389**, 220–227. https://doi.org/10.1016/j.foreco.2016.11.034

Cielava, L., Jonkus, D. & Paura, L. 2017. Lifetime milk productivity and quality in farms with different housing and feeding systems. *Agronomy Research* **15**(2), 369–375. https://doi.org/10.15159/AR.18.067

Dzhurovich, V.M., Vitakova, A.N., Mikhailov, A.P., Anokhina, P.K., Bogomolova, E.M. & Kurochkin, Yu.A. 1984. *Metodicheskie recomendatsii po virashivaniyu molodnyaka losey, soderzhaniyu i doeniyu losih* [Guidelines for the cultivation of young elks, the content and milking elks]. Kostroma, 27 pp. (in Russian)

Elokhina, A.V. & Elokhin, M.D. 2015. Change in the behavior of elks at Sumarokovo elk farm under the influence of tourist groups. *Salvation: problems, searches, solutions. Interregional scientific and practical conference.* Kostroma, pp. 133–136 (in Russian).

Found, R. & Cassady, C. 2019. Influences of Personality on Ungulate Migration and Management. *Frontiers in Ecology and Evolution* **7** https://www.frontiersin.org/articles/10.3389/fevo.2019.00438/full

Hartova, V. & Hart, J. 2018. Improvement of monitoring of cattle in outdoor enclosure using IQRF technology. *Agronomy Research* **16**(2), 410–415. https://doi.org/10.15159/AR.18.067

Knorre, E.P. 1961. Results and prospects of domestication of an elk. *Proceedings of the Pechoro-Ilychsky state reserve*, pp. 5–120 (in Russian).

Knorre, E.P. 1959. Ecology of an elk. *Proceedings of the Pechora-Ilychsky State Reserve*, pp. 5–120 (in Russian)

Kozhukhov, M.V. 1973. The results of twenty years of experimental work on the domestication of elks in Pechora-Ilychsky reserve. *Domestication of an elk*. Moscow, pp. 17–27 (in Russian).

Michlová, T., Dragounová, H., Seydlová, R. & Hejtmánková, A. 2016. The hygienic and nutritional quality of milk from Saanen goats bred in the Moravian-Silesian region. *Agronomy Research* **14**(S2), 1396–1406 https://doi.org/10.15159/AR.18.067

Minaev, A.N. & Purikov, A.V. 2015. Modern means of radio tracking of domesticated elks. *Salvation: problems, searches, solutions. Interregional scientific and practical conference.* Kostroma, pp. 140–152 (in Russian).

Mikhailov, A.P. 1973. The main tasks and the first results of experimental work on the domestication of elks at the Kostroma agricultural experimental station. *Domestication of an elk*. Moscow, 28–34 (in Russian).
Okunev, I.S., Koroleva, S.N., Gafurova, O.O. & Lapina, T.I. 2012. Parasites of elks at the Kostroma elk farm. *Veterinary pathology*. **1**. p. 123–126 (in Russian)
Savin, A.E. & Sokolov, A.N. 2015. Elk milk as a therapeutic product. *Crop farming: problems, searches, solutions Interregional scientific and practical conference*. Kostroma, pp. 15–19 (in Russian).
Shabrov, F.A. 2015. On the implementation of measures to increase the feed capacity of forestland for providing elks in the Sumarokovo elk farm. *Crop farming: problems, searches, solutions Interregional scientific and practical conference.* Kostroma, pp. 57–64 (in Russian).
Smirnov, A.V. 2015. The history of elk farming in Russia. *Crop farming: problems, searches, solutions. Interregional Scientific and Practical Conference.* Kostroma, pp. 3–6 (in Russian).
Sokolov, N.V. 2012. *Teoreticheskie sostavlyaushie odomashnivania losya evropeiskogo* [Theoretical components of the domestication of European elks]. Kostroma, 150 pp. (in Russian).
Stypinski, P. 2011. The Effect of Grassland-based Forages on Milk Quality and Quantity. *Agronomy Research* **9**(Special Issue II), 479–488. https://doi.org/10.15159/AR.18.067
Tatar, V., Mootse, H., Sats, A., Mahla, T., Kaart, T. & Poikalainen, V. 2015. Evaluation of size distribution of fat globules and fat and protein content in Estonian Goat milk. *Agronomy Research* **13**(4), 1112–1119. https://doi.org/10.15159/AR.18.067
Ugodskaya, E.K. & Sokolov, A.N. 2015. Milking elks, its composition and application prospects. *Elk farming: problems, searches, solutions. Interregional scientific-practical conference.* Kostroma, pp. 175–180 (in Russian).

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https://doi.org/10.15159/AR.20.029

Usability improvements of the Thermipig model for precision pig farming

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Abstract. Pig livestock farming systems encounter several economic and environmental challenges, connected with meat price decrease, sanitary norms, emissions etc. To deal with these issues, methods and models to assess the performance of a pig production system have been developed. For instance, Thermipig model represents the pig fattening room and simulates performances of pigs at the batch level, taking into account interactions between the individual variability of pigs, farmer's practices, room characteristics and outdoor climate conditions. The model requires some static basic inputs fulfilled in several spreadsheets (such as rooms, pigs, and dietary characteristics) but also data files for voluminous variable inputs (such as outdoor temperature or climate control box parameters) for further modelling and outcome producing. This leads to challenges in data providing by the farmers and have to be improved. This paper deals with the implementation of the separate modules of the developed data warehouse system for usability improvements of the Thermipig model. The idea is to substitute input from the data files with online data input and automated variable processing by the model using the python script for connection to the remote data warehouse. The data warehouse system is extended with ‘Property Sets’ section dealing with all the operations that can be performed to a set of input variables. This approach demonstrates the ability of the data warehouse to act as data supplier for the remote model. As well the outcome of the model is also transferable back to the data warehouse for evaluation. This work is done within the Era-Net SuSan PigSys project - Improving pig system performance through a whole system approach.

Key words: precision agriculture, precision livestock farming, modeling, Thermipig model, system usability, system integration.

INTRODUCTION

European pig production still continues to encounter economic and environmental challenges. Currently the maximum efficiency and productivity of an animal farm can be achieved by applying Precision Livestock Farming (PLF) (Porto et al., 2012; Pierpaoli et al., 2013). PLF aims to improve efficiency of production together with improving animal welfare (Banhazi et al., 2012).

Pig farms can be found everywhere in Europe (Marquer et al., 2014), but in each country different types of building and management rules can be applied. As well climatic conditions vary from country to country. All these factors influence the performance and welfare of the pigs and more generally the multiple performances of the pig fattening unit (Cecchin et al., 2019; Machado Filho, 2000). Profitability in commercial pork production is determined not only by daily gain and backfat thickness, but also by lean growth rates and levels of feed intake and feed conversion ratio (Paura & Jonkus, 2016). Accurate definition of the wide panel of local situations requires collection of precise information on climate, barn characteristics, indoor management rules, type of pigs and feeding strategies. Afterwards a modelling approach can be used to simulate and predict pig growth and behaviour as well as the behaviour of the fattening unit system through a representation of interactions among its components. Several models are available to simulate the growth performance of pigs, so-called 'Growth model', in different feeding or management conditions either at the average individual level (van Milgen et al., 2008; Niemi et al., 2010; NRC, 2012) or at the batch level (Cadéro et al., 2018). However, none of them deals with the interaction with ambient conditions. Therefore the growth model proposed by Cadéro et al. (2018) was merged to a bioclimatic model (Marcon et al., 2016), thus creating a new model called Thermipig (Brossard et al., 2019). Thermipig model is used in the present paper as a reference for possible usability and functionality improvements dedicated to further implementation of decision support systems for PLF purpose.

The aim of the development of the Thermipig model is to propose a tool that can help to identify target climate control box parameters of the fattening barn as well as insulation type, equipment installation or practices for improved sustainability of pig production under different climatic conditions. First simulations have been performed with a computer version of the model to evaluate the impact of theoretical technical options or indoor management rules on pigs' performance and energy use under different outdoor conditions.

The next step of the development of Thermipig model is to increase its usability through a user-friendly Web interface, integrated into a whole data warehouse system. This work is part of the Era-Net SuSan PigSys project (Improving pig system performance through a whole system approach).

DATA WAREHOUSE CONCEPT

Data warehouse (DW) is considered as a cloud-based data storage and processing unit, with capabilities to combine unlimited data sources like other existing systems and available on-farm generated data. The developed DW follows best practices in distributed and asynchronous data processing by utilizing multi-agent techniques in conjunction with real-time data warehousing approach (Komasilovs et al., 2019).

The DW architecture consists of various main components to ensure that information is stored for further analysis. After the data has been entered into the DW, the next step has to be the data analysis and decision making for getting added value from the data. Several potential models can be integrated into the DW to conclude on different aspects of pig status and variation of indoor conditions. Pig status models can include growth model and overall pig health model. Pig behaviour models can include criteria such as pig laying and standing behaviour model, aggressiveness and feeding behaviour. Description of indoor conditions is mainly based on a barn microclimate model. The DW idea and concept allow to integrate models based on the data collected within the DW or provide an API for other external system to get the data from the warehouse and then store the model output back to the warehouse. Within this work DW is extended with module for data input for external model called Thermipig.

GENERAL STRUCTURE OF THERMIPIG MODEL

The Thermipig model is of a multi-object type, mechanistic, dynamic, determinist and pig centred. The bioclimatic part of Thermipig is used to model the thermal exchanges at the room level. It represents and predicts the energy balance and direct energy consumption at the room level, and allows to calculate the ventilation rate and the input of heat required to achieve the expected ambient temperature. Therefore, it accounts for the interaction between the characteristics of the building in terms of room characteristics (size, insulation), equipment (fans, heater), parameters of the climate control box and characteristics of the group of pigs (number, weight, performance, heat production). The growth model part of Thermipig is an individual-based model adapted from the InraPorc model (van Milgen et al., 2008), combined with a generator of the within-room pig population and additional tools dealing with management issues such as mortality rate or delivery to slaughterhouse. It allows to calculate daily individual performance (weight, feed consumption) and heat production that are used as inputs by the bioclimatic model. Thermipig model allows for a good prediction of performance and ambient temperature (Brossard et al., 2019).

The model is written in Python language and several additional Python modules and Excel files are used to run the overall model. General structure of the Thermipig model execution is presented in Fig. 1.

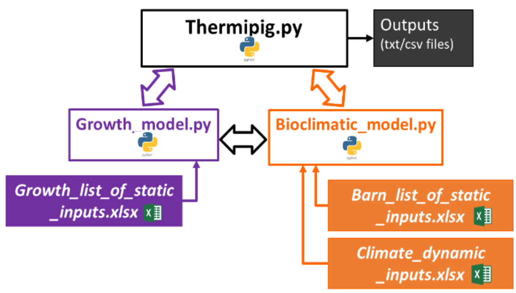

Figure 1. General structure of Thermipig model execution.

One of the main requirements for the model execution is inputting extensive amount of static and dynamic variables in the growth and bioclimatic modules. When the process is implemented locally on a computer, Excel files with multiple sheets are used to upload variable inputs and generate results. Growth model static inputs are related to management practices at the farm and includes information about shipment decisions, feeding strategy, batch management, room characteristics, animal characteristics and variability. The bioclimatic model static inputs are related to climate control parameters and include data describing the equipment available (fan system, heating system…), room dimensions and insulation. The bioclimatic dynamic inputs include hourly data on outdoor temperature and humidity, and dynamic changes in fan and heating system regulation setpoints (if automatically recorded by the equipment).

There are many disadvantages of using the Excel files for variable input. Firstly, the user is required to have the corresponding software, which is not free. Secondly, it is fastidious to input a lot of information when all the fields are difficult to observe simultaneously. In addition, the Excel files can be accidentally lost or deleted when they are stored on a local computer. Another challenge is to maintain and organise all the files in order to store and process the data describing multiple and different samples.

The aim of this paper is to present a developed user interface for substituting Excel data input with a user-friendly Web interface, which is integrated into the whole DW system to increase the model usability. In software engineering, usability is the degree to which a software can be used by specified consumers to achieve quantified objectives with effectiveness, efficiency, and satisfaction in a quantified context of use. There are cases when systems have adequate functionality, but without an appropriate usability the users will not be able to exploit it efficiently and will not adopt it. Furthermore, the output of Thermipig model is also changed from the file output to a web output directly.

USER INTERFACE FOR DATA INPUT

User interface for Thermipig model inputs is created as a part of the DW and is integrated in it. Technologies used for web interface development are Angular and Bootstrap (front-end) and Java 8, Spring Boot and MongoDB (back-end).

Developed DW platform is accessible online: https://pigsys.science.itf.llu.lv/. Any interested person can log in and test it.

The DW system is extended with ‘Property Sets’ section dealing with all the operations which can be performed to a set of input variables. Each system user can define multiple property sets, copy and delete them, edit the drafts and see the results of the modelling performed upon property set (the ones that have been processed by the Thermipig model). Available operations for each property set are demonstrated on Fig. 2.

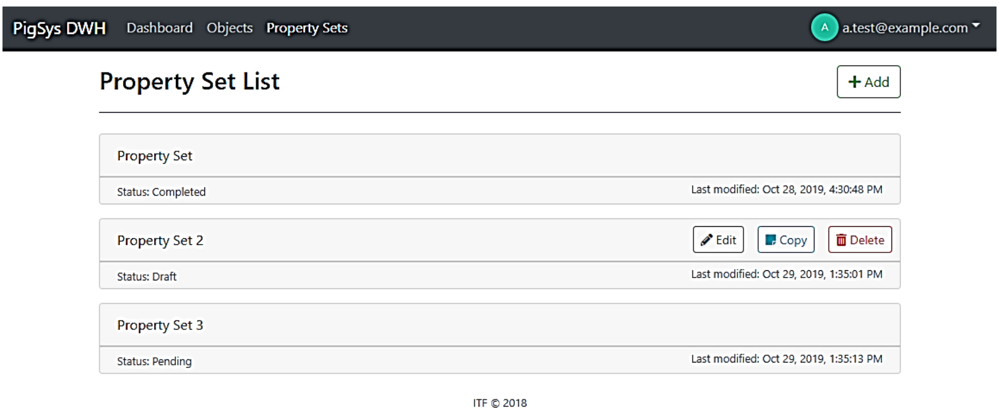

Figure 2. ‘Property Set’ section in PigSys DWH for data input.

When creating a new property set the only required field is the name of the set. All variable inputs are divided into groups to ease the process of filling the data and make it more organized. It is possible to either add properties one by one manually or add predefined sets of static properties and their default values automatically. In order to make the input process as easy as possible, inputs that can only have predefined values are represented by list boxes (Fig. 3).

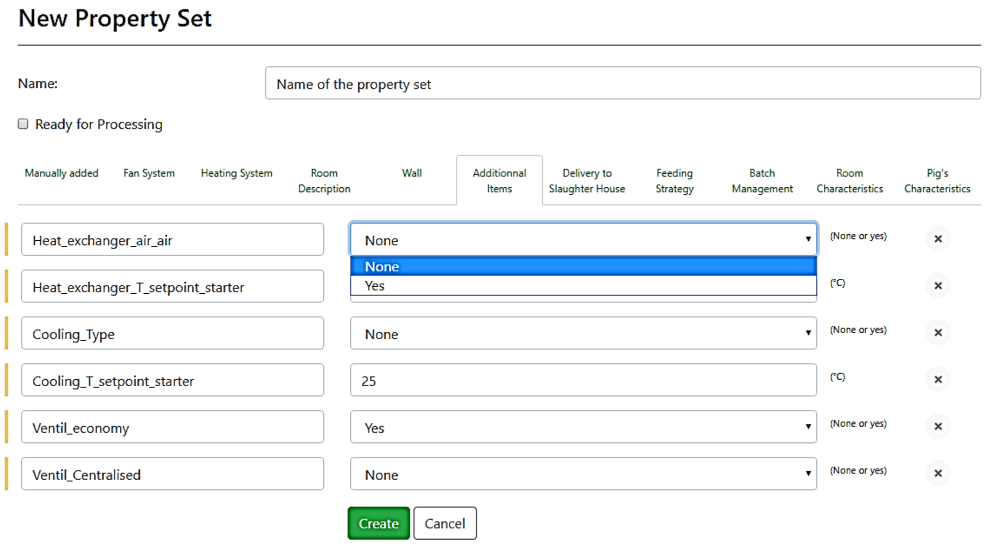

Figure 3. Creating a new property set.

A property set can be edited as long as it is a draft. To make the property set available for the Thermipig model to process, the user must check 'Ready for processing' changing status from 'Draft' to 'Pending'. Once the property set is saved with the status 'Pending' it becomes read-only and is processed by the model during next computation session. For usability purposes, when users want to define some other practices, such as the feeding strategy, the type of pig and/or regulation rules in a given room, a copying function for all property sets, including pending and completed, is available.

For Thermipig model calculations, a dataset of hourly climatic conditions (mainly outdoor temperature) is needed. Climatic data has been collected for several geographical locations in France, Sweden, Denmark, and Latvia and is embedded into the model. In the future users will be able to define the target location for the simulation and an appropriate historical dataset will be used.

PYTHON SCRIPT FOR CONNECTION TO THE DATA WAREHOUSE

Thermipig model was initially developed using Excel files for input variables. Then Python script used the Excel file, processed it by the rows and columns and assigned local Python variables to values defined in each specific cell in the Excel file. In this case, it was needed to use an additional library to ease the processing of the Excel files, like xlrd library. In the modified procedure Thermipig model will directly get the input values from the remote DW. Below is an example of the Python script snippets for connection to the remote DW and property set fetching. This procedure can be performed on-demand or scheduled according to user needs and computation power available for modelling. It consists of following logical steps:

- authentication of machine-to-machine application (the script) and token acquisition

```
headers = {'content-type': "application/json"}
payload = {
    "client_id": "...",
    "client_secret": "...",
    "audience": "pigsys-web-api",
    "grant_type": "client_credentials"
}
auth = requests.post('https://pigsys.eu.auth0.com/oauth/token',
                     json=payload, headers=headers).json()

headers = {
    'content-type': 'application/json',
    'Authorization': auth['token_type'] + ' ' + auth['access_token']
}
```

- requesting the system wide list of property sets available for processing

```
propertySetIds = requests.get(API_HOST + '/api/properties/processing',
                              headers=headers).json()
```

- in loop fetching parameters for a given property set

```
for id in propertySetIds:
    propertyData = requests.get(API_HOST + '/api/properties/processing/' + id,
                                headers=headers).json()
```

- processing the parameters through Thermipig model and sending results back to DW

```
requests.post(API_HOST + '/api/properties/processing',
              json={"id": id, "status": "In progress"},
              headers=headers)

# model
result = model(propertyData)
# end of model

requests.post(API_HOST + '/api/properties/processing',
              json={"id": id, "status": "Completed", "result": result},
              headers=headers)
```

After the Thermipig model has performed calculations and simulations, its outputs are transferred directly to the remote DW and are available in the user interface.

There are different outputs from the Thermipig model. One output set is related to the individual pig's performance including age, body weight, average daily gain, feed intake, protein and lipid content and deposition and mineral (N, P) retention and excretion. The other set is related to room ambient conditions including hourly temperature, ventilation and heating rates and energy consumption, hourly relative humidity and CO_2.

DISCUSSION AND CONCLUSIONS

This article is focused onto the demonstration of the approach applied to develop the data interface for an existing model and to integrate it into the previously developed DW platform.

Developed DW is an universal system as it allows connectivity to different multiple external systems, each of which can produce different data aimed for various livestock of PLF branches.

Proposed approach demonstrates the ability of joining geographically distributed solution, as presently the DW system and external Thermipig model are located in different institutess. Thermipig model itself is processed in France, but input variables and outputs are stored in the DW, deployed in Latvia. Especially for projects / applications developed in partnership, the geographically distributed solution allows to join external systems that can have their own particular development within each partner, provided that the links between modules are kept coherent. It allows also to join systems without ownership issues, each module being potentially hosted by its owner.

Implemented web interface for variable input eases the process of preparation for Thermipig calculations and therefore increases the usability of the model. In the future, it is planned to get feedbacks from the farmers concerning their evaluation of the proposed interface and of the approach for variable inputting process to the model.

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REFERENCES

Banhazi, T.M., Lehr, H., Black, J.L., Crabtree, H., Schofield, P., Tscharke, M. & Berckmans, D. 2012. Precision livestock farming: an international review of scientific and commercial aspects. *International Journal of Agricultural and Biological Engineering* **5**(3), 1–9.

Brossard, L., Cadero, A., Dourmad, J.Y., Renaudeau, D., Garcia-Launay, F., Marcon, M. & Quiniou, N. 2019. Combining a bioclimatic and a growth model to assess the effect of management practices and building ambiance on growing pig performances at the batch level. in Proc. 9th workshop 'Modelling nutrient digestion and utilization in farm animals' (MODNUT). *Advances in Animal Biosciences* **10**(2), 367.

Cadéro, A., Aubry, A., Brossard, L., Dourmad, J.Y., Salaün, Y. & Garcia-Launay, F. 2018. Modelling interactions between farmer practices and fattening pig performances with an individual-based model. *Animal* **12**(6), 1277–1286.

Cecchin, D., Ferraz, P.F.P., Campos, A.T., Sousa, F.A., Amaral, P.I.S., Castro, J.O., Conti, L. & da Cruz, V.M.F. 2019. Thermal comfort of pigs housed in different installations. *Agronomy Research* **17**(2), pp.378–384.

Komasilovs, V., Kviesis, A., Zacepins, A. & Bumanis, N. 2019. Development of the data warehouse architecture for processing and analysis of the raw pig production data. *AGROFOR* **3**(3).

Machado Filho, L.C.P. 2000. Pig welfare and meat quality A Brazilian view. In: conferencia internacional virtual sobre qualidade de carne suína, 1., 2000, Concórdia. Anais... Concórdia: *Embrapa Suínos e Aves*. pp. 34–40 (in Portuguese).

Marcon, M., Massabie, P., Kergourlay, F., Dourmad, J.Y. & Salaun, Y. 2016. MEDIBATE, a dynamic model of direct and indirect energy exchanges in pig barns for field decision support. *Journées de La Recherche Porcine En France* **48**, 177–182.

Marquer, P., Rabade, T. & Forti, R. 2014. Pig farming in the European Union, considerable variations from one Member State to another. *Eurostat Statistics in Focus* **15**(2013), 1–12.

van Milgen, J., Valancogne, A., Dubois, S., Dourmad, J.Y., Sève, B. & Noblet, J. 2008. InraPorc: A model and decision support tool for the nutrition of growing pigs. *Animal Feed Science and Technology* **143**(1–4), 387–405.

Niemi, J.K., Sevón-Aimonen, M.L., Pietola, K. & Stalder, K.J. 2010. The value of precision feeding technologies for grow-finish swine. *Livestock Science* **129**(1–3), 13–23.

NRC (National Research Council) 2012. Nutrient Requirements of Swine. Eleventh Revised Edition. National Academic Press, Washington, D.C. 20418 USA

Paura, L. & Jonkus, D. 2016. Use of automatic system for pig feed consumption control. *Agronomy Research* **14**(1), 160–166.

Pierpaoli, E., Carli, G., Pignatti, E. & Canavari, M. 2013. Drivers of Precision Agriculture Technologies Adoption: A Literature Review. *Procedia Technology*, **8**, 61–69.

Porto, S.M.C., Arcidiacono, C., Cascone, Anguzza, U., Barbari, M. & Simonini, S. 2012. Validation of an active RFID-based system to detect pigs housed in pens. *Journal of Food, Agriculture and Environment* **10**(2), 468–472.

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Influence of local extruded soybean cake and imported soybean meal on fattening pig productivity and pork quality

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Abstract. The aim of this study was to determine the influence of feeding local and imported soybean protein feeds to fattening pigs and examining it impact on the quality of pork. The trial was created with 40 pigs divided in two groups (20 in each). Pigs in the control group received imported soybean meal, in the trial group local farm grown in Latvia extruded soybean cake mixed in the compound feed. The diets were designed to be nutritionally equivalent. For fattening pigs each diet were available on an ad libitum basis to pens. During the study pigs were weighed three times at 84, 140 and 190 days at age. Feed consumption, pig carcass traits and meat chemical composition were determined. The final live weight in control group was 108.33 ± 2.904 kg and in trial group was 111.88 ± 2.793 kg there were no significant difference ($P > 0.05$). Average daily live weight gain in the all experimental period in control group was 0.779 ± 0.096 kg and in trial group was 0.822 ± 0.103 kg, there were no significant difference ($P > 0.05$). Feed consumption per kg of live weight in control group was 2.39 kg in trial group was 2.24 kg. Pig carcass traits and meat chemical composition were similar for both groups without significant differences ($P > 0.05$). Soybeans grown and processed in Latvia were equivalent to imported soybeans and gives good rates of pig growth and quality of pork.

Key words: fattening pig, pork quality, soybean protein.

INTRODUCTION

Precision feeding is a major breakthrough in pig nutrition and it is one of the most promising avenues to promote high-quality and safe pork, high animal welfare, and minimal impact on the environment (Pomar & Remus, 2019). Economic and environmental concerns have been forced the development of low crude protein (CP), amino acid (AA) fortified diets that deliver performance equivalent to diets with intact protein sources. However, shown in some studies, low CP diets have led to decreased performance, particularly in heavy weight finishing pigs. Decreasing dietary CP below 13% may compromise finishing pig growth and carcass performance (Soto et al., 2019). Amino acids given in excess will be deaminated and the resulting urea will be excreted in the urine. Finding a good balance between amino acids supply and amino acids requirement is very important for different reasons (Milgen & Dourmad, 2015). Soybean meal has long been considered an outstanding source of supplemental protein in diets for livestock and poultry. In fact, soybean meal is very often referred to as the 'gold standard' because other protein sources are compared to it. Soybean meal is rich in highly digestible protein, and the protein is composed of a superior blend of amino acids, the building blocks of body protein for livestock and poultry (Stein et al., 2013; Cromwell, 2017). Soybean meal represents two-thirds of total world output in protein feedstuffs (Oil-World, 2010). It is estimated that soybean accounts 85% of the protein supplements fed to pigs (Cortamira et al., 2000).

Several factors influence the concentration of amino acids present in soybean grains, such as climatic changes, weather, temperature, genetics, topography, and soil fertility (Degola et al., 2019).

Soybean growing in Latvia is a new growing industry with many challenges. The results of chemical composition of soybeans grown in different regions of Latvia showed that protein content in soybean samples determined from 32.7 to 40.7% fat content from 18.4 to 21.4% and significantly differed ($p < 0.05$) among growing places. Metabolizable energy calculated for pigs varied from 13.2 to 17.6 MJ kg^{-1} (Degola et al., 2019).

Dietary protein intake stimulates muscle protein synthesis. The muscle protein synthetic response to protein intake can vary substantially between different dietary protein types or sources (Gorissen et al., 2018). Dietary supplementation of protein and amino acids is important to promote normal and optimal growth for pigs (Son et al., 2019). Beyond their nutritional role as the source of amino acids for protein synthesis, they are instrumental in the regulation of food intake, glucose and lipid metabolism, bone metabolism and immune function (Jahan-Mihan et al., 2011). Nutritionally, amino acids are classified as essential or nonessential for animals based on their traditional role in protein synthesis. However, the critical regulatory roles for amino acids in metabolism have long been ignored. In fact, amino acids and their metabolites are regulators of cellular signal transduction, gene expression and the protein post-translational modification, especially in the intestine (Mou et al., 2019).

The term ideal protein can be defined as the protein which containing the minimum quantity of essential amino acids with maximum utilization to meet the exact nutritional amino acid requirements (Wang & Fuller, 1989). It refers to determine the required amounts of amino acids relative to lysine for maintenance, protein accretion, and growth performance of pigs (Milgen & Dourmad, 2015; Recharla et al., 2017). There is general agreement that ileal rather than fecal digestibility measurements represent more accurate estimates of amino acids availability in pig feeds (Sweich, 2017). Different ideal protein profiles have been proposed in in scientific literature for growing pigs and sows (Milgen & Dourmad, 2015).

Well balanced amino acid composition reduces odor by modulating the gut microbial community. Administration of pig diet formulated with the ideal protein concept may help improve gut fermentation as well as reduce the odor causing compounds in pig manure (Recharla et al., 2017).

Growth performance of pigs, carcass composition and quality of pork and pork products depend on multiple interactive effects of genotype (genetic background, presence of major genes hal and RN-), rearing conditions (feeding level, housing and environmental conditions, production system), preslaughter handling, and carcass and meat processing (Lebret, 2008). There are many factors affecting muscle fibre characteristics, including welfare, breed, gender, age, and others (Rehfeldt et al., 2004; Jeong et al., 2012;

Joo et al., 2013). One of the extrinsic factors is nutrition (Bee et al., 2007; Jeong et al., 2012). Although meat quality traits tend to be better in the group fed ad libitum (Lebedova et al., 2019).

Pigs on a high amino acid diet in late finishing pigs able to compensate to a large extent for amino acid restriction in growing and early finishing. Amino acid content in late finishing determined carcass quality (Millet et al., 2011).

The aim of the study was to compare feed with imported soybean protein to feed with soybean protein grown and produced in Latvia, determine the impact of feed on the quality of carcasses and meat.

MATERIALS AND METHODS

Animals and housing

The estimations were based on the ethical guidelines research was carried out in accordance with the Pig welfare requirements Cabinet Regulation No. 743. Yorkshire × Landrace cross breeds fattening pigs with start body weight 25 kg were selected for the trial. With the aim to carry out the studies two groups of pigs were formed 20 pigs in each group, per pen balanced for body weight and sex (10 female and 10castrated male). Pigs were selected from a commercial pig farm. Pigs were kept on concrete floors bedding was sawdust. Nipple drinker and 2 hole feeder were placed in each pen. The body weight of pigs was individually measured at the 84, 140 and 190 days at age. Diets in bouth groups at the trial was provided all times on an ad libitum basis to pens. The bodyweight and feed intake were determined to evalute average daily gain and average daily feed intake.

Dietary treatments and Performance Measures

The control diet included imported soybean meal, but in the trial group diets extruded soybean cake was made at the farm. Extruded soybean cake at farm was made from soya variety with early ripening ability (group 000) suitable for regions with lower (1,500–1,800 °C) sum of effective temperatures – 'Laulema' with crude protein content 43.36% (Degola et al., 2019). In the trial were calculated and prepared three diets for each group similar in crude protein content and to be isoenergetic for metabolizable energy. The rations contained barley, wheat, canola or soybean oil, fish meal, salt and trace element vitamin premix, phytase, depending of pig liveweight and age. Their amounts and choice of mixture were adjusted continuously over time depending on the actual weights of the pigs Table 1. The chemical compositions of the feed mixtures are presented in Table 2. Protein profiles in diets were calculated as the ratio of Lysine (Lysine 100%) to other amino acids.

Chemical analyses

Feed samples were tested in the Scientific laboratory of Agronomic analysis of Latvia. Samples of feed were milled through a 1-mm screen before analysis. Dry matter(DM), crude protein (CP), crude fibre (CF), fat, calcium (Ca), phosphorus (P), contents were analysed based on standard methodology (Degola et al. 2019). Amino acids were detected using amino acids analyzer. The identity and quantitative analysis of the amino acids were assessed by comparison with the retention times and peak areas of the standard amino acid mixture. The metabolizable energy (ME) were calculated based on tested parameters in accordance with Mc Donald et al., (2002). The meat samples were tested and quality parameters – pH, water, crude protein, fat (LVS ISO 1443:1973) content, and cholesterol content (BIOR-T-012-132-2011) were determined in laboratory of Food and Environmental Investigations (BIOR) in Latvia.

Table 1. Composition of diets for growing and fattening pigs

Traits	Control group			Trial group		
Pigs liveweights, (kg)	20–40	40–65	65–110	20–40	40–65	65–110
Ingredients,%						
Local wheat	64.8	67.16	72.13	39.31	40.0	43.28
Local barley	16.22	16.79	18.03	39.32	40.1	43.27
Imported soybean meal	12.22	9.55	6.14	-	-	-
Local extruded soybean cake	-	-	-	15.61	14.28	9.16
Fish meal	2.5	2.5	-	1.0	1.0	-
Premivit	3.2	3.0	3.2	3.76	3.53	3.29
Canola oil	1.0	1.0	0.5	-	-	-
Local soybean oil	-	-	-	1.0	1.0	1.0

Table 2. Chemical composition of diets for growing and fattening pigs

Traits	Control group			Trial group		
Pigs liveweights, (kg)	20–40	40–65	65–110	20–40	40–65	65–110
Nutrients						
Dry matter, (%)	87.8	87.8	87.6	88.8	88.7	88.3
Crude protein, (%)	18.8	17.9	15.4	18.2	17.4	15.2
Crude fiber, (%)	2.8	2.7	2.6	3.2	3.1	3.0
Fat, (%)	3.4	3.4	2.7	3.3	3.2	2.9
ME MJ, kg	13.5	13.5	13.3	13.4	13.4	13.3
Ca, (g)	8.4	7.9	8.2	9.6	9.1	8.5
P, (g)	4.8	4.6	4.1	5.3	5.2	4.9

Slaughter and carcass quality measurements

To determine carcass and meat parameters the finisher pigs at the 110 kg liveweight were slaughtered in commercial slaughterhouse. Carcass weights were fixed. Backfat depth (F) was measured at the head of the last rib, 6 cm from the mid back line, using a Introscope Optical Probe (Latvia reg.of the Cabinet of Ministers Nr. 307). For each individual pig the percentage of lean meat was calculated as: $66.6708 – 0.3493 \times F$. Muscle eye area was determined with the planimeter (Degola & Jonkus, 2018). The lenght of carcass was measured in a straight line from the forward edge of the first rib to the forward edge of the aitch bone and muscle eye area with the planimeter (Degola & Jonkus, 2018). Left side of carcases was divided into parts for determine weight of ham. For quality testing 24 hours after slaughter meat samples were taken from the *musculus longissimus lumborum et thoracis*.

Statistical analysis

Statistical analysis was performed according to the SAS/STAT 9.22 software package (2010). Data were reported as arithmetic means with the pooled SEM. The results of investigation were compared using Student's t-test. Statistical significance was evaluated at $P < 0.05$.

RESULTS AND DISCUSSION

Comparison of feed with imported soybean meal and feed with soybean cake grown and produced in Latvia, was made by determining the impact of feed on the quality of carcasses and meat. Pigs in the control group received imported soybean meal from 12.22% to 6.14% in the trial group local farm grown in Latvia extruded soybean cake 15.61% to 9.16% mixed in the compound feed. The diets were designed to be nutritionally equivalent.

The total amount of amino acids showed no essential difference in growing and fattening periods (Table 3).

Table 3. Composition of amino acids in diets for growing and fattening periods (% in dry matter)

Traits	Control group	Trial group	Control group	Trial group
Pigs liveweights, (kg)	40–65	40–65	65–110	65–110
Indispensable amino acid, (%)				
Arginine, (%)	0.86	0.87	0.80	0.83
Histidine, (%)	0.37	0.38	0.34	0.35
Izoleicine, (%)	0.58	0.60	0.53	0.55
Phenylalanine, (%)	0.75	0.76	0.68	0.69
Leicine, (%)	1.06	1.07	0.95	0.98
Lysine, (%)	0.88	0.94	0.79	0.92
Methionine, (%)	0.36	0.36	0.34	0.35
Threonine, (%)	0.57	0.57	0.51	0.53
Valine, (%)	0.72	0.71	0.64	0.70
Total Lys+Met+Thre	1.81	1.87	1.64	1.8
Total indispensable amino acids, (%)	6.15	6.26	5.58	5.9
Dispensable amino acid, (%)				
Alanine, (%)	0.68	0.67	0.62	0.63
Aspartic, (%)	1.16	1.22	1.04	1.12
Cysteine, (%)	0.26	0.27	0.23	0.23
Glycine, (%)	0.82	0.72	0.74	0.71
Glutamic, (%)	3.75	3.65	3.49	3.38
Histidine, (%)	0.37	0.38	0.34	0.35
Proline, (%)	1.24	1.25	1.21	1.21
Serine, (%)	0.74	0.73	0.67	0.68
Tyrosine, (%)	0.47	0.47	0.42	0.42
Total dispensable amino acids (%)	9.49	9.36	8.76	8.73
Total amino acids	15.27	15.24	14.0	14.28

It is also important to evaluate the individual results of the limiting essential amino acids in soybean-based poultry and swine feeds, i.e., methionine, lysine, and threonine (Goldflus, 2006).

Composition of indispensable amino acids amount in diets for growing and fattening periods at control and trial groups were similar for grower (40–65 kg) feed for finisher (65–110 kg) feed. Same relevance were founded for amount of dispensable amino acid in control and trial groups The sum of main limiting amino acids Lysine, Threonine and Methionine were similar for grower feeds in control group 1.81% in trial feed were1.86%. Sum of main limiting amino acids for finisher feeds in control group were 1.64% it was by 0.16% less than in trial group 1.80%.

Well known that the total amino acid amount from feedstuffs cannot be equally absorbed by the animal digestive tract is necessary evaluate composition of amino acids and amount of essential amino acids. In the ideal protein concept, researchers recommend the precise amount of digestible amino acids. In estimation was used InraPorc (Milgen & Dourmad, 2015) ideal protein profile for comparison composition of protein profiles in diets.

Table 4. Comparison of standardized Ileal digestibility protein profiles in diets with ideal protein profiles

Traits	Control group			Trial group			InraPorc[1]
Liveweight, (kg)	20–40	40–65	65–105	20–40	40–65	65–105	20–140
Lysine	100.0	100.0	100.0	100.0	100.0	100.0	100
Threonine	63.1	65.9	67.9	63.3	64.1	65.6	65
Methionine	43.3	46.2	40.0	42.4	43.0	36.0	30
Methionine+Cysteine	75.8	81.4	83.8	73.1	74.7	74.1	60
Tryptophan	23.1	24.2	26.0	23.5	24.0	24.6	18
Valine	78.1	82.3	88.8	77.5	79.3	87.5	70
Isoleucine	68.1	71.3	75.9	65.7	43.0	72.9	55
Leucine	112.9	130.4	143.0	122.2	124.9	137.4	100
Histidine	41.8	43.8	48.4	41.5	42.3	46.3	32
Phenylalanine	85.4	90.3	103.5	85.2	87.2	98.6	50
Phenylalanine +Tyrosine	141.3	148.7	167.6	140.7	143.7	160.7	95
Tyrosine	55.9	58.3	64.1	55.5	56.5	62.1	–
Cysteine	32.5	35.3	43.8	30.7	31.7	38.2	–

InraPorc[1] Ideal protein profile (Milgen & Dourmad 2015).

Results obtained of protein profiles in diets for growing and fattening periods in grower (40–65 kg) feeds showed threonine were less in control and trial groups compared to ideal protein profiles (InraPorc). In other groups threonine was in optimal relation. Threonine is often the second limiting amino acid in conventional commercial diets, and feeding pigs amino acid deficient diets limit protein deposition and affects tissue protein composition. The results of researchers Remus et al. (2019) show than amino acids requirements vary between individual pigs and cannot be accurately estimated based on traditional amino acids:Lysine ratio studies. The results of this trial indicate that pigs have great capacity to deal with excess and limited amino acids resources, by limiting protein deposition and changing amino acids composition differently among body tissues. Under limiting amino acids conditions, pigs modulate to some extent the utilization and retention of the limiting resource in order to maintain its natural functions in a normal manner.

Comparison of standardized Ileal digestibility protein profiles in diets with ideal protein profiles showed in trial group for feed (20–40 kg) Isoleucine was inadequate ratio it was insufficient. Milgen & Dourmad (2015) concluded valine deficiency decreases feed intake to a great extent and consequently gain. The same observation can be made for isoleucine. The content of the other amino acids was higher than that reported by InraPorc ideal protein profile.

All animals were maintained healthy and consumed provided experimental diets well. At the beginning of the investigation, the average pig start liveweight mass did not show any essential differences between groups, respectively in control group was 25.7 ± 0.31 kg and in trial, was 25.5 ± 0.23 kg.

At the age of 140 days the average liveweight of control group pigs was 66.56 ± 2.74 kg in the trial group was 68.88 ± 1.79 kg. Pigs from trial group in average were heavier by 3.5%. At the age of 190 days the average liveweight of control group pigs was 108.33 ± 2.74 kg, but in the trial group was 111.88 ± 1.79 kg. Pigs from trial group were heavier by 3.3%. There were no significant diferences on pigs average liveweight ($P > 0.05$).

Influence of diets on average daily gains showed, at period from 84 to 140 days in the control group was 0.743 ± 0.049 kg in trial group was by 6.0% higher 0.788 ± 0.031 kg average daily gain. The daily gain indices in fattening period from 140 to 190 days in control group was 0.853 ± 0.050 kg in trial group was by 2.9% higher 0.877 ± 0.032 kg. In all period of investigation from 84 to 190 days daily gain in control group was 0.779 ± 0.026 kg in trial group was by 5.5% higher 0.822 ± 0.026 kg.

At the final, the pigs which fed soybean cake grown in Latvia, showed by 3.3% higher live weight than pigs which fed mixed feed with imported soybean meal, there were no significant diferences on pigs average daily gain.

In estimation pigs from trial group consumed feed less by 2.70 kg than pigs in control group. Feed consumption per kg of liveweight gain for control group was 2.39 kg d^{-1} in trial group was by 6% better. Diets showed no significant effect on pig feed consumption and feed conversion rates between groups (Table 5).

Table 5. Influence of diets on feed consumption

Traits	Control group	Trial group
Feeding days	106	106
Feed consuption, (kg)	197.4	194.7
Feed consuption at day,(kg)	1.86	1.84
Feed conversion, (kg d^{-1})	2.39	2.24

The obtained values carcass weight, leght of carcass, muscle–eye area and ham weight (Table 6.) show better results at the trial group significant differences were not found between groups ($P > 0.05$).

Table 6. Influence of diet on pig carcass and meat traits

Traits	Control group	Trial group
Carcass weight, (kg)	78.5 ± 6.09	82.1 ± 6.47
Lenght of carcass, (cm)	103.2 ± 3.63	104.3 ± 4.89
Backfat, (mm)	11.0 ± 3.0	9.6 ± 2.5
Lean meat,(%)	61.60 ± 0.80	62.00 ± 0.72
Muscle-eye area, (cm^2)	62.40 ± 10.05	68.20 ± 14.93
Ham weight, (kg)	8.02 ± 1.23	8.65 ± 0.57
Moisture, (%)	72.50	72.40
Protein, (%)	22.40	22.20
Fat, (%)	4.05	4.65
pH	6.01	5.59
Cholesterol, (%)	50.50	45.90
Tryptophan, (g $100g^{-1}$)	0.282	0.294
Hydroxyproline, (%)	0.11	0.12
Tryptophan : Hydroxyproline ratio	2.56	2.45

According to the classification of pork, all pig carcasses were evaluated by the 'S' class. The meat analyses showed cholesterol countent in trial group was by 5% less than in control group. The others determined meat parameters between the groups were similar. Meat quality was not influenced by diets.

CONCLUSIONS

The results of the study showed that using of soybean cake grown and processed in Latvia can produce equivalent pig feed rations as using of imported soybean meal. Soybean grown in Latvia and processed in soybean cake can use in feed ration for pig during growing and fattening periods. The replacement of imported soybean protein by soybean protein grown in Latvia did not showed any negative impact on pig growth rates, quality of carcasses and meat traits. Economic evaluation is necessary to determine the effectiveness of soy produced in Latvia.

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REFERENCES

Bee, G., Calderini, M., Biolley, C., Guex, G., Herzog, W. & Lindemann, M.D. 2007. Changes in the histochemical properties and meat quality traits of porcine muscles during the growingfinishing period as affected by feed restriction, slaughter age, or slaughter weight. *J. Anim. Sci.* v. **85**, 1030–1045.

Cortamira, O., Gallego, A. & Kim, S.W. 2000. Evaluation of twice decorticated sunflower meal as a protein source compared with soybeanmeal in pig diets. *Asian Austral. J. Anim. Sci*. **13**(9), 1296–1303.

Cromwell, G,L. 2017. Soybean Meal – An Exceptional Protein Source. https://www.soymeal.org/soy-meal-articles/soybean-meal-an-exceptional-protein-source/, acessed 30. 01.2020.

Degola, L. & Jonkus, D. 2018. The influence of dietary inclusion of peas, faba bean and lupin as a replacement for soybean meal on pig performance and carcass traits. *Agronomy Research* **16**(2), 389–397.

Degola, L., Sterna, V., Jansons, I. & Zute, S. 2019. The nutrition value of soybeans grown in Latvia for pig feeding. *Agronomy Research* **17**(5), 1874–1880. https://doi.org/10.15159/AR.19.15

Goldflus, F.I.; Ceccantini, M.I.I. & Santos, W.I.I. 2006. Amino acid content of soybean samples collected in different Brazilian states – harvest 2003/2004. In *Brazilian Journal of Poultry Science* On-line version ISSN 1806-9061 Rev. Bras. Cienc. Avic. vol.**8** no.2 Campinas Apr./June 2006 http://dx.doi.org/10.1590/S1516-635X2006000200006 Acessed 28.01.2019

Gorissen, S.H.M., Crombag, J.J.R., Senden, J.M.G., Waterval, W.A.H., Bierau, J., Verdijk, L.B., & van Loon, L.J.C. 2018. Protein content and amino acid composition of commercially available plant-based protein isolates. *Amino Acids*. Volume **50**(12), 1685–1695.

Jahan-Mihan, A., Luhovy, BL, El Khoury, D. & Anderson, G.H. 2011. Dietary proteins as determinants of metabolic and physiologic functions of the gastrointestinal tract. *Nutrients*. May, **3**(5), 574–603.

Jeong, J.Y., Kim, G.D., Ha, D.M., Park, M.J., Park, B.C., Joo, S.T. & Lee, C. 2012. Relationships of muscle fiber characteristics to dietary energy density, slaughter weight, and muscle quality traits in finishing pigs. *Journal of Animal Science and Technology* **54**, 175–183.
Joo, S.T., Kim, G.D., Hwang, Y.H. & Ryu, Y.C. 2013. Control of fresh meat quality through manipulation of muscle fiber characteristics. *Meat Sci*. **95**, 828–836.
Lebedova, N., Stupka, R., Čitek, J., Okrouhla, M. & Zadinva, K. 2019. Effect of feed restriction on muscle fibre characteristics and meat uality traits in pigs. *Agronomy Research* **17**(1), 176–185. https://doi.org/10.15159/AR.19.004
Lebret, B. 2008. Effects of feeding and rearing systems on growth, carcass composition and meat quality in pigs. *Animal* **2**(10), 1548–1558.
LVS ISO 1443:1973. https://bior.lv/en/services/fat-determination-soxhlet-method, accessed 30.01.2020.
McDonald, P., Edwads, R.A., Greenhalgh, J.F.D. & Morgan, C.A. 2002. *Animal Nutrition. 6th Edition*. Pearson, UK, 669 pp.
Millet, S, Langendries, K, Aluwé, M. & De Brabander, DL. 2011. Effect of amino acid level in the pig diet during growing and early finishing on growth response during the late finishing phase of lean meat type gilts. *J Sci Food Agric* May, **91**(7), 1254-8. doi: 10.1002/jsfa.4307
Milgen, J. & Dourmad, J.Y. 2015. Concept and application of ideal protein for pigs. *J. Anim. Sci. Biotech*. **6**, 15. https://jasbsci.biomedcentral.com/articles/10.1186/s40104-015-0016-1 Acessed 28.01.2020.
Mou, Q., Yang, H.S., Yin, J.L. & Huang, P.F. 2019. Amino Acids Influencing Intestinal Development and Health of the Piglets. *Animals* (Basel). 2019 Jun; **9**(6), 302. Published online 2019 May 31. doi: 10.3390/ani9060302 PMCID: PMC6617173 PMID: 31159180 Acessed 15.01.2020.
Oil-World 2010. Major meals, world summary balances. Oil WorldWeekly **55**(3), 45.
Pomar, C. & Remus, A. 2019. Precision pig feeding: a breakthrough toward sustainability. *Animal Frontiers*, Volume **9**, Issue 2, April 2019, pp. 52–59.
Stein, H.H., Roth, J.A., Sotak, K.M. & Rojas, O.J. 2013. Nutritional value of soy products fed to pigs. Swine Focus #004.
https://nutrition.ansci.illinois.edu/sites/default/files/SwineFocus004.pdf[15.01.2020]
Recharla, N., Kim, K., Park, J., Jeong, J., Jeong, Y., Lee, H., Hwang, O., Ryu, J., Baek, Y., Oh, Y. & Parkcor, S. 2017. Effects of amino acid composition in pig diet on odorous compounds and microbial characteristics of swine. *J. Anim. Sci. Technol*. **59**, 28. Published online 2017 Dec 11. doi: 10.1186/s40781-017-0153- 5 Acessed 20.01.2020
Rehfeldt, C., Fiedler, I. & Stickland, N.C. 2004. Number and Size of Muscle Fibres in Relation to Meat Production. In: *Muscle development of livestock animals, edited by Te Pas*, M.F.W., Everts M.E., Haagsman, H.P. Lelystad, Netherlands, pp. 1–38.
Remus, A., Hauschild, L., Corrent, E., Létourneau-Montminy, M. & Pomar, C. 2019. Pigs receiving daily tailored diets using precision-feeding techniques have different threonine requirements than pigs fed in conventional phase-feeding systems. *Journal of Animal Science and Biotechnology*. Volume **10**, Article number: 16.
Son, A.R., Park, C.S, Park, K.R. & Kim, B.G. 2019. Amino acid digestibility in plant protein sources fed to growing pigs. *Asian-Australasian Journal of Animal Sciences* (AJAS) **32**(11), 1745–1752.
Soto, J.A., Tokach, M.D., Dritz, S., Woodworth, J.C., DeRouchey, J.M., Goodband, R.D. & Wu, F. 2019. Optimal dietary standardized ileal digestible lysine and crude protein concentration for growth and carcass performance in finishing pigs weighing greater than 100 kg. *Journal of Animal Science*, Volume **97**, 1701–1711.
Swiech, E. 2017. Alternative prediction methods of protein and energy evaluation of pig feeds. *Journal of Animal Science and Biotechnology*, Volume **8**, Article number: 39, acessed 30.01.2020
Wang, T.C. & Fuller, M.F. 1989. The optimum dietary amino acid pattern for growing pigs. 1. Experiments by amino acid deletion. *Br. J. Nutr*. **62**(1),77–89.

Agronomy Research **18**(S2), 1316–1322, 2020
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Longevity and milk production efficiency of Latvian local breeds during last decades

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Abstract. The aim of the study was to analyse the longevity and the amount of energy-corrected milk (ECM) per day of local dairy cattle breeds Latvian Brown (LB) and Latvian Blue (LZ). The study was based on the data of LB genetic resources (LB) 1770 and LZ 921 cows, which were born from January 1st, 2000 till December 31st year 2015. Milk productivity and longevity of the LB and LZ cows were analysed by birth year periods: 2000–2005, 2006–2010 and 2011–2015. LZ culled cows lifespan was in average 2,762.8 ± 55.14 days, or 7.6 years and it was significantly higher than for LB. The average lifespan of LB culling cows was 6.7 years. There are cows which had closed 7–12 lactations. On average, cows' lifespan decreased during analysed period. In general, LZ cows are characterized by higher length of productive life and milking days. LZ cows produced more EC milk during their productive life; however, they had the lower milking day ECM productivity than LB cows.

Key words: local breed, longevity, milk production efficiency.

INTRODUCTION

Latvian Brown (LB) is mentioned as a local cattle breed and Latvian Blue (LZ) as a local native breed in the DAD-IS list of the FAO database (FAO, 2019).

LB breed was formed in mid-19th century from local cows and Angler, then later in 19th century was improved by Danish Red bulls. At the beginning of the 20th century pedigree breeding was planned and milk recording was started, and some years later in the 1920's the herd-book was published. The LB is a red cattle breed, which was selected on higher milk productivity, a strong body and good udder shape. The LB breed is used for milk and meat production and it is well suited to local conditions and can graze on the poor pasture (FAO, 2019). Since the 1960's genetics from Danish Red, Angler, Brown Swiss, Red Holstein, Swedish Red-and-White have been used. The conservation of Latvian Brown stock was started in 2013 with only 134 cows; however, there are – 41 bulls' semen stocks in store at the artificial insemination stations (Porter et al., 2016). The LB breed cows with percentage of local breed genes at least 50% and only the Angler and Danish red breeds genetics used for improving are considered for inclusion in the genetic resources conservation program (National conservation programme of Latvian Brown breed dairy cows, 2019).

LZ breed originated from local cows on the coast of the Baltic Sea (FAO, 2019). Their coat colour is from light to dark blue (grey) and around the eyes have blue 'glasses' (Grīslis, 2006). In the 1920's blue cows as well as LB were included in the Latvian cows herd book. In the 1930's, in Latvia, we had only one purebred LZ farm in which in 1935 was started to use crossbreeding (Grīslis, 2006; Griņevičs & Grosvalds, 2011; Smiltina et al., 2015). LZ was officially accepted in 1995 as a breed to be conserved (FAO, 2019) and in 2000 a breeders association was established orientated on the planning and conservation of the very small and endangered population (Grīslis, 2006). The breed is well adapted to maritime and harsh climate and can graze on the poor pasture. LZ is found mainly in the northern and north-western part - coastal area of Latvia, in Kurzeme (FAO, 2019). In 2001, in the Agricultural Data Center (LDC) 224 LZ cows were registered, including 185 females and 39 males, as the LZ cows' population (Grīslis, 2006) and in 2013 they numbered 349 (Porter et al., 2016). LZ breed is at risk (FAO, 2019).

During the last decade the number of Holstein cows in Latvia is increasing and at the same time the number of red breeds' cows and cows which are classified as local is decreasing, especially LB genetic resources (LB) cows. Local cattle breeds are not only important for the conservation of genetic diversity, but are also economically effective on small farms (10 to 30 cows), because of higher longevity. According to Strandberg (1996) research, if longevity increases from three to four lactations, then profit per year will increase by 11–13%.

The aim of the study: to analyse the longevity traits and the amount of energy-corrected milk per day of local dairy cow breeds.

MATERIALS AND METHODS

The study was based on the data of Latvian Brown genetic resources 1770 and LZ 921 cows. For study purposes, from Latvian Agricultural Data Centre were collected data about cows' milk productivity of cows which were born from January 1st, 2000 till December 31st, 2015. The main criterion for including LB genetic resources cows in the research is percentage of genes of a local breed which has to be at least 50%. For LZ cows, the percentage of genes of a local breed could be 37.5% or higher because the main requirement during the conservation process of the breed was blue-gray colour and breed-typical characteristics (darker legs, white horns with black tips, stature around 130–135 cm). The analysed cows are from different parts of Latvia and are located in small farms with the stall housing systems and grazing in pastures.

Data about cows' birth, culling and first calving dates, milk yield (kg), milk fat and milk protein content (%) in full lactation and in milking day were included in the dataset. For the analysis of longevity, age at first calving, lifespan, length of productive life and a number of days milking were calculated. For analysis the efficiency of milk production, energy corrected milk (ECM) in one life day, in one productive life day and in one milking day for culling and lactating cows were calculated. Life length and ECM yield for lactating cows were recorded from the birth till November 1st year 2018.

The milk production efficiency in the paper is characterized with the amount of ECM per one life day, productive life day and per milking day.

Energy corrected milk (ECM) was calculated by formula:

$$ECM = milk\ yield, kg \times \frac{[(fat, \% \times 0.383) + (protein, \% \times 0.242) + 0.7832)]}{3.14}$$

Milk productivity and longevity of the LB and LZ cows were analyzed based on birth year periods. Data set was divided into three birth year periods: 2000–2005, 2006–2010 and 2011–2015.

Data in tables are presented as mean ± standard error of mean. A two-way analysis of variance (ANOVA) was used to determine the effect of factors – breed and period on cows' longevity and productivity traits. Pairwise comparisons were performed by Bonferroni test. Significant differences at $\alpha = 0.05$ in the tables were marked with different letters of the alphabet (a, b, and A, B). The data analysis was done by SPSS 23 program package.

RESULTS AND DISCUSSION

The number of LB and LZ cows used in the analysis is shown in Fig. 1. Analyzed cows were born from year 2000 to 2015 and calved from year 2001 to 2017. The number of LB continued to decrease through year 2000 to 2015. The number of LB and LZ breeds' cow's is decreasing since 2006 and it is relatively constant and similar during time period from year 2008 to 2015. Number of LB cows' in 2008 was noticeably lower than in earlier years.

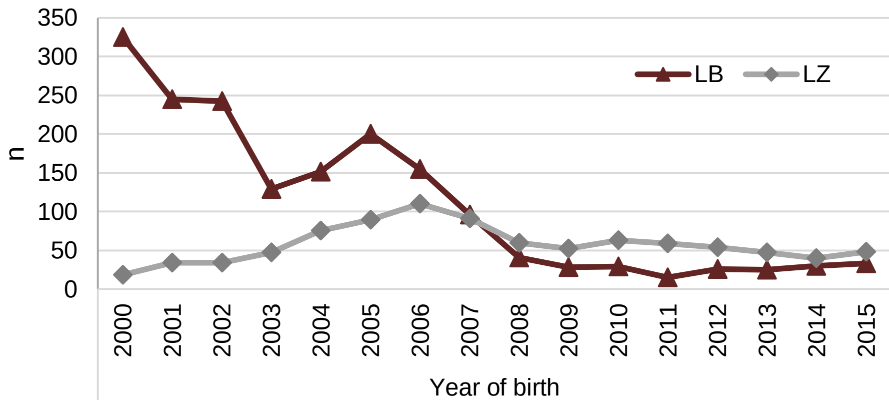

Figure 1. Number of LB and LZ cows by birth year.

Usually, local breeds have the small population size and breeding organizations need to control the rate of inbreeding. However, as study results in 2006 show (Grislis et al., 2005) the inbreeding level in LZ population was 1% and the average inbreeding of the inbred cows was 12.3%, the effective population size for LZ was Ne = 18 (Zutere et al., 2006). In the later investigation (Grīslis & Simkevica, 2018), 40% or 225 cows of the LZ population had the coefficient of inbreeding higher than 3%.

The local breeds have lower milk productivity; however, they are characterized by higher longevity. In total 0.7% of the LB and 2.4% of LZ breed cows, which were born between years 2000 to 2005, are still lactating and had closed 7–12 lactations at the end of 2018. From the first study period (2000–2005) 9 LB and 7 LZ cows had on average 8.9 ± 0.42 lactations. The proportion of LB and LZ cows which were born from 2006–2010 and are still lactating was more than 11%, and the proportion in 2011–2015 was around 70% (Fig. 2).

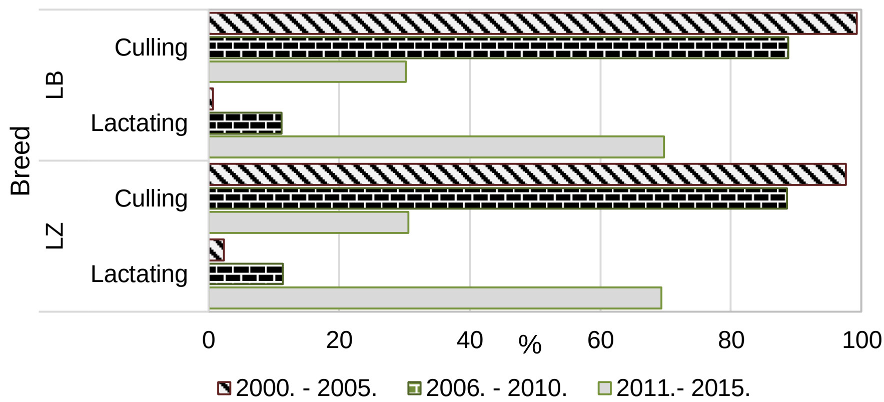

Figure 2. The distribution of lactating and culling LB and LZ cows by birth year periods.

On average, the analysed culling cows had a lifespan from 1,486.2 ± 150.4 to 2,762.8 ± 55.14 days and it is decreasing during the time (Table 1).

Table 1. The average longevity of culling LB and LZ breeds' cows by birth year periods

Period	Breed	n	Longevity traits			
			age at first calving, days	lifespan, days	length of productive life, days	milking days
2000-2005	LB	1,283	843.0 ± 4.35 ^{aA}	2,446.7 ± 26.22 ^{A}	1,603.7 ± 26.43 ^{A}	1,257.0 ± 21.52 ^{A}
	LZ	290	830.4 ± 9.15 ^{aA}	2,762.8 ± 55.14 ^{B}	1,932.4 ± 55.59 ^{B}	1,552.2 ± 45.25 ^{B}
2006-2010	LB	310	853.8 ± 8.85 ^{aA}	2,369.7 ± 53.33 ^{A}	1,515.9 ± 53.76 ^{A}	1,125.5 ± 43.77 ^{A}
	LZ	333	859.2 ± 8.54 ^{bA}	2,349.8 ± 51.46 ^{A}	1,490.6 ± 51.87 ^{A}	1,181.1 ± 42.23 ^{A}
2011-2015	LB	39	814.6 ± 24.95 ^{bA}	1,486.2 ± 150.4 ^{A}	671.6 ± 151.58 ^{A}	564.4 ± 123.40 ^{A}
	LZ	76	841.9 ± 17.87 ^{abA}	1,575.8 ± 107.7 ^{B}	733.9 ± 108.58 ^{A}	636.2 ± 88.40 ^{B}

$^{A; B}$ – significant differences between breeds in one period; $^{a;b}$ – age at first calving differs significantly between birth year periods for one breed.

2000–2005 birth year period cows had the lifespan an around 2,500 days. LZ cows' lifespan was 2,762.8 ± 55.14 days, or 7.6 years and significantly higher than for LB. The average lifespan of LB culling cows was 6.7 years. 2011–2015 birth year period LZ cows had the higher lifespan (1,575.8 days) than LB cows (1,486.2 days) ($P < 0.05$). In the investigation about Polish local breed cows' longevity, authors pointed out that the cows most frequently culled at the age of 8-9 years and the lowest lifespan was observed for Polish Red cows and it was 7.8 years (Adamczyk et. al., 2017). The LZ and LB cows had the lower lifespan compared to the Polish Red cows. LZ 2000–2005 birth year period cows are characterized by the higher length of productive life ($P < 0.05$) and milking days ($P < 0.05$) compared to the LB cows.

Age at first calving is an important factor in reducing production costs and economic return in the dairy sector (Eastham et al., 2018). The LB breed cows that were born between 2011–2015 had lower age at first calving (814.6 ± 24.95 days) compared to the cows born from the 2006 and 2010 birth year period ($P < 0.05$). The lowest age at first calving (830.4 ± 9.15 days) was observed for LZ breed cows, which were born in 2000–2005 analysed birth year period ($P < 0.05$).

As our previous investigation shows (Jonkus et al., 2018), the optimal age at first calving for the higher milk productivity was smaller than 25 months in LB breed group and 27–30 months in LZ breed group. Sawa et al. (2019) obtained that the optimal age at first calving in Black-and-White Polish Holstein-Friesian dairy cows' population was 22.1–26.0 months. The age at fist calving after 28 months is associated with decreases in lifetime milk production and increases in culling rate in the first production year due to low milk yield and udder diseases (Sawa et al., 2019).

For the milk production efficiency evaluation milk productivity during the lifetime, productive life time and milking day were evaluated (Table 2).

Table 2. The average lifetime ECM productivity of culling LB and LZ breed cows by birth year periods

Period	Breed	Milk productivity traits, kg			
		Lifetime ECM productivity	Life day ECM productivity	Productive life day ECM productivity	Milking day ECM productivity
2000-2005	LB	19,634.7 ± 388.23 ^{A}	7.3 ± 0.09 ^{A}	12.3 ± 0.11 ^{A}	15.1 ± 0.12 ^{A}
	LZ	24,179.9 ± 816.59 ^{B}	8.0 ±0.20 ^{A}	12.2 ± 0.23 ^{A}	14.8 ± 0.25 ^{A}
2006-2010	LB	19,144.9 ± 789.80 ^{A}	7.4 ± 0.19 ^{A}	13.0 ± 0.22 ^{A}	16.5 ± 0.24 ^{A}
	LZ	18,830.0 ± 762.04 ^{A}	7.3 ± 0.18 ^{A}	12.6 ± 0.21 ^{A}	15.2 ± 0.23 ^{B}
2011-2015	LB	9,537.9 ± 1,226.73 ^{A}	5.5 ± 0.53 ^{A}	14.5 ± 0.63 ^{A}	16.7 ± 0.66 ^{A}
	LZ	9,937.1 ± 1,595.12 ^{A}	5.7 ± 0.38 ^{A}	13.9 ± 0.45 ^{A}	15.5 ± 0.48 ^{B}

$^{A; B}$ – significant differences between breeds in one birth year period.

The lifetime ECM productivity of 2000–2005 birth year period cows was 19,634.7 kg for LB and 24,179.9 kg for LZ breed cows; however, cows which were born in 2011–2015 did not exceed an average lifetime milk yield – 10,000 kg ECM. LZ cows born in 2000–2005 period ($P < 0.05$) and 2011–2015 period produced more EC milk during their productive life than LB cows, although, the LZ cows had lower milking day ECM productivity. The lowest milk production 5.5 ± 0.53 kg ECM/life day was observed for LB cows and the highest one 8.0 ± 0.20 kg ECM life day for LB cows.

In general, LZ culling cows had longer lifetime and higher lifetime productivity, but lower productive life day and milking day ECM productivity. This can be explained by the fact that LZ cows had significantly higher average milking days (1,275.8 ± 30.0) at all periods than LB cows (1,215.4 ± 19.60) ($P < 0.05$).

In our previous research, there was found out, that Latvian brown cows' lifetime productivity and life day productivity was around 15,000 kg and milking day ECM productivity were in average 9.6 kg in small and 11.9 kg in large size farms therefore farm size is showing effect on dairy cow efficiency (Cielava et al., 2014). In our further investigation the life day milk productivity of Latvian brown cows was lower in the stall housing than it was in loose housing (accordingly 8.2 kg and 9.9 kg ECM per one life day) (Cielava et al., 2017). During the last decade the lifetime and life day productivity have been decreasing due to the shorter lifespan and length of productive life.

The milk production intensity of lactating cows was analysed and significantly higher life day ECM productivity (11.7 ± 1.08 kg) was obtained from 9 still producing LB cows which were born in 2000–2005 (Fig. 3).

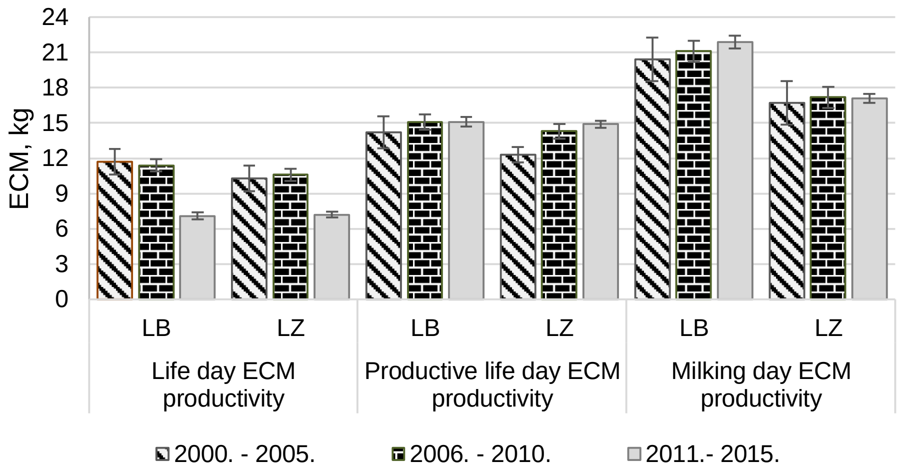

Figure 3. The average lifetime ECM productivity of lactating LB and LZ breed cows depending on their birth year periods.

ECM productivity in one productive life day and one milking day did not differ significantly between study periods, but it significantly differed between breeds ($P < 0.05$). From the LB breed cows in one milking day 3.7–4.8 ECM kg more than from LZ breed cows were obtained in all study periods.

In comparison with lactating LB and LZ cows, a significantly higher daily ECM yield was obtained in all study periods than with culling cows (Table 2, Fig. 3).

Taking into account the small number of local cows population in Latvia and their keeping purpose, low milk productivity is not a reason for the culling of these cows. The main goal is to get as many non-inbred calves as possible to preserve genetic diversity.

CONCLUSIONS

LZ breed was associated with higher longevity (lifespan, length of productive life, milking days) traits than LB in Latvian dairy herds. From culling and now lactating LB breed cows significantly higher daily ECM yield were obtained in all study periods than it was LZ cows.

The present study shows the number of local LB and LZ breeds cows' with percentage of genes of a local breed through 2000 to 2015 was decreased. This indicates that local breeds have a small population number and breeding organizations need to control the rate of inbreeding.

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REFERENCES

Adamczyk, K., Szarek, J., Majewska, A., Jagusiak, W. & Gil, Z. 2017. Factors affecting longevity of cows with high share of Polish local breeds' genes. *Animal Science Papers and Reports* **35**(1), 35–46.

Cielava, L., Jonkus, D. & Paura, L. 2014. Effect of farm size on the productivity and longevity of Latvian Brown cows. In: *Ressearch for Rural Development 2014: annual 20th international scientific conference proceedings*. Latvia University of Agriculture, Jelgava, **1**, 95–99.

Cielava, L., Jonkus, D. & Paura, L. 2017. Lifetime milk productivity and quality in farms with different housing and feeding systems. *Agronomy Research* **15**(2), 369–375.

Eastham, N.T., Coates, A., Cripps, P., Richardson, H., Smith, R. & Oikonomou, G. 2018. Associations between age at first calving and subsequent lactation performance in UK Holstein and Holstein-Friesian dairy cows. PloS one **13**(6), pp. 1–13.

FAO. ©2019. Domestic Animal Diversity Information System (DAD-IS). Available at: http://www.fao.org/dad-is [Accessed: 2019, October 13].

Griņēvičs, I & Grosvalds, I. 2011. Creation of Latvian cow breeds and disappearance of the Latvian Blue Cow Available at: http://priede.bf.lu.lv/konf/apsek/ zoo/2011/Grinevics_Grosvalds_Blue_Cow.pdf [Accessed: 2019, October 25] (in Latvian).

Grīslis, Z. 2006. Blue cows in Vidzeme. Jelgava. BŠSA "Zilā govs", 1–36 (in Latvian).

Grislis, Z., Markey, L. & Zutere, R. 2005. The inbreeding analysis in Latvian Blue cow population. In: *Proc. of the 11th Baltic animal breeding and genetics conference*. Palanga, Lithuania, 65–69.

Grīslis, Z. & Šimkevica, D. 2018. Latvian Blue selection. Biedrība Šķirnes saglabāšanas apvienība 'Zilā govs', Jelgava, 1–43 (in Latvian).

Jonkus, D., Cielava, L. & Petrovska, S. 2018. Dairy cow longevity in voluntary milking system. In: *Proc. of the 26th NJF congress "Agriculture for the next 100 years*". Kaunas, Lithuania, 40–45.

National conservation programme of Latvian Brown breed dairy cows. 2019. Available at: https://www.ldc.gov.lv/upload/doc/doc20.pdf. [Accessed: 2020, January 17].

Porter, V., Alderson, L., Hall, S.J.G. & Sponenberg, D.P. 2016. *Mason's World Encyclopedia of Livestock Breeds and Breeding*. CABI, UK. 226. pp.

Sawa, A., Siatka, K. & Krežel-Czopek, S. 2019. Effect of Age at First Calving on First Lactation Milk Yield, Lifetime Milk production and Longevity of Cows. *Annals of Animal Science* **19**(1), 189–200. doi: 10.2478/aoas-2018-0044

Smiltina, D., Balins, A. & Grislis, Z. 2015. A review of Latvian Blue (LZ) cows from the list of animal genetic resources in Latvia. *Acta Biologica Universitatis Daugavpiliensis* **15**(1) 165–177.

Strandberg, E. 1996. Breeding for longevity in dairy cows. In: *Progress in Dairy Science*. Phillips, C.J.C. (ed.). Oxon, CAB International, Wallingford, 125–144.

Zutere, R., Grislis, Z. & Sjakste, T. 2006. Breeding programs in Latvian livestock. In: *Proc. of the 12th Baltic animal breeding conference.* Jurmala, Latvia, 6–13.

Agronomy Research **18**(S2), 1323–1335, 2020
https://doi.org/10.15159/AR.20.095

Modelling of hot-air and vacuum drying of persimmon fruit (*Diospyros kaki*) using computational intelligence methods

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Abstract. The study evaluated the feasibility of applying computational intelligence methods as a non-destructive technique in describing the drying behaviour of persimmon fruit using vacuum drying (VD) and hot-air-drying (HAD) methods and to compare the results with thin layer mathematical models. Drying temperatures were 50, 60 and 70 °C. Kinetic models were developed using semi-theoretical thin layer models and computational intelligence methods: multi-layer feed-forward artificial neural network (ANN) and support vector regression (SVR). The statistical indicators of coefficient of determination (R^2) and root mean square error (RMSE) were used to assess the suitability of the models. The thin-layer mathematical models namely page and logarithmic accurately described the drying kinetics of persimmon slices with the highest R^2 of 0.9999 and lowest RMSE of 0.0031. ANN showed R^2 and RMSE values of 1.0000 and 0.0003, while SVR showed R^2 of 0.9999 and RMSE of 0.0004. The validation results indicated good agreement between the predicted values obtained from the computational intelligence methods and the experimental moisture ratio data. Based on the study results, computational intelligence methods can reliably be used to describe the drying process of persimmon fruit.

Key words: persimmon fruit, drying methods, computational intelligence methods, artificial neural network model, support vector regression model.

INTRODUCTION

Persimmon (*Diospyros kaki*) is an edible fruit that grows in subtropical and warm temperate climates and it is considered an important fruit in many countries such as China, Japan and Korea and Turkey. It has a high nutritional value such as vitamin A, C, carotenoid and phenolic compounds (Bozkir et al., 2019). The high moisture content of the fruit leads to rapid deterioration even at refrigerator temperatures. Therefore, drying is considered as one of the processes to increase persimmon shelf life. This indicates that persimmon can be used as an ingredient in products such as breakfast cereals and snacks (Doymaz, 2012).

Drying of agricultural products causes the enzymatic reactions to be inactivated as a result of heat and mass transfer leading to a reduction of the moisture content inside the product (Tomsone et al., 2018). Drying methods such as hot-air drying (HAD), freeze-drying (FD), vacuum drying (VD), microwave drying (MWD) and infrared drying (IRD) have been used in drying agricultural products (Doymaz, 2012; Karaman et al., 2014). However, HAD is the most commonly used technique due to the uniformity of the dried product and non-toxicity (Onwude et al., 2016b; Ozola & Kampuse, 2018). In the VD method, the use of low temperatures in the absence of oxygen can preserve heat-sensitive and easily oxidizable foods where discoloration and decomposition of the flavor and some nutritional substances are prevented (Tekin & Baslar, 2018; Karasu et al., 2019). Both HAD and VD can also affect the physical and phytochemical properties of the food products. Hence, the determination of the optimum operating parameters, drying conditions and the determination of suitable drying models are important for achieving the nutritional value along with minimum product cost and maximum yield (Tomsone et al., 2018).

Some studies have explored several thin-layer drying models to describe the drying processes of fruits and vegetables. The thin-layer drying models are empirical, semi-theoretical and theoretical models based on the assumption of mass diffusivity, conductivity and geometry (Akoy, 2014; Aboltins et al., 2018). Theoretical models have followed the fundamentals of mass and heat transfer laws during the drying process and their parameters have a physical meaning (Górnicki et al., 2020). In practical, according to (Kaleta & Górnicki, 2010) theoretical models normally take a long time due to the complexity of the diffusion equations governing the process. Semi-theoretical models offer a compromise between theory and ease of application and they are deduced from simplified versions of Fick's second law of diffusion (Ashtiani et al., 2017). Empirical models are basically built between the direct relationship between the curve of moisture content and drying time and does not need to consider the theory during the drying process (Kaleta et al., 2013). These models, however, do not produce accurate results (Onwude et al., 2016b). Thus, researchers are considering new drying modelling approaches or computational tools.

Computational tools such as artificial neural networks (ANN) and support vector regression (SVR) are considered as complex tools for complex systems and dynamic modelling (Khaled et al., 2018). Application of ANN and SVR offer many advantages compared to conventional modeling techniques due to the learning ability, increased flexibility, online non-destructive measurements, reduced assumptions, suitability to the nonlinear process and tolerance of incomplete data (Rodríguez et al., 2014). The difference between ANN and SVR is fundamentally based on how the non-linear data is classified. For example, ANN applies a multi-layer connection and different activation functions to deal with nonlinear problems. While SVM employs nonlinear mapping to make the data linear separable using kernel functions (Khaled et al., 2020).

ANN and SVR have been successfully applied in modelling and optimizing the drying processes of fruits and vegetables such as pomelo (Kırbaş et al., 2019), ginkgo biloba seeds (Bai et al., 2018), mushroom (Omari et al., 2018), celeriac slices (Beigi & Ahmadi, 2018), pepper (Jafari et al., 2016), eggplant (Bahmani et al., 2016), tea leaves (Xie et al., 2014), tomato (Movagharnejad & Nikzad, 2007). In view of this, ANN and SVR techniques can be applied to the drying kinetics of persimmon fruit under HAD and VD, and this knowledge is limited in the literature. This modelling technique can also be useful for assessing the drying parameters in real conditions, optimum processing conditions and drying efficiency.

The objectives of this study are to evaluate the feasibility of applying ANN and SVR modelling as a non-destructive technique in describing the drying behaviour of persimmon fruit under different drying conditions and to compare the results with thin layer mathematical models. Also, to investigate the color change kinetics of persimmon slices under different drying conditions during the VD and HAD.

MATERIALS AND METHODS

Samples preparation and moisture content determination

Persimmon fruits (*Diospyros kaki*) bought from a market in Prague, Czech Republic, were used for the experiment. A total of 30 samples were selected based on similar physical appearances (shape color and size). Prior to each experiment, the samples were peeled and washed under running tap water. Then they were sliced into thickness and diameter of 5 mm and 56 mm, respectively, using a Sencor slicer (SFS 4050SS, Czech Republic). The persimmon fruit with a sliced section of dimension 56 mm and thickness of 5 mm used in the study is shown in Fig. 1. The initial moisture content of the fresh samples was determined as 3.98 kg kg^{-1} (dry basis), according to ASABE standard (ASAE, 2005), by drying 25 g of selected samples at 70 °C for 24 h using the conventional oven.

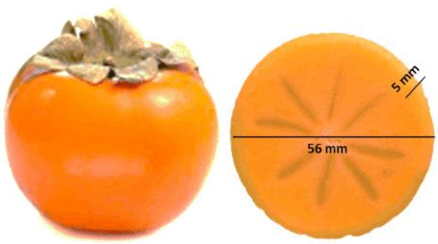

Figure 1. Persimmon fruit with a sliced section of diameter 56 mm and thickness of 5 mm.

Drying experiments

In this study, two different drying processes were used include VD and HAD to dry the persimmon sliced samples.

Vacuum drying

The VD technique was carried out using a laboratory-scale drying unit (l 450 Goldbrunn, Poland) as shown in Fig. 2. For VD, the vacuum was regulated by a vacuum pump (VE 135, RoHS, China) at a 50 mbar ultimate pressure and 2 Ls^{-1} pump speed. It is worth noting that the pressure was monitored through the vacuum gauge which was unstable during the drying process. This problem was solved manually by fixing the pressure at approximately 50 mbar. This meant that when the

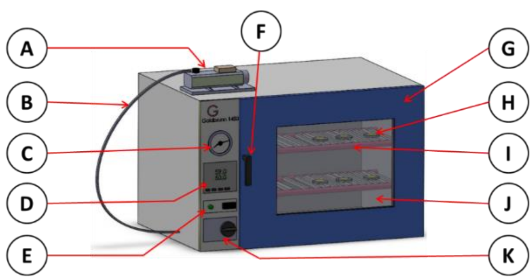

Figure 2. Schematic diagram of the vacuum dryer; A: vacuum pump; B: pipe; C: vacuum gauge; D: temperature regulator; E: ON/OFF master switch; F: door handle; G: door; H: sample; I: shelves; J: inspection window and K: vacuum valve.

pressure increased above or decreased below 50 mbar, the pump was opened for the adjustment. The VD operates by heating the samples with a conduction heat from a heater plate in the container. The vacuum pump reduces the pressure around the sample to be dried and further ensures less atmospheric pressure. This decreases the boiling point of the water inside that product and thereby increases the rate of evaporation significantly. The sliced samples were dried at three temperatures (50 °C, 60 °C, and 70 °C). Prior to the experiments, the VD was set-up to the required temperature for 30 min to enable the dryer temperature to reach equilibrium with the surrounding air temperature. The weight of the persimmon sample was measured 1 hour interval using a digital scale (HR-250AZ, A&D Company Limited) weighing balance of 252 g 0.1 mg^{-1} precision. The weight of the samples was measured during the drying process every 1 hour. The experiments were carried out in triplicate and the average values used in further analyses.

Hot-air drying

A laboratory-scale convective HAD (UF 110, Memmert, Germany) was used. Similar to VD, three temperatures (50 °C, 60 °C, and 70 °C) with a constant air velocity of 1.10 ms^{-1}, until constant weight between two successive readings was attained. The air velocity was measured using a Thermo–Anemometer (Model 451104, EXTECH Instruments, Taiwan). Before starting the experiments, the HAD was set-up to the required temperature for 30 min to enable the dryer temperature to reach equilibrium with the surrounding air temperature. Similar to the VD, the weight of the persimmon sample was measured 1 hour interval using a digital scale weighing balance, whereby the samples were removed from the dryer and measured and then returned to the dryer. The mass interval was expressed as the moisture ratio as illustrated in Fig. 4. The experiments were conducted in triplicate and the average values used in further analyses.

Drying kinetics

The variation in moisture content during VD and HAD techniques was expressed in the form of moisture ratio (dimensionless) as described in Eq. 1.

$$MR = \frac{(M_t - M_e)}{(M_o - M_e)} \quad (1)$$

where M_t, M_e and M_o are the moisture content of the samples at time t, equilibrium moisture content and initial moisture content, respectively.

According to Aghbashlo et al. (2009), M_e values did not change because they were relatively low compared to M_t and M_o values, resulting in negligible error during simplification, thus the moisture ratio was expressed as shown in Eq. 2:

$$MR = \frac{M_t}{M_o} \quad (2)$$

Mathematical modelling

The experimental drying data measured were fitted to 5 selected thin-layer drying models. The selected mathematical models, namely, Newton, Page, Logarithmic, Two-term and Modified Henderson and Pabis as listed in Table 1 using the non–linear least squares regression analysis using Sigma Plot software (Version 12.0, Systat Software Inc., California, USA). The use of these models gives a better prediction with fewer assumptions (Onwude et al., 2018).

Table 1. Thin layer mathematical drying models

Model no.	Model name	Model expression	Reference
1.	Newton model	MR = exp(-*kt*)	(Lewis, 1921)
2.	Page model	MR = exp(-kt^n)	(Page, 1949)
3.	Logarithmic model	MR= aexp(-*kt*)+*c*	(Yagcioglu, 1999)
4.	Two-term model	MR=*a*exp(-k_1t)+*b*exp(-k_2t)	(Henderson, 1974)
5.	Modified Henderson and Pabis model	MR=*a*exp(-*kt*)+*b*exp(-*gt*)+*c*exp(-*ht*)	(Karathanos, 1999)

Computational intelligence methods

Artificial neural network

Artificial Neural Network (ANN) is a powerful and complex modelling tool for processing model information based on biological neural networks (Khaled et al., 2018). In general, ANNs are created with three layers (input, hidden and output layer). In this study, a multilayer feed-forward network structure was used with three input parameters (drying techniques, temperature and drying time), 1–2 hidden layers and one output parameter (moisture ratio) as shown in Fig. 3. Algorithms applied to the training of the model were the Levenberg-Marquardt back propagation transfer function choice and sigmoid function as given in Eq. 3.

$$f(x) = \frac{1}{1 + e^{-x}} \tag{3}$$

The datasets were prepared by randomly dividing the data into training (70%) and testing (30%). The total number of data used in this study was 137 with 96 was used to build the model and 41 was used for testing the model. The chosen hidden layer architectures were [3], [6], [3, 3], [6, 6] matrix, where for example, [3, 3] represents the 2 hidden layers with 6 neurons (Fig. 3). The software Weka 3.6 (Hamilton, New Zealand) was used to analyse the ANN model. In this study, the error was used as the criteria to stop training to prevent the overtraining of ANN structure. This means that when the error against the iterations shows no change and it's saturated, then the ANN training is stopped.

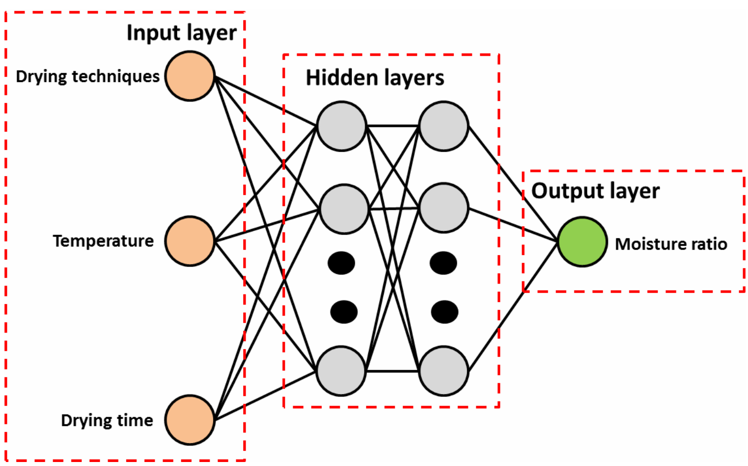

Figure 3. Artificial Neural networks topology.

Support vector regression

Support Vector Regression (SVR) is a supervised learning model which combines theoretical solutions with numerical algorithms used for regression method (Das & Akpinar, 2018). SVR as a regression method is considered as an effective approach due to its capability of capturing nonlinear relationship in the feature space. The SVR used for the moisture ratio values was determined by the SMOreg sequence in the Waikato Environment for Knowledge Analysis (WEKA) software, whereby, the SMOreg implements SVR for regression. The three input variables used for the SVR model were drying techniques, temperature and drying time with the output as the moisture ratio. In addition, two filter types were applied, namely normalize and standardize in order to determine how/if the data need to be transformed. Furthermore, three different kernel models: polynomial, Pearson universal and Radial Basis Function (RBF) were used to construct the predictive model of the calculated moisture ratio values.

Color measurements

Color is one of the most important quality evaluation attributes for fruits and vegetables during drying. For the color measurements, first the image of fresh (reference) and dried samples obtained from VD and HAD methods were captured using smartphone camera (Oppo F7, Dongguan, China). The smartphone camera consisted of a 16 mega-pixel charged-coupled device (CCD). Persimmon slices were put in a glass plate located on the white paper as a background during image capture for more focus, and the distance between sample and smartphone camera was set-up to be 22 cm vertically. Then the images were transferred to ImageJ software, which is an open-source software available to the public domain (http://rsb.info.nih.gov/ij/). Also, it can operate on Linux, Mac OS X, and Windows, in both 32-bit and 64-bit modes. ImageJ has different tools to analyse the images. In this study, ImageJ was used to determine the color parameters: lightness ($L*$), redness/greenness ($a*$) and yellowness/blueness ($b*$). The total color difference (ΔE) was estimated based on Eq. 4. For each sample, three replications were performed.

$$\Delta E = \sqrt{(L^* - L_o^*)^2 + (a^* - a_o^*)^2 + (b^* - b_o^*)^2} \tag{4}$$

where L_o^*, a_o^* and b_o^* indicate the reference values of fresh samples.

Statistical analysis for mean comparison

Statistical analysis was performed using the Statistical Analysis System software (SAS version 9.2, Institute, Inc., Cary, N.C.). Duncan test was used to compare the mean significant differences between quality attributes ($L*$, $a*$, $b*$ and ΔE) for different drying time intervals and at different drying techniques (VD and HAD). The findings of replicate measurements were presented with mean ± standard error values. The fit accuracy of experimental data to the thin layer, ANN and SVR models was determined by the coefficient of determination (R^2) and root mean square error (RMSE). They are computed mathematically as given in Eqs 5 and 6:

$$R^2 = 1 - \frac{\sum_{i=1}^{N}(V_{pred} - V_{exp})^2}{\sum_{i=1}^{N}(V_{pred} - V_m)^2} \tag{5}$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(V_{pred} - V_{exp})^2}{N}} \tag{6}$$

where V_{pred} is the predicted value, V_{exp} is the actual observation from experimental data, V_m is the mean of the actual observation, and N is number of observations.

From the values of R^2 and RMSE, the higher the value of R^2 and the lower the RMSE value, the better the goodness of fit.

RESULTS AND DISCUSSION

Behaviour of drying process of persimmon fruit slices

The variations of moisture ratio with time for VD and HAD techniques at different temperatures (50 °C, 60 °C, and 70 °C) are illustrated in Fig. 4. The moisture ratio of the samples for all techniques decreased with an increase in drying time. The drying rates for VD and HAD methods occurred in the falling rate period. Based on Fig. 4, a, it is clear that the drying time thus reduces as the drying temperature increases. The moisture ratio values of 0.18 and 0.22 were determined at a drying time of 170 min and at temperatures of 70 °C and 60 °C. At a drying time of 360 min was found the moisture ratio of 0.22 at 50 °C. Similar results were observed using HAD (Fig. 4, b). The results indicate that the moisture transfer rate from the inner layers to its surface thus increases as the drying air temperature increases. The rate of moisture evaporation at the surface of the persimmon sample to the atmosphere also increases as the temperature increases, leading to the higher drying rate. For this, the drying time for persimmon samples using HAD was different from VD. The results are in agreement with other researchers on the drying behaviour of various varieties of persimmon (Çalışkan & Dirim, 2015; Bozkir et al., 2019).

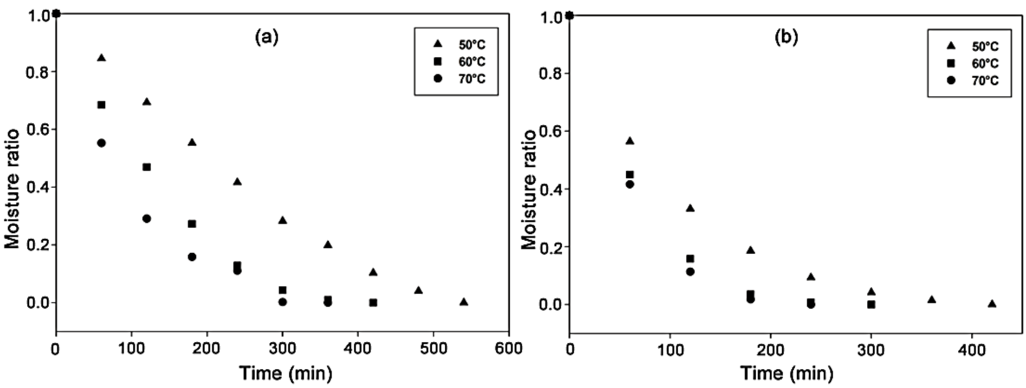

Figure 4. Drying characteristics of persimmon fruit sliced samples; [a] VD and [b] HAD.

Comparison between the mathematical modelling

The mathematical models were applied to describe the drying kinetic of persimmon slices during VD and HAR methods. Table 2 shows the selected mathematical models that fitted the experimental moisture content data. Although all the selected five models adequately fitted the experimental data, the logarithmic model sufficiently described the drying kinetics of the samples with R^2 values between 0.9964 and 0.9980 and RMSE values between 0.0146 and 0.0206 for VD technique at all drying temperatures. For HAD technique, Page and logarithmic models significantly described the drying kinetics of the samples with R^2 values between 0.9979 and 0.9999 RMSE values between 0.0031 and 0.0171. The validation of the logarithmic model by comparing the predicted moisture data and those obtained from the experiments is presented in Fig. 5. The moisture ratio data predicted using logarithmic model lied closely along a straight regression line for different drying conditions indicating the suitability of the model for describing the VD and HAD behaviours of persimmon fruit samples. Onwude et al., (2018) also reported the adequacy of Page and logarithmic models for predicting the drying kinetics of sweet potato. Similarly, Younis et al. (2018) indicated the appropriateness of page and logarithmic models for describing the drying performance of garlic slices.

Table 2. Statistical evaluation of the mathematical drying models for persimmon samples of VD and HAD

Drying method	Temp °C	Model no	Model parameters	R^2	RMSE
VD	50	1	k = 0.2491	0.9577	0.0682
		2	k = 0.1224, n = 1.4734	0.9940	0.2559
		3	a = 1.5532, k = 0.1229, c = -0.5369	0.9980	0.0146
		4	a = 0.5606, k_1 = 0.2663, b = 0.5108, k_2 = 0.2663	0.9650	0.0620
		5	a = 0.3715, k = 0.2663, b = 0.3610, g = 0.2663, c = 0.3388, h = 0.2663	0.9650	0.0620
	60	1	k = 0.4503	0.9821	0.0457
		2	k = 0.3292, n = 1.3069	0.9960	0.0217
		3	a = 1.1435, k = 0.3445, c = -0.1331	0.9964	0.0206
		4	a = 0.5347, k_1 = 0.4632, b = 0.4988, k_2 = 0.4632	0.9836	0.0438
		5	a = 0.3531, k = 0.4632, b = 0.3503, g = 0.4632, c = 0.3300, h = 0.4632	0.9836	0.0438
	70	1	k = 0.6145	0.9957	0.0220
		2	k = 0.5871, n = 1.0608	0.9963	0.0205
		3	a = 1.0325, k = 0.5613, c = -0.0345	0.9974	0.0173
		4	a = 0.5045, k_1 = 0.6171, b = 0.5003, k_2 = 0.6171	0.9958	0.0219
		5	a = 0.3329, k = 0.6171, b = 0.3408, g = 0.6171, c = 0.3312, h = 0.6171	0.9958	0.0219
HAD	50	1	k = 0.5737	0.9986	0.0120
		2	k = 0.5514, n = 1.0481	0.9990	0.0103
		3	a = 1.0228, k = 0.5349, c = -0.0255	0.9998	0.0047
		4	a = 0.5027, k_1 = 0.5753, b = 0.5005, k_2 = 0.5753	0.9987	0.0120
		5	a = 0.3315, k = 0.5753, b = 0.3405, g = 0.5753, c = 0.3313, h = 0.5753	0.9987	0.0120
	60	1	k = 0.8838	0.9957	0.0235
		2	k = 0.7945, n = 1.2519	0.9998	0.0047
		3	a = 1.0409, k = 0.8064, c = -0.0359	0.9982	0.0152
		4	a = 0.5142, k_1 = 0.8898, b = 0.4950, k_2 = 0.8898	0.9958	0.0232
		5	a = 0.3401, k = 0.8898, b = 0.3409, g = 0.8898, c = 0.3282, h = 0.8898	0.9958	0.0232
	70	1	k = 0.9781	0.9943	0.0284
		2	k = 0.8745, n = 1.3348	0.9999	0.0031
		3	a = 1.0564, k = 0.8581, c = -0.0522	0.9979	0.0171
		4	a = 0.5158, k_1 = 0.9840, b = 0.4928, k_2 = 0.9840	0.9944	0.0281
		5	a = 0.3409, k = 0.9840, b = 0.3408, g = 0.9840, c = 0.3270, h = 0.9840	0.9944	0.0281

Temp: Temperature.

Artificial neural network (ANN)

Time, temperature and drying techniques were used to predict moisture ratio using ANN model. Table 3 shows the statistical results related to the training and validation of the multilayer feed-forward network structure of experimental data. The training data set were used to assess the optimum number of neurons and hidden layers for multilayer neural network modelling for determining the best predictive power. The results illustrated that the architecture with 2 hidden layers and 6 neurons [3, 3], obtained the best results as compared to those of 1 hidden layer [3, 6 and 9 neurons] and 2 hidden layers (12 and 18 neurons), respectively. Moreover, the networks were found to be susceptible to the number of neurons in their hidden layers. Thus, smaller neurons led to under-fitting, while too many neurons contributed to overfitting. From the results produced from ANN model, the highest R^2 and the lowest RMSE values of 1.0000 and 0.0003, respectively as listed in Table 3. These results indicated that ANN found higher results compared to page model (R^2 = 0.9999 and RMSE = 0.0031) as shown in Table 2. Zenoozian et al. (2014) found that 2 hidden layers and 30 neurons adequately predicted the moisture changes during the drying kinetics of pumpkin. ANN with 2 hidden layers has also been successful in predicting the drying behaviour of other fruits and vegetables such as pumpkin, pepper, apple slices during microwave-vacuum drying (Nadian et al., 2014; Jafari et al., 2016; Onwude et al., 2016a).

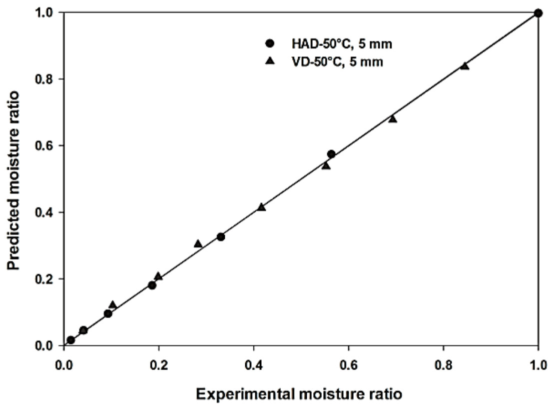

Figure 5. Predicted versus experimental moisture ratio data for logarithmic model at 50 °C for VD and HAD methods.

Table 3. ANN model statistical evaluation

No. hidden layer	No. Neurons	Training		Testing	
		R^2	RMSE	R^2	RMSE
1	3	0.9982	0.0167	0.9966	0.0290
1	6	0.9992	0.0038	0.9879	0.0363
1	9	0.9986	0.0209	0.9876	0.0819
2	**3.3**	1.0000	0.0003	0.9978	0.0089
2	6.6	0.9999	0.0013	0.9969	0.0275
2	9.9	0.9996	0.0019	0.9988	0.0269

Support vector regression (SVR)

The statistical results related to the training and validation of the SVR of persimmon drying experimental data are given in Table 4. Similar to ANN model, the training data set was used to evaluate the best filter and kernel type modelling for determining the best predictive power. The results showed that the normalize and standardize filter types with pearson universal kernel obtained the best results as compared to those of normalize with polynomial and RBF kernel and also standardize with polynomial, and RBF kernel, respectively. From the results of Table 2 and Table 4, it is clear that the SVR model produced the same value of R^2 (0.9999) as compared to the highest values of theoretical mathematical models. However, the SVR model found the lowest RMSE of 0.0013 as compared to the lowest value of RMSE (0.0031) using page model from the theoretical mathematical models. Few studies used SVR as a model in drying techniques. Das & Akpinar, (2018) applied SVR to investigate pear drying performance by different ways of convective heat transfer. The authors applied normalization and standardization filter to the target attribute with three kernel models namely polynomial kernel, pearson universal kernel and RBF kernel. They found that polynomial kernel found the lowest RMSE of 0.3351. Generally, more efficient results for computational intelligence methods can be obtained when the parameters of ANN and SVR are optimized (Khaled et al., 2018).

Table 4. Statistical results for SVR model

Filter type	Kernel type	Training		Testing	
		R^2	RMSE	R^2	RMSE
Normalize	Polynomial kernel	0.9080	0.1538	0.8718	0.1601
Normalize	**Pearson universal kernel**	**0.9999**	0.0013	0.9303	0.1197
Normalize	RBF kernel	0.9976	0.0262	0.9364	0.2056
Standardize	Polynomial kernel	0.9728	0.0780	0.8717	0.1604
Standardize	**Pearson universal kernel**	**0.9999**	0.0004	0.9361	0.1094
Standardize	RBF kernel	0.9973	0.0271	0.9360	0.1098

Color measurements

The quality properties of persimmon fruit slices during VD and HAD methods were determined based on the change in color parameters of L^*, a^*, and b^* and the total color change ΔE as given in Table 5. It can be seen clearly from Table 5 that that the lightness (L^*) of the samples using VD and HAD methods decreased significantly ($p \leq 0.05$) compared to the fresh samples. However, there was no significant difference between the lightness of samples using VD at 60 °C and 70 °C and also HAD from 50 to 70 °C for fresh samples. Lightness of samples using VD at 50 °C was significantly different compared to the fresh samples. Using VD significantly ($p \leq 0.05$) reduced the lightness of samples compared to HAD. VD at 50 °C showed the lowest lightness values of 52.560 ± 3.680. The redness/greenness (a^*) of samples reduced significantly ($p \leq 0.05$) compared to fresh samples. There was no significant difference between the redness/greenness of samples using VD at 50 °C and HAD at 50 °C in comparison with fresh samples. Generally, the values of redness/greenness (a^*) using HAD were higher than VD, except the value using VD at 60 °C. The lowest value of redness/greenness (a^*) was 11.684 ± 0.259 using HAD at 60 °C. Similar results were found for the yellowness/blueness (b^*), where the color of all dried samples reduced significantly ($p \leq 0.05$) compared to the fresh samples. In addition, there was no significant difference between the b^* of fresh samples with those of dried samples using VD at 60 °C and 70 °C, and HAD from 50 to 70 °C respectively. Dried samples using VD at 50 °C showed yellowness/blueness mean value of 56.040 ± 3.410. The values of total color change using the VD method were higher compared to the HAD method. The highest value of 17.790 ± 4.100 was found for VD at 50 °C. This could be due to the mechanisms involved in VD which thus gradually sucks the air compared to HAD. Generally, the change of color properties of the fresh samples under different drying methods is due to the increased sample temperature resulting in increased enzymatic and non-enzymatic chemical reactions of the product (Nozad et al., 2016).

Table 5. Drying methods and color parameters of persimmon fruit.

Drying method	Temp ˚C	Color properties for different sample thickness			
		L*	a*	b*	ΔE
	Fresh	61.425 ± 2.533^{a}	26.282 ± 0.747^{a}	65.698 ± 2.126^{a}	-
VD	50	52.560 ± 3.680^{b}	$20.69 \pm 1.95bc^{ab}$	56.040 ± 3.410^{b}	17.790 ± 4.100^{a}
	60	56.161 ± 3.476^{ab}	16.280 ± 3.598^{bc}	61.073 ± 2.834^{ab}	15.859 ± 1.822^{ab}
	70	59.646 ± 0.413^{ab}	12.804 ± 0.764^{c}	62.265 ± 0.076^{ab}	14.568 ± 0.669^{ab}
HAD	50	56.930 ± 1.152^{ab}	23.438 ± 0.258^{a}	61.762 ± 1.088^{ab}	9.528 ± 1.568^{b}
	60	60.250 ± 2.229^{a}	11.684 ± 0.259^{c}	64.831 ± 2.231^{a}	14.928 ± 0.680^{ab}
	70	59.846 ± 1.149^{ab}	17.259 ± 1.164^{bc}	64.491 ± 0.750^{a}	10.140 ± 0.405^{b}

Temp: Temperature. Data represents the mean and three replicates (±standard error). Different letters at the same column indicates statistical difference for Duncan test, $p < 0.05$ for drying temperature.

CONCLUSIONS

The study investigated the potential of using computational intelligence as a modelling tool for predicting the drying process of persimmon fruit slices (samples). The results showed that VD and HAD had a significant effect on the drying kinetics and color properties of samples. As increase in drying temperature influenced the drying kinetics using HAD and VD. Dried samples using HAD showed significant color attributes compared to VD. The thin-layer modelling results showed that Page and logarithmic model can adequately ($R^2 = 0.9999$) described the drying kinetics of samples. Accurate results were found using ANN ($R^2 = 1.0000$) model. Computational intelligence methods and theoretical models gave similar results. However, the ANN and SVR models are able to describe a wider range of experimental data whereas the application of theoretical models is limited to specific experimental conditions in most cases. Thus, the ANN and SVR models may be considered as suitable alternative modelling methods for describing the drying behaviour of samples. On the other hand, computational intelligence methods can be successfully applied to industrial drying processes and operations as well as online monitoring and control.

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REFERENCES

Aboltins, A., Rubina, T. & Palabinskis, J. 2018. Shrinkage effect on diffusion coefficient during carrot drying. *Agronomy Reseach* **16**(S2), 1301–1311.

Aghbashlo, M., Kianmehr, M.H., Khani, S., Ghasemi, M. & others. 2009. Mathematical modelling of thin-layer drying of carrot. *International Agrophysics* **23**(4), 313–317.

Akoy, E.O.M. 2014. Experimental characterization and modeling of thin-layer drying of mango slices. *International Food Research Journal* **21**(5).

ASAE. 2005. Moisture measurement – unground grain and seeds. *American Society of Agricultural and Biological Engineers* 2–4.

Ashtiani, S.M., Salarikia, A. & Golzarian, M.R. 2017. Analyzing drying characteristics and modeling of thin layers of peppermint leaves under hot-air and infrared treatments. *Information Processing in Agriculture* **4**(2), 128–139.

Bahmani, A., Jafari, S.M., Shahidi, S.-A. & Dehnad, D. 2016. Mass transfer kinetics of eggplant during osmotic dehydration by neural networks. *Journal of Food Processing and Preservation* **40(**5), 815–827.

Bai, J., Xiao, H., Ma, H. & Zhou, C. 2018. Artificial neural network modeling of drying kinetics and color changes of ginkgo biloba seeds during microwave drying process. *Journal of Food Quality* **2018**, 1–9.

Beigi, M. & Ahmadi, I. 2018. Artificial neural networks modeling of kinetic curves of celeriac (Apium graveolens L) in vacuum drying. *Food Science and Technology* **2061**, 1–6.

Bozkir, H., Rayman, A., Serdar, E., Metin, G. & Baysal, T. 2019. Ultrasonics - sonochemistry influence of ultrasound and osmotic dehydration pretreatments on drying and quality properties of persimmon fruit. *Ultrasonics - Sonochemistry 54*(December 2018), 135–141.

Çalışkan, G. & Nur Dirim, E. 2015. Freeze drying kinetics of persimmon puree. *GIDA*, **40**(1), 9–14.

Das, M. & Akpinar, E.K. 2018. Investigation of pear drying performance by different methods and regression of convective heat transfer coefficient with support vector machine. *Applied Sciences* **8**, 2–16.

Doymaz, I. 2012. Evaluation of some thin-layer drying models of persimmon slices (*Diospyros kaki L*). *Energy Conversion & Management* **56**, 199–205.

Górnicki, K., Kaleta, A. & Choińska, A. 2020. Suitable model for thin-layer drying of root vegetables and onion. *International Agrophysics* **34**(1), 79–86.

Henderson, S.M. 1974. Progress in developing the thin layer drying equation. *Transactions of the ASAE* **17**(6), 1167–1168.

Jafari, S.M., Ghanbari, V., Ganje, M. & Dehnad, D. 2016. Modeling the drying kinetics of green bell pepper in a heat pump assisted fluidized bed dryer. *Journal of Food Quality* **39**, 98–108.

Kaleta, A. & Górnicki, K. 2010. Some remarks on evaluation of drying models of red beet particles. *Energy Conversion and Management* **51**(12), 2967–2978.

Kaleta, A., Górnicki, K., Winiczenko, R. & Chojnacka, A. 2013. Evaluation of drying models of apple (var. Ligol) dried in a fluidized bed dryer. *Energy Conversion and Management* **67**, 179–185.

Karaman, S., Toker, O.S., Çam, M., Hayta, M., Kayacier, A., Karaman, S., … Kayacier, A. 2014. Bioactive and physicochemical properties of persimmon as affected by drying methods. *Drying Technology* **32**, 258–267.

Karasu, S., Akcicek, A. & Kayacan, S. 2019. Effects of different drying methods on drying kinetics, microstructure, color, and the rehydration ratio of minced meat. *Foods* **8**, 2–14.

Karathanos, V.T. 1999. Determination of water content of dried fruits by drying kinetics. *Journal of Food Engineering* **39**(4), 337–344.

Khaled, A.Y., Aziz, S.A., Bejo, S.K., Nawi, N.M., Jamaludin, D., Ul, N. & Ibrahim, A. 2020. A comparative study on dimensionality reduction of dielectric spectral data for the classi fi cation of basal stem rot (BSR) disease in oil palm. *Computers and Electronics in Agriculture* **170**, 105288.

Khaled, A.Y., Aziz, S.A., Bejo, S.K., Nawi, N.M. & Seman, I.A. 2018. Spectral features selection and classification of oil palm leaves infected by Basal stem rot (BSR) disease using dielectric spectroscopy. *Computer and Electronics in Agriculture* **144**, 297–309.

Kırbaş, İ., Doğuş, A., Şirin, C. & Usta, H. 2019. Modeling and developing a smart interface for various drying methods of pomelo fruit (Citrus maxima) peel using machine learning approaches. *Computer and Electronics in Agriculture* **165**(June), 2–8.

Lewis, W. 1921. The rate of drying of solid materials. *The Journal of Industrial and Engineering Chemistry 13*(5), 427–432.

Movagharnejad, K. & Nikzad, M. 2007. Modeling of tomato drying using artificial neural network. *Computer and Electronics in Agriculture* **59**, 78–85.

Nadian, M.H., Rafiee, S., Aghbashlo, M., Hosseinpour, S. & Mohtasebi, S.S. 2014. Continuous real-time monitoring and neural network modelling of apple slices color changes during hot air Drying. *Food and Bioproducts Processing*, pp. 1–40.

Nozad, M., Khojastehpour, M. & Tabasizadeh, M. 2016. Characterization of hot-air drying and infrared drying of spearmint (Mentha spicata L) leaves. *Journal of Food Measurement and Characterization* **10**, 466–473.

Omari, A., Behroozi-Khazaei, N. & Sharifian, F. 2018. Drying kinetic and artificial neural network modeling of mushroom drying process in microwave-hot air dryer. *Food Process Engineering* **41**(7), 1–10.

Onwude, D.I., Hashim, N., Abdan, K., Janius, R. & Chen, G. 2018. Modelling the mid-infrared drying of sweet potato : kinetics, mass and heat transfer parameters, and energy consumption. *Heat and Mass Transfer* **54**, 2917–2933.

Onwude, D.I., Hashim, N., Janius, R.B., Nawi, N. & Abdan, K. 2016a. Modelling the convective drying process of pumpkin (Cucurbita moschata) using an artificial neural network. *International Food Research Journal* **23**(Suppl), S237–S243.

Onwude, D.I., Hashim, N., Janius, R.B., Nawi, N.M. & Abdan, K. 2016b. Modeling the thin-layer drying of fruits and vegetables: A review. *Comprehensive Reviews in Food Science and Food Safety* **15**(3), 599–618.

Ozola, L. & Kampuse, S. 2018. The influence of drying method to the changes of bioactive compounds in lingonberry by-products. *Agronomy Reseach* **16**(4), 1781–1795.

Page, G. 1949. *Factors influencing the maximum rates of air drying shelled corn in thin layers*. Purdue University, ProQuest Dissertations Publ., West Latayette, IN, USA.

Rodríguez, J., Clemente, G., Sanjuán, N. & Bon, J. 2014. Modelling drying kinetics of thyme (Thymus vulgaris L.): Theoretical and empirical models, and neural networks. *Food Science and Technology International* **20**(1), 13–22.

Tekin, Z.H. & Baslar, M. 2018. The effect of ultrasound-assisted vacuum drying on the drying rate and quality of red peppers. Journal of Thermal Analysis and Calorimetry **132**(2), 1131–1143.

Tomsone, L., Kince, T. & Ozola, L. 2018. Effect of drying technologies on bioactive compounds maintenance in pumpkin by-products. *Agronomy Reseach* **16**(4), 1728–1741.

Xie, C., Li, X., Shao, Y. & He, Y. 2014. Color measurement of tea leaves at different drying periods using hyperspectral imaging technique. *PloS One* (61201073), 1–15.

Yagcioglu, A. 1999. Drying characteristic of laurel leaves under different conditions. In Drying Characteristics of Laurel Leaves under Different Conditions. *Proceedings of the 7th International Congress on Agricultural Mechanization and Energy*, Adana, 26-27 May 1999, pp. 565–569.

Younis, M., Abdelkarim, D. & El-abdein, A.Z. 2018. Saudi journal of biological sciences kinetics and mathematical modeling of infrared thin-layer drying of garlic slices. *Saudi Journal of Biological Sciences* **25**(2), 332–338.

Zenoozian, M.S., Devahastin, S., Razavi, M.A., Shahidi, F. & Poreza, H.R. 2014. Use of artificial neural network and image analysis to predict physical properties of osmotically dehydrated pumpkin. *Drying Technology* **26**, 37–41.

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The biological basis for the use of protein growth stimulant made from cattle split for wheat foliar feeding and disease suppression

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Abstract. The new modern preparation – protein growth stimulant – was generated in accordance with technology of employees of Saint-Petersburg ITMO University and Saint-Petersburg State Agrarian University. Biological activity of the preparation was determined by measurements of 20 indicators of the wheat productivity. In addition, 16 indicators of different types of pathogenesis were determined. These can be formed at distribution of *Helminthosporium* root rot, wheat rust species, powdery mildew and wheat leaf blotch. The use of the protein growth stimulant promoted increase of potential yield in 80% of samples. In comparison with the control, 15 wheat varieties, treated with the preparation, showed an increase in the main productivity indicators: the length of the spike, the number of spikelets per spike, the weight of 1,000 grains, the productive tilling capacity and the general bushiness. The intensity of *Helminthosporium* root rot development decreased 11.9% (it was found in 53.3% of samples) and the wheat leaf blotch by 15.6% (in 66.7% of samples). The wheat brown rust development intensity decreased insignificantly (3.6% compared to the control). In the same time, values of the pathogen pustule area decreased at average by 79.8%. There was an increase in total nitrogen in wheat leaves at 92% of samples. As was revealed, the effectiveness of the protein growth stimulant largely depends on the wheat variety. To conclude, the prospect of using the new effective protein growth stimulant to increase productivity of wheat and protection from diseases was shown.

Key words: diseases of wheat, growth stimulants, soft wheat, the elements of productivity.

INTRODUCTION

Environmental safety of food production is one of the main requirements for agricultural products of modern world agroindustrial sector. Intensive nature management with its chemicalization and receipts of toxic substances in agrocenoses causes degradation of soil cover and destruction of microflora. All these factors lead to an increased content of toxic substances and compounds in crops of cultivated plants. Russia, along with many economically developed countries, revises the concept of agricultural production development, shifts it in the direction of reducing the external anthropogenic impact on agrocenoses and for creating an enabling environment for the realization of their own potential (Pavlyushin & Lysov, 2019). Organic agriculture is one of the forms for realization of this task. It assumes application of natural growth stimulants as inducers of plant resistance to diseases (Čepulienė & Jodaugienė, 2018; Kolesnikov et al., 2018; Siah et al., 2018; Khan et al., 2019) and yield increase.

Active study of the effect of amino acid fertilizing on plants begun in the 70–80´s of the last century and it continues to the present (Che et al., 2011; Sudisha et al., 2011; Hammad & Ali, 2014). Many scientists have noted that amino acids activate the mechanisms of plant growth after exposure to stress and low temperature (Soro, 1985), improve the pollen fertility and the formation of fruit ovaries (Marcucci, 1984), increase the ability of assimilation of nutrients (Stoyanov, 1981), crop yield (Gulewicz et al., 1997) and pest and disease resistance (Kovacs et al., 1986; Wang et al., 2019). Biostimulants (amino acids and yeast) have a positive effect on most of the physiological signs and wheat crop yield elements, if wheat has experienced water stress during drought. This contributes to overcoming the harmful effects of high temperatures on crops (Hammad & Ali, 2014).

A number of studies have found that plants are able to quickly and better absorb natural α-amino acids (from which proteins are built) of optically active L-configuration. L-α-amino acids are easily absorbed by plants and quickly incorporated into the metabolism as their own (Kretovich, 1986). Glutamate is a universal amino acid and is central to both mammals and higher plants. As a result of post–harvest treatment of pear fruits with L–glutamate (L-Glu), there was a significant inhibition of the development of blue mold on them caused by *Penicillium expansum Link ex. Thom.* at different storage temperatures (Jin et al., 2019). The combined use of L–glutamate (or glycine) complexes with copper-containing bactericides reduced the side toxic effects of copper on plants (Niu et al., 2018). Biochemists have found that unsaturated amino acids (UnsAA (BCAAs)) affect the activity of various biological processes. However, the mechanisms of their action on plant cells are poorly understood, so their research is currently underway (Roblin et al., 2016).

In Russia, such amino-containing preparations are registered as an anti-stress agent – Megaphol; stimulator of harmonious development- Viva, and stimulator of root formation- Radipharm (Valagro). Despite the effectiveness of the action, their production and application did not have a large-scale use in crop production due to economic reasons. Currently, a promising direction is the creation of new domestic biological plant growth stimulants obtained by chemical hydrolysis from by-products of slaughter animals (Kutsakova et al., 2013, RF patent No. 2533037). When using this stimulant (hereinafter named as growth stimulant) on fruit and berry crops, their earlier maturation, increase in productivity and improving the quality of fruit and strengthening the protective mechanisms of phytoimmunity was noted (Kremenevskaya et al., 2017). The preparation is undergoing for active testing in the School of Biotechnology and Cryogenic Systems, ITMO University at the Department of Plant protection and quarantine of Saint-Petersburg State Agrarian University. It is derived from a by-product of cattle processing (list of cattle shins). The mechanism of action of amino acids containing preparations assumes their good digestibility by plants and absence of phytotoxicity. Glutamic acid, cysteine, glycine, histidine, lysine are included in the complex form of chelated compounds with trace elements. Tyrosine, arginine, alanine, proline, serine, threonine and valine in its composition stimulate plant metabolism and enhance the adaptive potential of plants to environmental factors (Bityuckij, 1999).

The aim of the study wass to substantiate the prospects of using the protein growth stimulant from a by-product of cattle processing to increase the yield and quality of grain and to reduce the injuriousness of wheat pathogens.

MATERIALS AND METHODS

Production of the protein growth stimulator

Canned beef split was used to receive the protein hydrolysate as the stimulator. Hydrolysis was carried out according to the research methods described by Kremenevskaya et al., 2017. The composition of raw materials was always monitored to avoid possible negative divergences and their impact on the finished product. The hydrolysate was dried by the use of the semi-industrial unit (Kutsakova, 2004). Studies of the amino acid composition of the stimulant were carried out in two-dimensional liquid chromatography system using Nexera-e software (Shimadzu Corporation, Japan) in the Leningradskaya inter-regional veterinary laboratory of feed and grain safety and quality. The moisture content of the samples was calculated, using the universal humidity analyzer MX-50 (AND, Japan); the sodium chloride concentration was measured using the PAL-SALT Mohr salinometer (ATAGO, Japan), and the pH and calcium ion content were measured using the I – 510 microprocessor ionomer (Aquilon company, Russia).

Plant material and location of the experiment

Experimental research was carried out in the experimental field of Pushkin laboratories of N.I. Vavilov Federal research Center of the all-Russian Institute of plant genetic resources (VIR). The field experiment to determine the effect of protein growth stimulant on the productivity and intensity of wheat diseases was performed on 15 samples of wheat of different origin: LP–588–1–06, c–65446 (catalog number) (Germany); Uralosibirskaya, c – 65244 (Russia, Omsk region); c–32666 (Russia, Sverdlovsk region); Orenburgskaya 22, i–147624 (Russia, Orenburg region); Pamyati Judina, c–65243 (Russia, Irkutsk region); Leningradskaya 97, c–62935 (Russia, Leningrad region); Amurskaya Krasnokoloska, c–32095 (Russia, Amur region); Ulianovskaya 100, c–65250 (Russia, Ulyanovsk region); Kampanin, c–65445 (Germany); Vasilisa, c–65443 (Belarus); Tyumenskaya 30, c–65248 (Russia, Tyumen region); Krasnouphimskaya 110, c–65478; Tuliaykovskaya 108, c–65452 (Russia, Samara region); Tuliaykovskaya 110, c–65454 (Russia, Samara region); Tyumenskaya 29, c–65247 (Russia, Tyumen region). These samples were provided for study by the VIR wheat genetic resources Department.

Treatment of plants with the protein stimulant and the wheat productivity analysis

Prophylactic spraying of wheat with a growth stimulant was carried out in three-fold repetition: the phases of tillering, stem elongation, and beginning of ear formation.

The concentration of the aqueous solution of the stimulant was 195 mg L^{-1}. The phases of plant development were determined by the generally accepted Zadoks scale (Zadoks et al., 1974). Wheat productivity was studied in three-fold repetition in the phase of earing-flowering and maturation by a set of indicators characterizing the morphological characteristics of plants and the structure of yield. In the earing–flowering phase, a complex of plant indicators was studied: productive and general bushiness (pieces), plant phase (digital code, Zadoks scale), flag and pre-flag leaf area (cm^{-2}), height of plants (cm) length of spike (cm), number of spikelets in the spike (piece), weight of spike (g). In addition, the number and length of roots (the main embryonic root, embryonic coleoptile and roots) extending from the epicotyl was determined. The indices of root mass and vegetative part of plants were calculated. The sample size for each variant of the experiment was 10–15 plants, which provided a reliable result for measuring the complex of the indicators at $P > 0.95$.

In the maturation phase (phase of full ripeness), the structure of the following wheat yield indicators was studied: number of the spikelets per spike; the spike length, cm; the spike weight; the grain weight per spike; the number of grains per spike; and the 1,000 grains weight. The potential biological yield of a single wheat plant (g $plant^{-1}$) was estimated in accordance with the values of the productive bushiness of one plant and the grain weight per spike. The potential yield (Y_p) of wheat varieties in relation to the area of sowing (t ha^{-1}) was calculated by the productive bushiness, the grain weight per spike, and the number of plants sown per one m^2 according to the original formula:

$$Y_p = M_k \cdot К_p \cdot P_p \cdot 10{,}000 \qquad (1)$$

where M_k – grain weight per spike (t); K_p – productive bushiness of the sample; P_p – sowing density (number of plants per one m^2).

In estimation the grain weight per spike and productive bushiness for each variant of the experiment, the amount of sampling was 10–15 plants.

The total nitrogen content in the flag leaves of soft wheat was calculated at the beginning of flowering stage using of the photoelectrometric method (Ermakov, 1987) in accordance with the GOST 10846-91.

Methods for assessing the intensity of wheat root rot

Assessment of the degree of damage to plants by root rot was carried out in laboratory conditions in the phase when wheat finished tillering and earing- lowering according to the scale: 0 – epicotyl without lesion, 1 – single spots on epicotyl; 2 – strong defeat; 3 – fatal defeat, a plant is died. In each variant of the experiment, 20 plants were evaluated. The development of root rot according to the variants of the experiment was determined, taking into account the weighted average of the degree of plant damage (Popov, 2011):

$$R_r = \frac{\sum(\mathrm{a} \cdot b) \cdot 100}{A \cdot K} \qquad (2)$$

where R_r – development of root rot; a – number of plants with the same signs of damage; b – the corresponding score; A – number of plants registered (healthy and sick); K – the highest score of the accounting scale.

From each experimental plot, 20 plants with the roots were dug out randomly. Underground plant parts were carefully removed from the soil and then washed in running water. The main germinal roots, germinal and coleoptile roots, and nodal roots were visually analyzed. The root rot development was evaluated in a natural infectious background. The main causative agent of the disease was *Bipolaris sorokiniana (Sacc.) Shoem*.

Methods for assessing the intensity of wheat foliar diseases

The development intensity of the pathogens of wheat leaves was taken into account in the following phases and stages of wheat ontogenesis: the phase of wheat tillering (finished tillering), the phase of flag leaf (the leaf-tube formation, the last leaf sheath opening), the phase of earings (the end of the earings), phase of flowerings (the start of flowerings and the end of the flowering), maturation (milk ripeness of grain; wax ripeness). The intensity of the flag and pre-flag wheat leaves defeat by the powdery mildew (*Blumeria graminis* Speer.) was assessed visually according to the degree of plant damage (Fig. 1), as well as additional indicators – the number and area of powdery mildew stains.

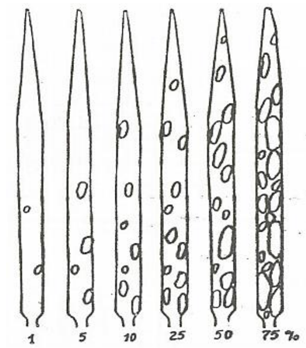

Figure 1. The account scale for the intensity of cereals defeat by the powdery mildew.

The number of powdery mildew stains was determined by counting them, based on the area of the wheat leaf: $S_п = 0.7 \cdot L \cdot S$, (where 0.7 is the narrowing factor, L – length of leaf (cm), S – width of leaf (cm)). When a large number of spots with a touch of powdery mildew on the leaf was registered, their number was first counted in one cm^2 of the leaf, and then it was transferred to the value to the entire area of the wheat leaf. For each variant of the experiment, the values of the above indicators of the wheat powdery mildew pathogenesis were determined by the results of the analysis of 15 flag and 15 pre-flag leaves.

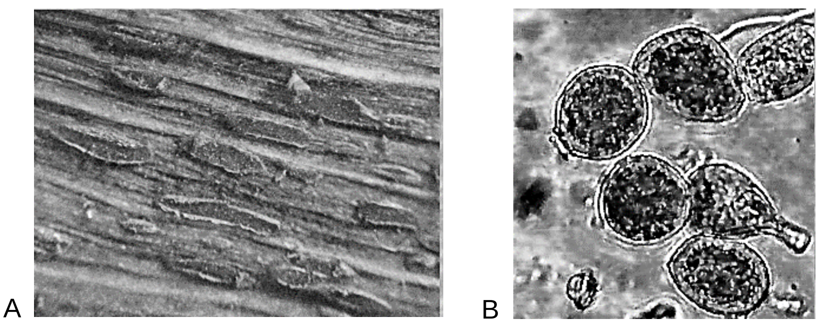

Figure 2. Symptoms of wheat brown rust on the soft wheat leaves (A – uredospore pustules arranged on the flag leaf randomly, magnification of 16^x; B – spherical uredospores, magnification of 800^x).

Defeat of wheat flag and pre-flag leaves by brown rust *Puccinia recondita Rob. ex Desm. f. sp. tritici Eriks.* (Fig. 2) was counted on the Peterson scale (Fig. 3). The number of pustules per leaf and the area of the pustule were used as an additional phytopathological parameters. The number of pustules was determined by counting them on wheat leaves using the MBS–10 microscope (Lytkarino Optical Glass Factory, Russia).

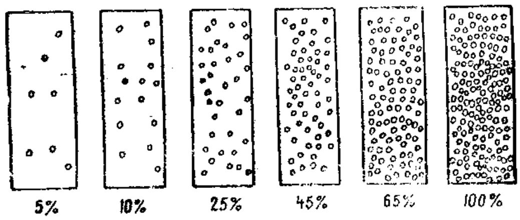

Figure 3. The Peterson account scale for the plant defeat by the brown rust (Peterson et al, 1948; The methods…,1988).

The degree of sample damage by wheat leaf blotch (*Stagonospora nodorum Castell.et Germano*) was determined according to the visual scale of James (James, 1971). For each experimental variant, the intensity of leaf blotch development (Fig. 4) was characterized by the results of the analysis of 15 flag and 15 pre-flag leaves.

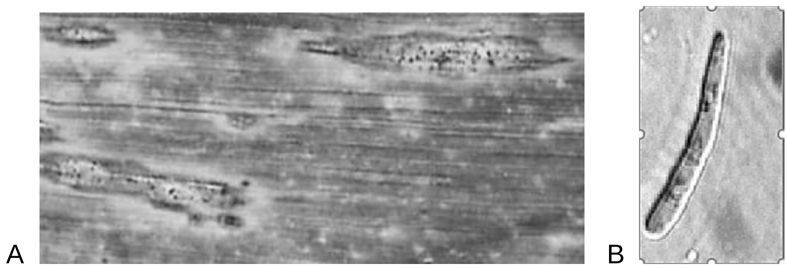

Figure 4. Symptoms of wheat leaf blotch on the soft wheat leaves (A – spots with pycnidia, magnification of 16^x; B – pycnidiospore, magnification of 800^x).

The intensity of wheat defeat by yellow rust (*Puccinia striiformis* West. (*Puccinia glumarum* Eriks. et Henn.) (Fig. 5) was assessed on the Manners scale (Fig. 6); and, in addition, visually, with using a microscope MBS-10 (Lytkarino Optical Glass Plant, Russia). The number of pustules (total per leaf), number of yellow rust stripes with pustules, the length of stripes with pustules, the area of the pustule and their number in the stripe were used as an additional indicators of pathogenesis.

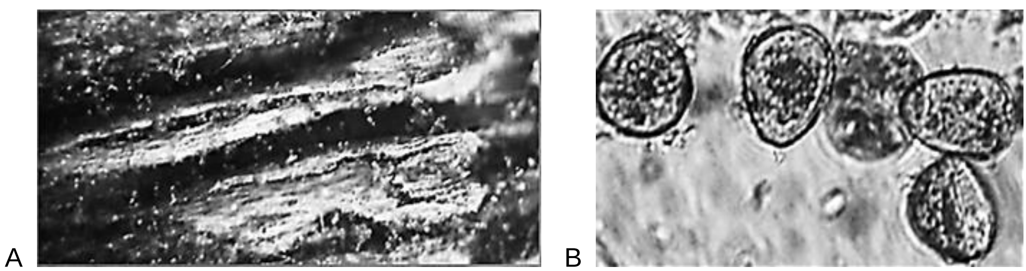

Figure 5. The symptoms of wheat yellow rust on the leaves of soft wheat (A – uredospore pustules in strips on the flag leaf, magnification of 56^x; B – rounded uredospores, magnification of 800^x).

For each variant of the experiment, the values of the above indicators of yellow rust pathogenesis were determined by the results of the analysis of 15 flag and 15 pre-flag leaves.

The size of the powdery mildew stains and rust's pustules (brown & yellow rust), which were formed in pathogenesis on wheat leaves, was determined using eyepiece and stage micrometers.

The values of pustule area and powdery mildew stains (infectious structures $S_{i.s.}$ were calculated under the assumption on their elliptic form using the expression:

$$S_{i.s.} = m \cdot \pi \cdot a \cdot b \qquad (3)$$

where a and b – the values of the semiaxes of the ellipse (in the lines of the eyepiece micrometer), m – the scale factor of the microscope.

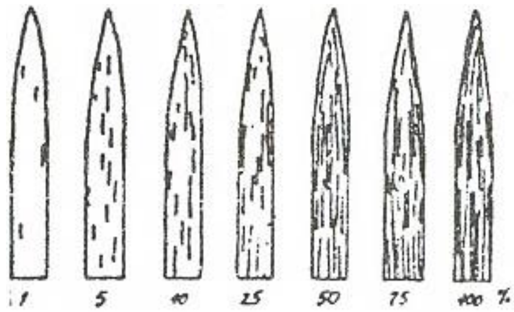

Figure 6. The Manners account scale for the degree of plant defeat by the yellow rust (Babayantc et al., 1988).

The use of this complex of pathogenesis indicators allowed to expand the range of statistical analysis methods applicable to the study and to increase the accuracy of the experiment in determining the biological effectiveness of a protein growth stimulant. Statistical analysis of the results was carried out in the programs SPSS 21.0, Statistica 6.0 and Excel 2016. In the calculations, the methods of parametric statistics were used (based on *mean* and their *standard errors ±SE*; and *95% confidence intervals* and the *Student's t-test*).

RESULTS AND DISCUSSION

Effect of the protein growth stimulant on the wheat yield

The main active ingredient in the preparation, which has a stimulating effect, is the aminoacid glycine (1.20 μmol mg^{-1}). Other significant amino acids in the stimulant were: proline (0.63 μmol mg^{-1}), alanine (0.58 μmol mg^{-1}), glutamic acid (0.41 μmol mg^{-1}) and aspartic acid (0.155 μmol mg^{-1}). The concentration of the aqueous solution of the stimulant was 0.195 g L^{-1}.

At the first stage of the study, the comparison of wheat productivity indicators in the variants of the experiment was carried out: when treated with a protein growth stimulant and without treatment (control group). Growth stimulant increased the yield in 80% of samples (Fig. 7). Compared with the control (untreated samples), growth stimulator had the greatest effect on the biological yield of five wheat varieties: Tuliaykovskaya 108, c–65452 (103%, t = 2.6); Pamyati Judina, c–65243 (86.8%, t = 2.8); Krasnouphimskaya 110, c–65478 (77.9%, t = 2.9); Uralosibirskaya, c – 65244 (56.4%, t = 3.1); Orenburgskaya 22, i–147624 (43.2%, t = 2.0), where t is the *Student's criterion*.

It should be noted that highly productive varieties take out a large amount of nutrients from the soil and consume a lot of water. Therefore, such varieties require a high level of agricultural technology. If there are no such conditions, a potentially more productive variety not only does not give a yield increase, but can also lose to another variety, less productive, but also less demanding to the cultivation conditions.

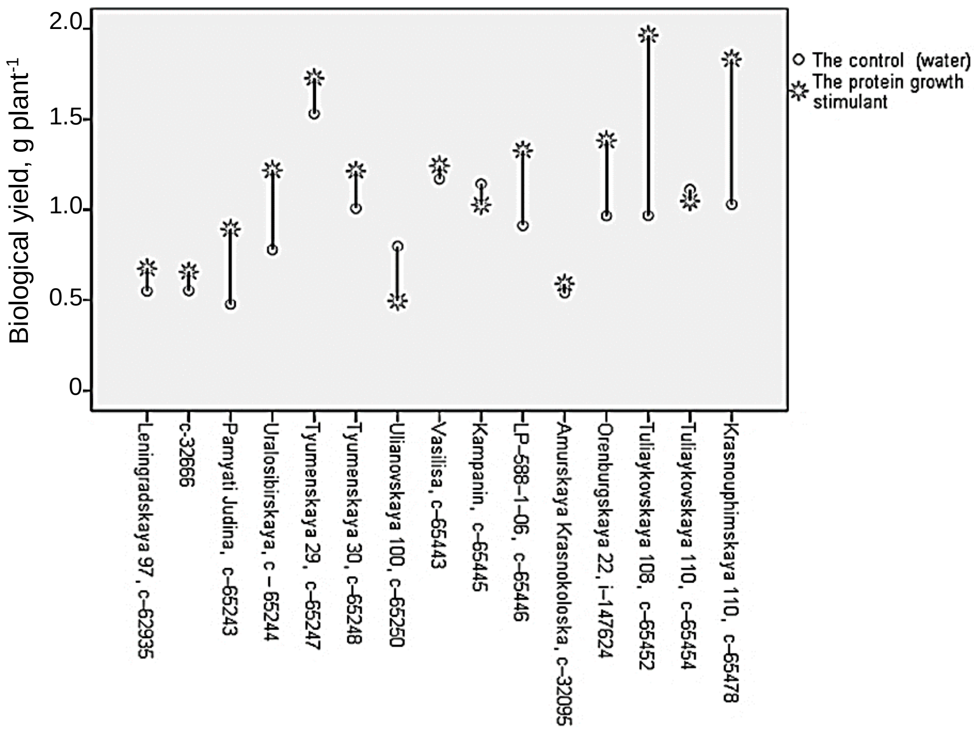

Figure 7. Changes in the wheat biological yield when using the protein growth stimulator.

If the growth stimulant was used, the increase of the main indicators of wheat productivity was noted as follows:

- productive bushiness – 15.4% (found in 46.7% of samples, but the increase in the values of the indicator was not statistically significant);
- total bushiness – 22.5% (revealed in 60.0% of samples, statistically significant in 26.7% of samples);
- spike length – 17.9% (revealed in 73.3% of samples, statistically significant in 46.7% of samples);
- the number of spikelets per spike – 16.1% (revealed in 73.3% of samples, statistically significant in 46.7% of samples);
- weight of 1,000 grains – 11.5% (73.3% of samples, statistically significant in 33.3% of samples);
- spike weight – 32.9% (found in 66.7% of samples, statistically significant in 40.0% of samples).

General and productive bushiness usually makes up for density in the field and it is a useful biological adaptation of plants to environmental conditions. When using a protein growth stimulant, its predominant effect on the overall bushiness of plants was revealed. The most important indicators that directly determine the wheat yield are the spike length, the number of spikelets per spike, the 1,000 grains weight and the spike weight. To the greatest extent, the plant growth stimulant had an impact on the growth of the spike length and the number of spikelets per spike. In the analysis of positive and reliably positive changes (according to the *Student's test* at $P < 0.05$), protein growth stimulant exerted the most effect on the spike weight, the 1,000 grains weight, the number of grains per spike, the number of spikelets per spike, the spike length and the total tillering (Fig. 8).

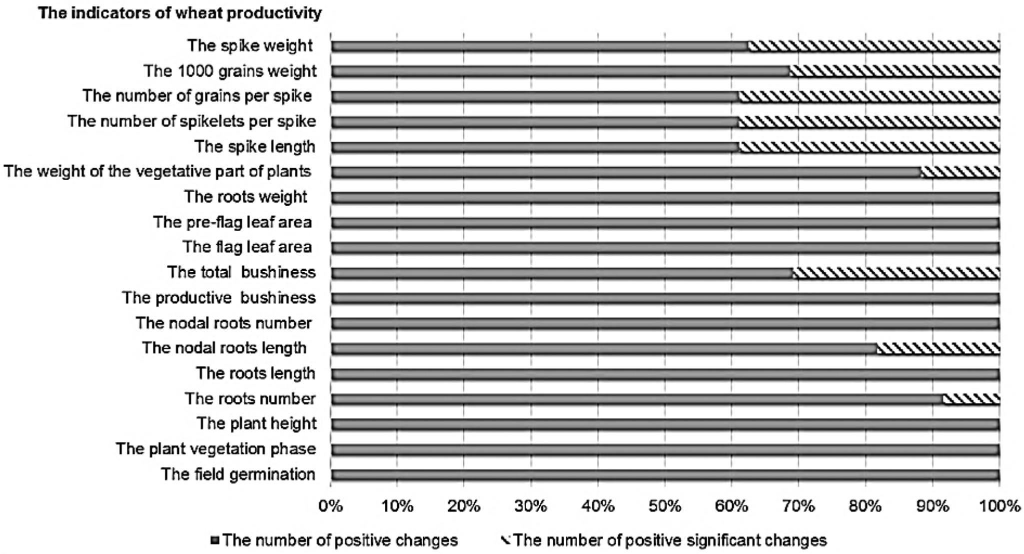

Figure 8. The number of changes in the wheat productivity indicators values when using the protein growth stimulator compared to the control (%).

The growth of the wheat flag leaf area directly correlates with the increase in photosynthetic activity of the plant and determines the increase in the yield and is also related to the spike length and the number of spikelets per spike. In addition, the growth stimulant caused the acceleration of plant development in the ontogenesis phases (by 5.9% in 66.7% of samples); increase in plant height (11.5% in 86.7% of samples), flag leaf area (17.4% in 60.0% of samples), pre-flag leaf area (8.9% in 33.3% of samples), and weight of roots (by 34.9% in 80.0% of samples). Wheat resistance to lodging is directly related to the height of plants. However, short-stemmed wheat forms suffer from drought and, in particular, they are not adapted to cultivation in continental conditions of some regions of Russia, for example Western Siberia.

It should be noted that the total N content in the flag leaves during the period of flowering is able to have significant influence on yield of wheat. In the number of experiments, a close positive relationship was observed between the grain protein content and N content of the flag leaves, especially at nitrogen evaluation in the flowering – the beginning of grain formation. A close negative correlation was revealed between the concentration of amino acids in wheat leaf juice and the concentration of gliadins and glutenins; a close positive correlation was found between the concentration of amino acids in leaf juice and the content of water-soluble proteins, globulins, non-extractable proteins, as well as the acid and alkaline proteases activity (Novikov, 2017). Significantly, foliar treatment with the protein growth stimulant caused an increase in the total N in wheat leaves in 92% of samples by an average of 84.6%.

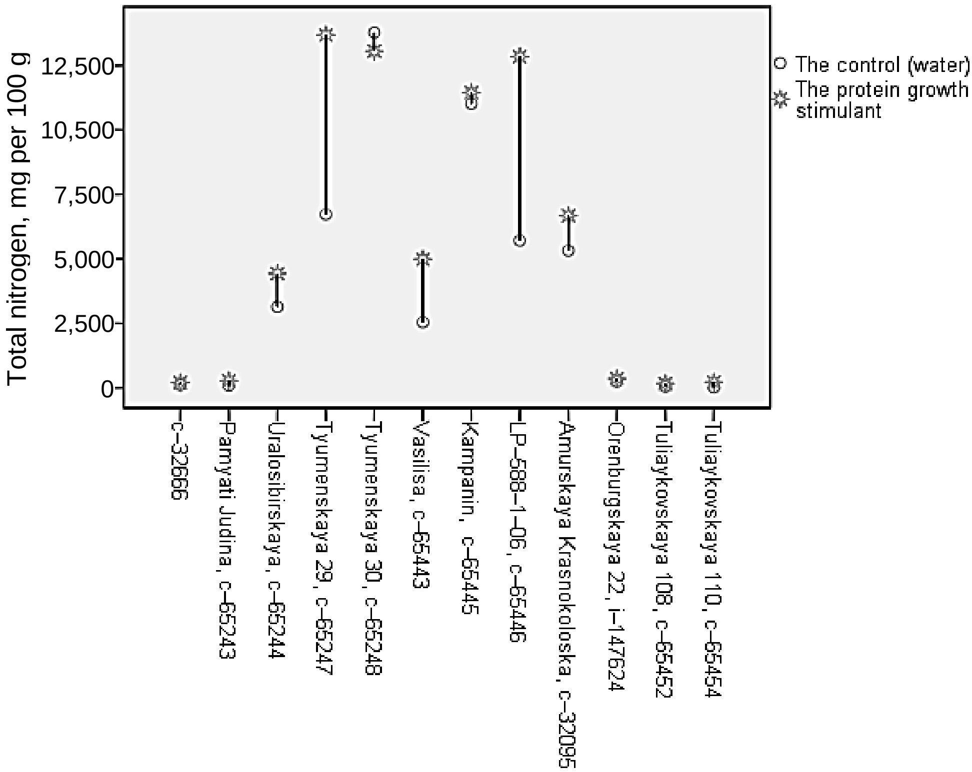

Figure 9. Changes of the total nitrogen content in the flag leaves at the beginning of wheat flowering when using the protein growth stimulator.

Effect of the protein growth stimulant on suppression of wheat diseases

In the second stage of the study, the effect of protein growth stimulant on the intensity of wheat diseases was studied. The use of the stimulator showed that the intensity of *Helminthosporium* root rot decreased by 11.9% in 53.3% of the samples. To the greatest extent (22%), a decrease in the development of the disease was observed on wheat cultivar Pamyati Judina, c–65243, and 17% on cultivar Tyumenskaya 29, c-65247. *Helminthosporium* rot of wheat belongs to the group of the most harmful diseases of ecological and parasitic nature, i.e. pathogen is present in the soil and affects weakened plants. It can be assumed that the use of the growth stimulant increased the adaptive potential of plants to external environmental factors, including this disease.

When plants were treated with the growth stimulant, there was a decrease in the wheat brown rust development intensity in 40% of the studied samples by 3.6%. In addition, use of growth stimulant caused a decrease in the values of the pustule area of wheat brown rust in 60% of the samples (an average at 79.8%) – the most important indicator of varieties to disease resistance. In this regard, it can be assumed that the protein stimulator of plant growth contributed to the activation of phytoimmunity reactions to the disease and after a number of additional experiments, it could be recommended as an inducer of wheat resistance to brown rust.

Use of growth stimulant decreased the intensity of wheat leaf blotch development on the pre-flag leaves 15.6%, compared to the control. This pattern was found in 66.7% of samples. The greatest degree of leaf blotch reduction with the use of a growth stimulant was registered in varieties Krasnouphimskaya 110, c–65478 (37.5%, t = 3.0) and Tuliaykovskaya 110, c–65454 (36%, t = 7.9). In recent years, wheat leaf blotch has been included in the list of especially dangerous diseases, in particular for wheat. This is the reason of emergency situations in crop production. With moderate development of the disease, crop losses can be 10–15%, when epiphytotics is 30–40%. One of the reasons for the ubiquitous wheat leaf blotch infection is the lack of varieties resistant to the disease. The use of the protein growth stimulant insignificantly reduced the disease development in more than half of the studied wheat varieties.

It should be noted, that the intensity of wheat infection by pathogens substantially affects the elements of productivity and the structure of wheat yield. The mathematical models describing the injuriousness of wheat pathogens, including in mixed infections, was previously developed by the authors.

According to the analysis of 250 soft wheat samples (Kolesnikova & Kolesnikov, 2012; Kolesnikov et al, 2012), it was found that the dependence of yield loss (by the grain weight per spike of one plant, %), Y_z from the brown rust pustule area $S_{b.p.}$, the numbers of pustules $N_{b.p.}$, and wheat flag leaf area $S_{l.z.}$ can be described by a multivariate linear regression equation: $Y_z = –134.654\ S_{b.p.} – 0.023\ N_{b.p.} – 0.021\ S_{l.z.}$ (R^2 = 0.67; F = 7.67; P = 0.05). The dependence of the wheat yield loss (by the grain weight per spike of one plant, %) from the number of pustules can be expressed by the equation: $Y_z = –0.219\ N_{b.p.}$ (R^2 = 0.48; F = 52.03; P = 0.00). The number of pustules of the pathogen $N_{b.p.}$ can be determined, based on the data of the conditional development of the disease R_b.: $N_{п.б.} = 3.931\ R_b$. According to the model, an increase in disease development by 1% (5 pustules per leaf) led to crop losses 1.1%; by 25% - to crop losses of 21.88%. The size of the pustule area, as a factor of wheat resistance to the disease, significantly affects the value of wheat yield loss Y_z (by the grain weight per spike, %). The dependence of Y_z on the brown rust pustule area S_{bp} can be expressed by the equation: $Y_z = –327,405\ S_{б.п}$ (R^2 = 0,598; F = 19,36; P = 0.001) (Kolesnikova & Kolesnikov, 2012).

A regression equation describing the relationship between the development of brown rust R_b, wheat leaf blotch R_s and yield loss per plant (by the number of grains per spike Yn.z. and by grain weight Yz.), has the form: $Y_{n.z} = –1.673R_б –2.033R_s$ (F = 13.81, P = 0.04) and $Y_{z.} = –1.662R_б–2.032R_s$ (F = 9.04, P = 0.008). An increase in the development of a diseases complex by 1% led to the following decrease in yield: $Y_{n.z}$ = 3.71% and $Y_{z.}$ = 3.7%.

CONCLUSIONS

This study has shown the prospects of using the protein growth stimulant to increase productivity and protect wheat from diseases. However, the effectiveness of the growth stimulant depended heavily on the variety. When using the growth stimulator, a significant increase in the potential yield of wheat varieties of mainly Russian selection with a relatively high adaptive potential to the agroecological conditions of the Leningrad region was revealed. The maximum increase in potential yield (103%) compared to the control was registered on the variety Tuliaykovskaya 108, c–65452 (Russia, Samara region), which is determined by the maximum productivity of the variety in comparison with other cultivars. Reliable effect of the preparation was based on increase of the length of spike, number of spikelets per spike, number of grains per spike, weight of 1,000 grains. In addition, for this variety, foliar spraying of plants with a growth stimulant caused a significant decrease in the wheat leaf blotch intensity (36.3%). To the greatest extent, the growth stimulant caused a significant increase in the spike length, the number of grains per spike and the grain weight per spike on the cultivar Pamyati Judina, c–65243 (Russia, Irkutsk region) (44.3%, 66.7%, 68.9%, respectively); the number of spikelets per spike on the cultivar Tyumenskaya 30 c–65248 (21.2%); the 1000 grains weight on the cultivar Uralosibirskaya, c – 65244 (Russia, Omsk region) (22.9%); and the grain weight per spike on the cultivar Orenburgskaya 22, i–147624 (Russia, Orenburg region) (49.3%).

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REFERENCES

Babayanc, L, Meshterhazi, A., Vekhter, F., Neklesa, N., Dubinina, L., Omel'chenko, L., Klechkovskaya, E., Slyusarenko, A., Bartosh, P. 1988. *The methods of infection background creation and estimation of wheat and barley resistance to main diseases*. Prague, 322 pp.

Bityuckij, N.P. 1999. *Microelements and plant*. St. Petersburg state University publishing house, Saint-Petersburg, 156 pp. (in Russian).

Čepulienė, R. & Jodaugienė D. 2018. Influences of biological preparations on soil properties in the spring wheat crop. In Raupelienė, A. (ed): *Proceedings of the 8th International Scientific Conference Rural Development 2017*. Aleksandras Stulginskis University, Kaunas, pp. 31–36. http://doi.org/10.15544/RD.2017.013

Che, Y.Z., Li, Y.R., Zou, H.S., Zou, L.F., Zhang, B. & Chen, G.Y., 2011. A novel antimicrobial protein for plant protection consisting of a *Xanthomonas oryzae* harpin and active domains of cecropin A and melittin. *Microbial Biotechnology* **4**(6), 777–793. doi:10.1111/j.1751-7915.2011.00281.x

Gulewicz, K., Aniszewski, T. & Cwojdziński, W. 1997. Effects of some selected lupin biopreparations on the yields of winter wheat (*Triticum aestivum L.* ssp. *vulgare* Vill) and potato (*Solanum tuberosum L.*). *Industrial Crops and Products* **6**(1), 9–16. https://doi.org/10.1016/S0926–6690(96)00171–9

Ermakov, A.I. 1987. *Methods of biochemical research of plants*. Leningrad, Agropromizdat, 430 pp. (In Russian).
Hammad, S.A.R., &. Ali, O.A.M., 2014. Physiological and biochemical studies on drought tolerance of wheat plants by application of amino acids and yeast extract. *Annals of Agricultural Sciences* **59**(1), 133–145. https://doi.org/10.1016/j.aoas.2014.06.018
James, W.C. 1971. An illustrated series of assessment for plant diseases preparation and usage. *Canadian Plant Disease Survey* **51**(2), 39–65.
Jin, L., Cai, Y., Sun C., Huang Y. & Yu T. 2019. Exogenous L–glutamate treatment could induce resistance against *Penicillium expansum* in pear fruit by activating defense–related proteins and amino acids metabolism. *Postharvest Biology and Technology* **150**, 148–157. https://doi.org/10.1016/j.postharvbio.2018.11.009
Khan, S., Basra S.M.A., Nawaz M., Hussain I. & Foidl, N. 2019. Combined application of moringa leaf extract and chemical growth–promoters enhances the plant growth and productivity of wheat crop (*Triticum aestivum L.*). *South African Journal of Botany*. Available online 21 March 2019. https://doi.org/10.1016/j.sajb.2019.01.007
Kolesnikov, L.E., Pavlova, M.N. & Rykhlova, K.V. 2012. Influence of causative agents of diseases of leaves on productivity and economic efficiency of cultivation of spring wheat. *Izvestiya Saint–Petersburg State Agrarian University* **29**, 19–23 (in Russian).
Kolesnikov, L.E., Novikova, I.I., Surin, V.G., Popova E.V., Priyatkin, N.S. & Kolesnikova, Yu.R. 2018. Estimation of the efficiency of the combined application of chitosan and microbial antagonists for the protection of spring soft wheat from diseases by spectrometric analysis. *Applied Biochemistry and Microbiology* **54(**5), 546–552 (in Russian).
Kolesnikova, Y.R. & Kolesnikov, L.E. 2012. The productivity of spring soft wheat and its limitation by leaf disease agents. *Izvestiya Saint–Petersburg State Agrarian University* **27**, 60–67 (in Russian).
Kovacs, A.I., Maini, P. & De Leonardis, A. 1986. Nematostatic effect of biostimulant Siapton. In *Atti Giornate Fitopatologia*, Clueb Ed. Bo, Riva del Garda, pp. 415–424 (in Italian).
Kremenevskaya, M., Sosnina, O., Semenova, A., Udina, I. & Glazova, A. 2017. Meat industry by–products for berry crops and food production quality improvement. *Agronomy Research* **15**(S2), 1330–1347.
Kretovich, V.L. 1986. *Plant Biochemistry*. Higher school, Moscow, 503 pp.
Kutsakova, V.E. 2004. Drying of liquid and pasty products in a modified spouted bed of inert particles. *Drying Technology* **22**(10), 2343–2350.
Kutsakova, V.E., Frolov, S.V., Kremenevskaya, M.I. & Marchenko, V.I. 2013. Patent 2533037 RF: MPK C05 F 1/00, A 01 N 33/00. A method for producing a protein stimulator of plant growth and development. № 201334879/13; appl. 24.08.13; publ. 20.11.2014. – Bull. No. 32, 6 pp.
Marcucci, M.C. 1984. The influence of storage and of organic nutrients on the germination of pollen and fruit set of apple and pear. *Acta Horticulturae*: *Flowering and Fruit Set in Fruit Trees* **149**, 117–122.
Niu, J., Guo, D., Zhang, W., Tang, J., Tang, G., Yang, J., Wang, W., Huo, H., Jiang, N. & Cao, Y. 2018. Preparation and characterization of nanosilica copper (II) complexes of amino acids. *Journal of Hazardous Materials* **358**, 207–215. https://doi.org/10.1016/j.jhazmat.2018.06.067
Novikov, N.N. 2017. The new method for diagnostics of nitrogen nutrition and forecasting the quality of wheat grain. *Izvestiya TSHA*. **5**, 29–40 (in Russian).
Pavlyushin, V.A. & Lysov, A.K. 2019. Phytosanitary safety of agro–ecological systems and remote phytosanitary monitoring in plant protection. *Sovremennye problemy distantsionnogo zondirovaniya zemli iz kosmosa* **16**(3), 69–78 (in Russian).

Peterson, R.F., Campbell, A.B. & Hannah, A.E. 1948. A diagrammatic scale for estimating rust intensity of leaves and stem of cereals. *Canadian Journal of Research Section C* **26**, 496–500. doi:10.1139/cjr48c-033

Popov, Y.V. 2011. Method for the estimation of the root rots development in cereals. *Plant protection and quarantine* **8**, 45–47 (in Russian).

Roblin, G., Laduranty, J., Bonmort, J., Aidene, M. & Chollet, J.F. 2016. Unsaturated amino acids derived from isoleucine trigger early membrane effects on plant cells. *Plant Physiology and Biochemistry* **107**, 67–74. https://doi.org/10.1016/j.plaphy.2016.05.025

Siah A. Magnin–Robert M., Randoux B., Choma C., Rivière C., Halama P. & Reignault P. 2018. Natural agents inducing plant resistance against pests and diseases. *Sustainable Development and Biodiversity. Natural Antimicrobial Agents* **19**, 121–159.

Soro, R. 1985. Stimulation of orange trees. *Agricola Vergel* **4**, 166–169 (in Spanish).

Stoyanov, I. 1981. Restoration of maize plants after Magnesium starvation with the help of Magnesium and Siapton. In *Proceeding of 3rd International Symposium on Plant Growth Regulators*, Varna, Bulgaria, pp. 602–606.

Sudisha, J., Kumar, A., Amruthesh, K.N., Niranjana, S. R. & Shetty, H.S. 2011. Elicitation of resistance and defense related enzymes by raw cow milk and amino acids in pearl millet against downy mildew disease caused by *Sclerospora graminicola*. *Crop Protection* **30**(7), 794–801. https://doi.org/10.1016/j.cropro.2011.02.010

Wang, S., Bao, L., Song, D., Wang, J., Cao, X. & Ke, S. 2019. Amino acid–oriented poly–substituted heterocyclic tetramic acid derivatives as potential antifungal agents. *European Journal of Medicinal Chemistry* **179**, 567–575. https://doi.org/10.1016/j.ejmech.2019.06.078

Zadoks, J.C., Chang, T.T. & Konzak, C.F. 1974. A Decimal Code for the Growth Stages of Cereals. *Weed Research* **14**, 415–421.

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https://doi.org/10.15159/AR.20.090

Model for the bee apiary location evaluation

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Abstract. Honeybees are predominant and ecologically as well as economically important group of pollinators in most geographical regions. As a result of analysing current situation in studies and practices, a conclusion was drawn that beekeeping sector is in decline. The identified reasons for this are land-use intensification, monocropping, pesticide poisoning, colony diseases, parasites and adverse climate. One of the solutions is to find a proper bee colony harvesting location and use luring methods to attract bees to this location. Usually beekeepers choose the apiary location based on their own previous experience and sometimes the position is not optimal for the bees. This can be explained by different flowering periods, variation of resources at the known fields, as well as other factors. This research presents a model for evaluation of possible apiary locations, taking into account resource availability estimation in different surrounding agricultural fields. Authors propose a model for real agricultural field location digitization and evaluation of possible apiary location by fusing information about available field resources. To achieve this, several steps have to be completed, such as selection of fields of interest, converting selection to polygons for further calculations, defining the potential values and coefficients for amount of resources depending on type of crops and season and calculation of harvesting locations. As the outcome of the model, heat map of possible apiary locations are presented to the end-user (beekeeper) in the visual way. Based on the outcome, beekeepers can plan the optimal placement of the apiary and change it in the case of need. The Python language was used for the model development. Model can be extended to use additional factors and values to increase the precision for field resource evaluation. In addition, input from users (farmers, agricultural specialists, etc.) about external factors, that can affect the apiary location can be taken into account. This work is conducted within the Horizon 2020 FET project HIVEOPOLIS (Nr.824069 – Futuristic beehives for a smart metropolis).

Key words: precision beekeeping, smart apiary location, harvesting location evaluation, HIVEOPOLIS.

INTRODUCTION

Pollination is an essential ecosystem service, and bees are critical to the rich diversity of fruits, vegetables, and nuts humans eat (Bolshakova & Niño, 2018). Bees help to preserve wild biodiversity for 90% of crop species, and sustain the health and vitality of human food production and pollinators are required for producing of 15 to

30% of the human food supply (Greenleaf & Kremen, 2006). The value of the European honey bee, *Apis mellifera L.* to pollination services is estimated at $217 billion globally and $20 billion in the United States annually (Frankie et al., 2014). For the last decade, annual bee and hive losses have considerably increased. Declines in pollinator populations could have serious economic repercussions, including rising food costs and potential crop failures (Potts et al., 2016). Without the bee pollination, many of our favourite healthy foods, such as almonds, avocados, apples, watermelons, would be at risk. The loss of honeybees would also affect the meat and dairy industries as many of the crops requiring bee pollination serve as food for animals. Without honeybees, farmers would have to use other, more expensive, but less effective pollination techniques and consumers would pay the price. Thanks to the progress in information and communication technologies, new tools and services are available worldwide to manage and constantly monitor the bee colonies (Komasilovs et al., 2019). Precision apiculture (called also Precision Beekeeping) is developed and is defined as an apiary management strategy based on the monitoring of individual bee colonies to minimize resource consumption and maximize the productivity of bees (Zacepins et al., 2015). Idea of the Precision Apiculture is to monitor the main bee colony parameters in real life and make on-time decisions. One of the important managemental decisions for the beekeeper is to select the best placement location for apiary. Optimal location will allow bee colonies to forage on higher amount of the resources with minimal energy consumption. As it is stated by (Vlad et al., 2012) to ensure maximum productivity and continuous honey gathering, beekeepers move their beehives closer to the honey resources. It can be almost considered as a requirement in order to stay competitive in the honey market and to ensure sustainability. The importance of the appropriate site selection, taking into account the range the bees can fly and the food sources available, is also emphasized in (Poelsma, 2019). There is also a web tool ('Honey bee forage map') available (https://www.beepods.com/honey-bee-forage-map/), that allows one to observe the nearest locations (area) the bees can visit within a specific radius (e.g., 2 km or more), however this tool does not provide any information about possible food sources and optimal placement for beehives.

Apiary location selection is usually based on beekeepers' previous experience, according to the flowering calendar of different crops and plants, or the limited availability of physical space. Beekeepers can also be informed by the local farmers who need the pollination service. Unfortunately, apiary location does not guarantee that bees will fly to the field wanted by beekeeper, therefore it is required to guide bees to designated place for optimal resource harvesting. There are several methods for manual bee colony attraction to specific location (field). One of the methods is to place sugar syrup at the field to attract bees, resulting in bees' memory pattern development, which in return ensures that bees will prioritize this particular field for their next gather trips. However, this technique is prohibited in some countries. There is also an idea of directing bees to nearby acacia trees by placing acacia flowers at the hive entrance; however, this beekeepers' idea has not been scientifically confirmed.

Sometimes beekeepers want to harvest honey from specific places, pursuing single-source honey – e.g. linden bloom or spruce honeydew – to obtain honey with distinct flavour, as such honey is more valuable on the market (Crane & Walker, 1985; Persano Oddo et al., 2004).

In some countries, for example in Indonesia (Gratzer et al., 2019), migratory beekeeping is very common, and beekeepers are forced to change the apiary location often to provide food sources for their bees. By utilizing semi-automated decision support system for identifying optimal apiary location, beekeepers could be able to decrease the expenses and effort related to migrating the bee colonies. In addition, it could result in increase of the potential amount of forage resources. The optimal number of bee colonies placed in a particular location or region is also questionable, as it introduces in-between colony resource distribution challenges and competition. For example, natural colony density as determined for both Palearctic and Nearctic forests was established at 0.5 colonies per km^2 (Galton, 1971; Visscher & Seeley, 1982). Other studies show the numbers of natural density of 0.11–0.14 honey bee colonies km^{-2} (Oleksa, Gawroński & Tofilski, 2013; Kohl & Rutschmann, 2018).

In the future, with increase of automation and robotics, implementation of such solutions to the beekeeping sector may result in possibility to automatically direct bees to a specific location. There are already researches and prototypes addressing this objective (Landgraf et al., 2018). This technology would allow guiding the bees to location beekeepers consider to be optimal, and pollinate only the necessary fields, excluding potentially dangerous for the bees (containing pesticides), unwanted fields and limiting risk factors linked to flying paths of the bees. Therefore, it will be necessary to gather information about the available resources in the fields, possible dangerous places, potential flying paths, etc.

The aim of this research is to develop a model, which can be afterwards integrated in a wider platform, which potentially will provide support for beekeepers in finding and selecting good apiary locations, and in future, could also be integrated into a system of futuristic hives with the aim to autonomously find the best harvesting location. Proposed model for the bee apiary location evaluation is the first stage of the complex data fusion solution for beekeeping needs, which would be developed and built in the future.

This research is conducted within the Horizon 2020 FET programme project HIVEOPOLIS (https://www.hiveopolis.eu/). Collection of hives, technologies and humans is called Hiveopolis in our concept. HIVEOPOLIS technology will be integrated in a way that it provides a synergistic added value to the colony, to its owner and to the society in general.

DEVELOPMENT OF THE MODEL FOR BEE APIARY LOCATION EVALUATION

This section presents a model for evaluation and selection of possible apiary locations utilizing aerial and satellite images of agricultural fields. The model development process can be divided into two main steps. In the first step, the fields in the aerial image of the region are annotated with a polygons and an estimated value of resources on that field. As the result, authors obtain a semantically annotated map, which can be used for automatic evaluation. Based on this semantic map, in the second step, the method calculates a value function assigning each location on the map an estimated amount of resources to be collected at that location. This value function is then used to estimate favourable apiary locations as local maximums. Model is developed in Python language using several libraries, including Matplotlib (Hunter, 2007), NumPy (van der

Walt, Colbert and Varoquaux, 2011), Shapely (Gillies, 2007) Python package for computational geometry.

Further, the method is discussed and illustrated in more detail. To develop the proposed model several steps have to be completed:

1. Get the region of interest from the map

- At this point the system is intended to be used by a beekeeper. User (beekeeper) should choose the needed image and crop the region of interest to work with (see Fig. 1). Authors used Google Maps for selection of images of terrains and regions. Used part of the map can be seen here: https://www.google.com/maps/@56.5696785,23.4593229,5558m/data=!3m1!1e3. It is assumed, that dimension of the region of interest is 10 km to 10 km. Region of interest can be also different if needed.

$W = 10{,}000$

where W – map region width (m).

Figure 1. Example of terrain map used for model evaluation.

Figure 2. Example of some defined polygons on the map.

2. Define polygons which are representing agricultural fields or possible sources of resources for bees

- At this moment, this task is completed manually using specifically developed basic web interface. User should mark all the vertices (see Fig. 2 for demonstration of some defined polygons) of each polygon and the tool will extract their coordinates.
- In the example, there are 56 polygons defined within selected region.

$$\forall f_n \in F \rightarrow p_n = O(f_n) \quad (1)$$

where F – fields from regions of interest; $O(f)$ – polygon outlining the field.

3. Transfer real image to semantically annotated map of polygons

- Based on the coordinates generated from previous stage, the map annotated with chosen polygons is built (see Fig. 3 and Fig. 4). This map can be further processed in many different ways, applying different parameters for the polygons and implementing other layers (for example roads).

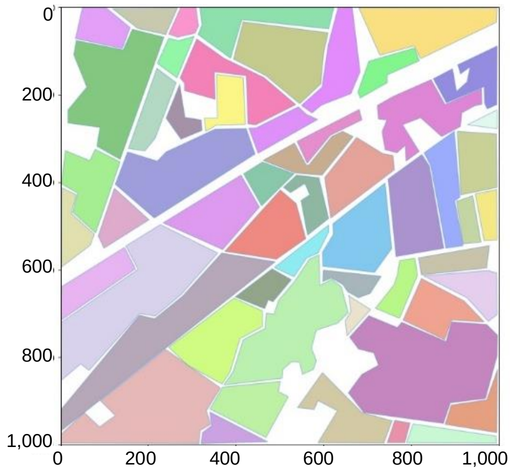

Figure 3. Generated digitized map of marked fields and regions.

Figure 4. Digital map combined with the real map.

4. Define each polygon value

- One of the options for processing the polygons is to define field values and assign them to already generated polygons. Practically, field values should be related to the theoretical amount of resources available for bee forage. It is a very challenging task to evaluate the exact amount of resources available at a foraging location. It is possible to use the information about specific plants and crops and their indices describing pollen and/or nectar production. In the provided example, polygons' values are assigned randomly to demonstrate the calculation method itself, therefore values can be different from the real situation. It is assumed that five different agricultural plants are growing in the selected region. The field nectar production is from 20 kg to 100 kg from one hectare.

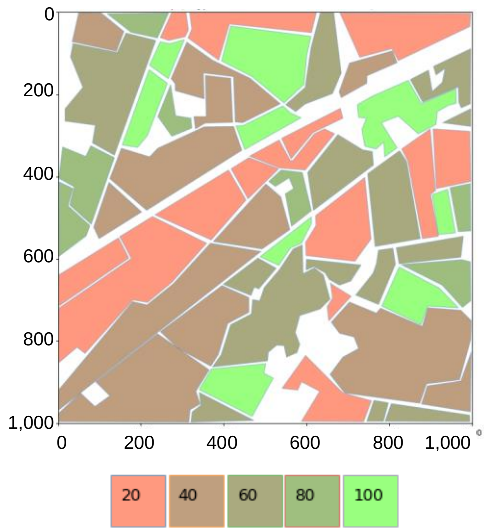

Figure 5. Encoding of the fields.

$$v_n = V(p_n) = R \in \{20,40,60,80,100\} \quad (2)$$

where *V(p)* – plant honey production index in a given field (represented by polygon)
R – uniform random distribution over a set of values.

- To visually differentiate the fields by their value, colour encoding is implemented, ranging from bright red to bright green, with five colour steps. The region with the highest value will have its polygon coloured bright green (see Fig. 5).

5. Calculate all possible positions of the bee apiary

- Within this stage all possible apiary locations are considered, calculated and evaluated. This is completed by going through the annotated map in a sliding window mode and multiplying the area of polygons (fields), which is within the possible bee colony flying region, with polygon value. Flying distance of the bee colony is considered within the radius of 3 km from the colony location (Prešern, Mihelič & Kobal, 2019). Another source states that productive flying radius of the bee colony should not exceed 2 km (Кривцов and Лебедев, 2019). This parameter can be adjusted by the user if needed. In the authors' case, the radius of 3 km is chosen. Authors assume the harvesting region of the bee colony to be a circular region around the apiary location. Authors also assume that each point within the harvesting region is equally likely to be reached by the bees. In order to estimate the value of a given location, the values of all field polygons that intersect with the harvesting region are integrated and weighted with the area of intersection.

$$C(x_c, y_c) = \{(x, y): (x - x_c)^2 + (y - y_c)^2 \leq r^2\} \quad (3)$$

where C – harvesting area of a colony (circle) placed at (x_c, y_c) coordinates; r – harvesting distance (r = 3,000 in the example).

- Other parameters used in the model are:
  - ✓ 2.5 kg of foraged nectar is required for a colony to produce a 1 kg of honey (Гребенников, 2005).
  - ✓ Honeybees are foraging only approximately 35% of maximal possible field nectar (Нарчук and Морева, 2016).

$$\forall x, y \in \{1..W\} \rightarrow H(x, y) = hk \sum_n V(p_n) * C(x, y) \cap p_n \vee \quad (4)$$

where *H(x, y)* – total potential harvest for single colony placed at (x, y); k – honeybees foraging efficiency (k = 0.35 used in example); h – nectar to honey production rate (h = 0.4 used in example.

6. Visualise resource availability using the heatmap

- For better demonstration of the calculation outcome the field resource availability heat map is generated and shown to the user (see Fig. 6). Resource availability is calculated for each possible apiary location.

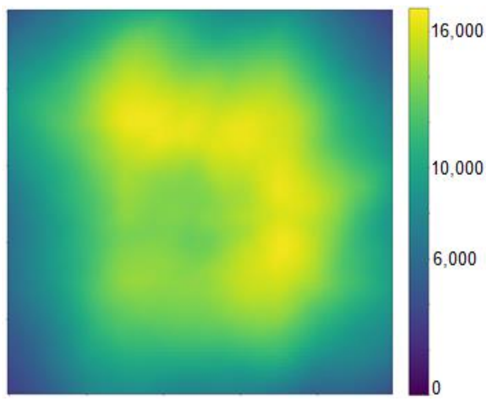

Figure 6. Heat map of resource availability in each possible point.

7. Choose the best place for the apiary location

- Based on the value function, the system generates a heat map and proposes the best possible apiary locations.
- In addition, several parameters were considered:
  - ✓ One bee colony consumes up to 90 kg of honey for their local needs (Лебедев and Кривцов, 2019);

- ✓ Maximum number of bee colonies in one location is equal to 70;
- ✓ Minimum number of bee colonies in one location is equal to 15;
- ✓ Average amount of honey production per colony is equal to 60 kg.

$$argmax_{x,y} H(x,y)$$

subject to

$$H(x,y) \geq H_{colony}$$
$$15 \leq N(x,y) \leq 70$$

where H_{colony} – amount of honey needed for the colony survival; $N(x,y)$ – number of colonies placed at a location (x, y).

Based on this parameters the model calculates the best possible apiary locations and demonstrates the apiary location to the end user, where the number represents the maximum hive count in the one apiary. In this particular example, there are three locations where maximum number of colonies is possible (number 70) and five additional locations with less number (less than 70) of colonies (see Fig. 7).

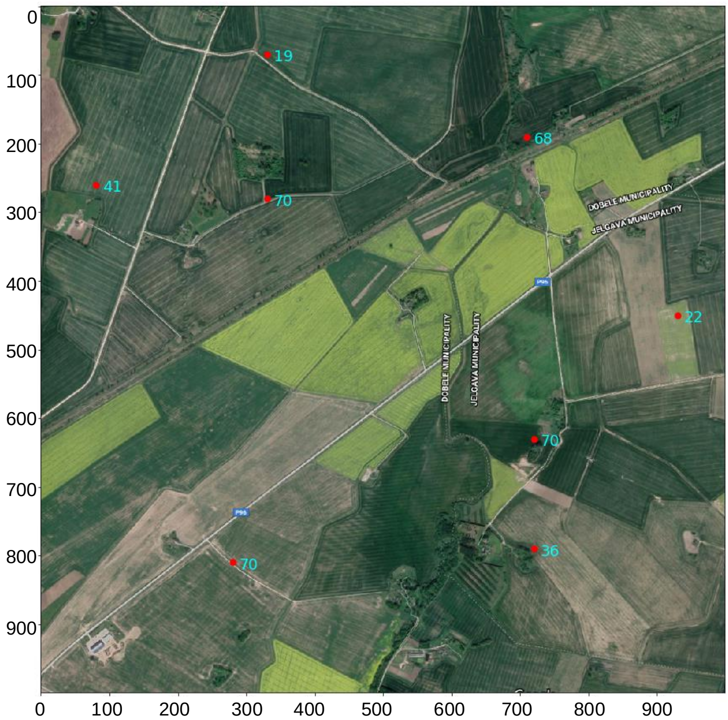

Figure 7. Demonstration of optimal apiary locations.

When the apiary location is chosen, it is needed to check if the location is viable, e.g. it is not on the road or at some restricted place (like field is a private property, and it is not allowed to enter and place bee colonies, etc.). At this point, this stage is not automatized, but is planned to be improved in the future.

PLANNED FUTURE IMPROVEMENTS OF THE MODEL

In the future work, it is planned to automate some model development stages, like definition of polygons, which could be time consuming task in case there are many fields to describe in a chosen image. Potentially, the machine learning techniques can be applied to solve this task.

In the future, the model presented in the previous section will be integrated in a more general system. Authors propose to develop augmented map to show potential places for bee colony nectar/pollen foraging with some useful additional attributes, like amount of food resources, the quality of the food base, some additional data (for example the name of a crop or plant growing on the field). Information to be used by augmented map can come from different data sources already available or generated in the future.

One of the main data sources should be supplied by the farmers, regarding flowering of the field, and/or usage of substances (chemicals, pesticides) that can potentially harm the bees.

In the future, the output of the augmented map can be autonomously taken by the futuristic bee hives for planning a nectar collection.

CONCLUSIONS

Authors propose a model that can be used by beekeepers to choose the optimal place for apiary location and to plan transportation of hives, when potential resources of one field will come to an end.

The model is implemented in Python language and could be improved in the future by adding additional parameters for polygons to better describe the real-life situation.

The model development is in its early stage and requires further development and evaluation in real world conditions.

With the implementation of Precision Beekeeping and autonomous beekeeping, futuristic bee colonies could be able to use the information provided by the model by themselves to plan the foraging location and its intensity.

In this work, the model for an apiary location evaluation is developed for use by potential users, but in the future, it is planned to build up an interface to provide the evaluation not for human user but for machine or for augmented organism, like futuristic beehives.

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REFERENCES

Bolshakova, V.L. & Niño, E.L. 2018. *Bees in the Neighborhood: Best Practices for Urban Beekeepers*, *Bees in the Neighborhood: Best Practices for Urban Beekeepers*. doi: 10.3733/ucanr.8596.

Crane, E. & Walker, P. 1985. 'Important honeydew sources and their honeys', *Bee World*, 66(3), pp. 105–112. doi: 10.1080/0005772X.1985.11098832

Frankie, G.W., Thorp, R.W., Coville, R.E. & Ertter, B. 2014. *California Bees and Blooms: A Guide for Gardeners and Naturalists*. Heyday. 320 pp.

Galton, D. 1971. *Survey of a thousand years of beekeeping in Russia*. Bee Research Association, London.

Gillies, S. 2007. 'Shapely: manipulation and analysis of geometric objects'. Available at: https://github.com/Toblerity/Shapely.

Gratzer, K., Susilo, F., Purnomo, D., Fiedler, S. & Brodschneider, R. 2019. 'Challenges for Beekeeping in Indonesia with Autochthonous and Introduced Bees', *Bee World* **96**(2), pp. 40–44. doi: 10.1080/0005772X.2019.1571211

Greenleaf, S.S. & Kremen, C. 2006. 'Wild bees enhance honey bees' pollination of hybrid sunflower', *Proceedings of the National Academy of Sciences of the United States of America* **103**(37), pp. 13890–13895. doi: 10.1073/pnas.0600929103

Hunter, J.D. 2007. 'Matplotlib: A 2D Graphics Environment', *Computing in Science & Engineering* **9**(3), pp. 90–95. doi: 10.1109/MCSE.2007.55

Kohl, P.L. & Rutschmann, B. 2018. 'The neglected bee trees: European beech forests as a home for feral honey bee colonies', *Peer J*, 2018(4). doi: 10.7717/peerj.4602

Komasilovs, V., Zacepins, A., Kviesis, A., Fiedler, S. & Kirchner, S. 2019. 'Modular sensory hardware and data processing solution for implementation of the precision beekeeping', *Agronomy Research* **17**(2), pp. 509–517. doi: 10.15159/AR.19.038

Landgraf, T., Bierbach, D., Kirbach, A., Cusing, R., Oertel, M., Lehmann, K., Greggers, U., Menzel, R. & Rojas, R. 2018. 'Dancing Honey bee Robot Elicits Dance-Following and Recruits Foragers'. Available at: http://arxiv.org/abs/1803.07126

Oleksa, A., Gawroński, R. & Tofilski, A. 2013. 'Rural avenues as a refuge for feral honey bee population', *Journal of Insect Conservation* **17**(3), pp. 465–472. doi: 10.1007/s10841-012-9528-6

Persano Oddo, L., Piana, L., Bogdanov, S., Bentabol, A., Gotsiou, P., Kerkvliet, J., … & von der Ohe, K. 2004. 'Botanical species giving unifloral honey in Europe', *Apidologie* **35**(Suppl. 1), pp. S82–S93. doi: 10.1051/apido:2004045

Poelsma, F. 2019. *Choosing the best location for your beehives*. Available at: https://www.vatorex.ch/en/choosing-the-best-location-for-your-beehives-part-1/.

Potts, S.G., Imperatriz-Fonseca, V., Ngo, H.T., Aizen, M.A., Biesmeijer, J.C., Breeze, T.D., … & Vanbergen, A.J. 2016. 'Safeguarding pollinators and their values to human well-being', *Nature* **540**(7632), pp. 220–229. doi: 10.1038/nature20588

Prešern, J., Mihelič, J. & Kobal, M. 2019. 'Growing stock of nectar- and honeydew-producing tree species determines the beekeepers' profit', *Forest Ecology and Management* **448**, pp. 490–498. doi: 10.1016/j.foreco.2019.06.031

Visscher, P.K. & Seeley, T D. 1982. 'Foraging strategy of honeybee colonies in a temperate deciduous forest.', *Ecology* **63**(6), pp. 1790–1801. doi: 10.2307/1940121

Vlad, V., Ion, N., Cojocaru, G., Ion, V. & Lorent, A. 2012. 'Model and support system prototype for scheduling the beehive emplacement to agricultural and forest mellferous resources', *Scientific Papers A. Agronomy*, LV–2012, pp. 410–415.

van der Walt, S., Colbert, S.C. & Varoquaux, G. 2011. 'The NumPy Array: A Structure for Efficient Numerical Computation', *Computing in Science & Engineering* **13**(2), pp. 22–30. doi: 10.1109/MCSE.2011.37

Zacepins, A., Brusbardis, V., Meitalovs, J. & Stalidzans, E. 2015. 'Challenges in the development of Precision Beekeeping', *Biosystems Engineering* **130**, pp. 60–71. doi: 10.1016/j.biosystemseng.2014.12.001.

Гребенников, Е.А. 2005. *Все о меде*. Минск: Книжный Дом.

Кривцов, Н. И. & Лебедев, В. И. 2019. *Технологии содержания пчелиных семей.* Юрайт.

Лебедев, В.& Кривцов, Н. 2019. *Beekeeping: bee colony breeding and keeping (Пчеловодство: разведение и содержание пчелиных семей). Litres.* (in Russian).

Нарчук, Э.П. & Морева, Л.Я. 2016. 'Nectar as a renewable natural resource', *Biosfera* **8**(3), pp. 301–314.

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Developing of modern cultivation technology of organic potatoes

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Abstract. Medium term field experiment demonstrates the effectiveness of biological ways to increase potatoes yield on the sod-podzolic, light loamy soil.
The effects of the following two groups of factors were studied:
1. The level of mineral nutrition provided by compost;
2. Microbiological pant protection product (bio preparations): Flavobacterin which has N fixer attribute and Vitaplan and Kartofen, which have bio fungicide features.
Potatoes varieties 'Nevsky' and 'Udacha' were cultivated. The compost was applied in the dose range from 0 to 160 kg N ha^{-1}. Treatment with bio-preparations was carried out by potato tubers during planting and leaves during the growing season. Weeds were removed mechanically. Weather conditions had a significant impact on the formation of the crop. The average yield of standard potato tubers for 2017–2018 ranged from 14.1 to 29.3 t ha^{-1}. The use of both microbiological preparations and compost gave approximately the same effect and increased yields by 35–37% compared to the control. The use of compost together with bio-preparations provided an output of 27.6–29.3 t ha^{-1} of potatoes. In 2019, the joint use of compost and bio-preparations allowed to reach a yield of 40 tons ha^{-1} of marketable potatoes. On the basis of the obtained data, the mathematical dependences of the yield of potatoes on the dose of compost, the type of biological preparation and the complex indicator of the year conditions were determined.

Key words: crop rotation, potatoes, organic fertilizers, microbiological drugs, field experiment.

INTRODUCTION

The modern agricultural food system is quite productive, but it creates many environmental problems, such as reduced biodiversity, soil degradation, eutrophication of water bodies, the release of large amounts of greenhouse gases, and a number of others (Foley et al., 2005; Ponisio et al., 2015). One of the most widely practiced and studied alternative to intensive farming is organic farming. Organic production is actively developing around the world. According to the last annual IFOAM report (2018), in 2017 the number of countries where organic production is carried out was 181, and where there is government regulation - 93. The value of the global organic market in the same year reached 90 billion euros. Although there is an opinion about the low productivity of agricultural systems that do not use synthetic fertilizers and pesticides, they are more profitable and environmentally friendly and provide the same or more nutritious products that don't contain pesticide residues compared to conventional agriculture. A review by Reganold et al. (2016) and meta-analysis by Ponisio et al. (2015) demonstrated that when using crop rotation in organic farming, including fields with perennial legumes, the yield gap is only 8–9%. Feiziene et al. (2016) experimentally confirmed that in organic crop rotations, including red clover, it is an active storage of carbon and nitrogen in the soil humus compounds and a significant reduction in greenhouse gas emissions compared with intensive agriculture. Similar results are presented by Müller-Lindenlauf (2009), who emphasizes that the increase in soil carbon and nitrogen reserves in organic production provides greater sustainability of crop productivity compared to conventional agriculture. Obviously, it is the use of composts, presence of legumes in the crop rotation and the softer mechanical soil treatment that contributes to the preservation of organic matter in the soil (Huhta & Minin, 2014).

However, Ponisio et al. (2015) emphasize that the time has come to invest in analytically accurate, agroecological and socio-economic studies aimed at bridging yield gaps between sustainable and conventional agriculture (when they arise), identifying barriers to the introduction of sustainable methods and improving the living conditions of the rural poor. At the same time, one must be guided by taking into account the various interests of farmers, including social ones, which intend to engage in organic farming (Raudsepp-Hearne C et al., 2010; Escobar et al., 2019).

In Russia, Federal Law N 280 dated 03.08.2018 '*On organic products and on amendments to certain legislative acts of the Russian Federation*' was adopted in August 2019 and entered into force on January 1, 2020. There are also four federal regulatory documents that determine the requirements for the production and delivery of organic products to consumers. It should be noted that these requirements generally comply with similar requirements adopted by IFOAM and the European Union.

The scientific basis is an adaptive, landscape approach aimed at a more complete use of natural processes and cycles, as far as possible closing biogeochemical cycles and so cutting the cost of non-renewable resources, preserving biodiversity and, at the same time providing high-quality food (Zhuchenko, 2008). Organic farming is well-suited to small-scale producers who will be able to enter the market with high-quality local products produced with minimal negative impact on the environment which are attractive to local consumers. This requires the use of modern, alternative agricultural technologies based on a synthesis of the latest achievements of biological and engineering science and adapted to local conditions (Seufert et al., 2012; Popov et al., 2018). However, according to the Union of Organic Agriculture, most Russian agricultural producers are not familiar with effective methods of organic farming.

Potato is the third most important food crop, after rice and wheat, with a total production of more than 300 million metric tons, as established by the International Potato Center (2017) in Lima, Peru. The Russian Federation ranks third in the world in the production of potatoes, which is cultivated in all major regions of the country. Potato is a very flexible crop that is why it is widely distributed. Potatoes were cultivated in the North-Western zone of the Russian Federation in the first half of the eighteenth century. However, intensive potato cultivation technologies began to be developed and used by agricultural producers in the seventies of the twentieth century.

To date, a number of instructions and recommendations have been issued on the cultivation of potatoes for the main zones of the country (Loginov et al., 2009; Starovoitov et al., 2013). The natural conditions of the North-Western region of the Russian Federation are characterized mainly by low fertility soils, increased rainfall in the second half of the growing season, and the appearance of potato late blight in some years. In accordance with this, the instructions recommend the introduction of increased doses of artificial fertilizers for potatoes, possibly together with organic ones, and regular treatment with pesticides, which contradicts the requirements for the production of organic potatoes. In this regard, the goal of our work is to formulate the technology for competitive production of organic potatoes in this area using the achievements of agricultural sciences and information technology.

MATERIALS AND METHODS

An experimental field crop rotation with elements of organic farming started in the Experimental Field of the Institute in 2016. The experiments were conducted on Humose, Coarse Loamy, Gleyic Sod-Podzolic soil, developed on calcareous moraine, with a weakly acidic reaction and medium to high levels of available P and K. The crop rotation consists of following crops: potatoes; red beat; barley with subseeding of clover and timothy; 2 years of perennial grasses; on the 3rd year grasses will be moved and rye will be sowing. Rye will be mowed in the spring and plowed into the soil, as green fertilizer. Plot size was 5.6×11 m = 61.6 m^2, with four replications in a complete randomized design.

This article presents data obtained in the plots with potato crops.

Three factors studied in the experiment were following:

1. Organic fertilizers (composts) to improve the mineral nutrition of potatoes;
2. Culture of microorganisms - nitrogen fixers (Flavobacterin preparation), which can contribute to the production of additional biological nitrogen by potatoes;
3. The effect of bio fungicides: Extrasol (2016), Vitaplan SP (2017–2018) and Kartofen SP (2019).

The studies were carried out with two types of compost based on chicken manure and prepared industrially:

- Compost produced in the IAEP bio convector (BIOGUM);
- Compost produced in the bio convector of Biozem LLC (KMN).

They are characterized by a high dry matter content (about 50%) and a high content of nitrogen and phosphorus (Table 1). BIOGUM was used in the experiment of 2017–2019, and KMN - in 2016 and 2017.

Table 1. Composts composition in field experiments

	Type of compost	Dry matter, %	N mg kg^{-1}	P mg kg^{-1}
1	BIOGUM	48.5	2.20	0.81
2	KMN	54.2	1.74	1.33

The compost doses used in the experiments were calculated on nitrogen (0; 40; 80; 160 kg N ha^{-1}) and corresponded to different levels of the planned potato productivity. Organic fertilizers were applied before ridging, followed by embedding the fertilizer by disking.

Two potatoes varieties were cultivated: variety ‘Nevsky’ in 2016 and variety ‘Udacha’ in 2017–2019. Potato variety ‘Nevsky’ is a traditional variety, medium early, relatively resistant to many diseases. The ‘Udacha’ potato variety is a new variety, relatively resistant to late blight, perfectly adapts to various soils and weather conditions, it characterized by good taste and smoothness of the tuber surface (Shabanov, 2016). Potatoes for cultivation were obtained from the seed farm (super elite and elite classes).

Biological fungicides based on *Bacillus subtilis* named as Vitaplan SP (strain VCM-B-2604D) and Kartofen SP (strain VCM-B-2605D) developed by Russian Research Institute of Plant Protection) were used in the experiment to protect potatoes from diseases. Potatoes were treated with bio-preparations at the time of planting and by foliar spray during the growing season. For this purpose, a specially designed sprayer was installed on the planter and on the cultivator. Inter-row cultivation was carried out regularly, starting from the second week after planting, using an experimental specimen of a row-crop cultivator of an original design that provides deep loosing of inter rows. Weed vegetation was removed mechanically using small rotary harrow BRU-0.7 harrows mounted on the cultivator.

For operational work with experimental data, a specialized process management information system was created, into which all the information obtained in the experiments was entered. Observations of the growth rate of biomass (by the phases of plant development), phytosanitary conditions and soil properties were carried out regularly. Soil samples were taken from the arable horizon (0–25 cm) four times per season. Following characteristics were determined: soil moisture, pH, nitrate and ammonium content.

Analytical studies were performed at the IAEP Chemical Analytical Laboratory in accordance with the relevant GOSTs. Determination of nitrates and ammonium in soil was carried out using the ion metric method, GOST 26951-86.

Statistical processing of research results was carried out using the Statistic program.

Theory and modelling

The main way to organize competitive production of organic products is to ensure high productivity in optimal agro-technical terms with high accuracy and minimal material and energy resources. The solution to this problem is possible by creating and using an Information Management System (IMS). The IMS will collect operational information about weather conditions and the state of the soil environment and vegetation cover. On this basis and with the use of it knowledge base on plant development, depending on the prevailing conditions and methods of technological impact on this development, the IMS will recommend the choice of operations and the time of its implementation to farmer. In the future, the IMS will send commands directly to robotic agricultural equipment. Field studies are carried out to provide the future IMS with information on the dependence of the formation of potato biomass and its chemical composition on the prevailing weather and soil conditions and agrotechnical effects (Table 2). Obviously, this kind of research is also carried out by other scientists (Timoshina 2017), therefore, all available information will be used to form the information base of the future IMS.

Table 2. Scheme of the main technological processes of potatoes cultivation and of factors affecting them

No.	Technological process	Factors determining the choice of operations	Time interval selection
1	Autumn soil cultivation	1.1. Type of previous crop and the time of it harvest; 1.2. Soil moisture.	Before soil freezes
2	Spring soil cultivation	2.1. Type of autumn soil cultivation; 2.2. Soil moisture and temperature; 2.3. Soil density.	
3	Fertilization and incorporation	3.1. According analysis of soil and fertilizer; 3.2. Soil moisture and soil physics status.	As close to sowing time as possible
4	Ridging and potatoes sowing	4.1. Characteristic of variety; 4.2. Soil moisture and temperature.	In steady warm weather
5	Crop protection against diseases and pests,	5.1. Phytosanitary condition of agroecosystem.	At potatoes sowing; During potatoes vegetation
6	Potato planting care	6.1. Weather conditions; 6.2. Soil physical condition; 6.3. Weeds.	During potatoes vegetation
7	Harvesting	7.1. Weather conditions; 7.2. Soil physical condition.	When the potatoes are ripe

RESULTS AND DISCUSSION

The weather conditions in 2016–2019 are presented in Table 3. They significantly differed among themselves during critical periods of development of potato plants. Potato planting took place in the last week of May during all years and the first seedlings appeared, usually after two weeks. Weather conditions in May were characterized by high variability of both temperature and precipitation, which created various temperature and humidity conditions in the soil at the beginning of the development of potatoes. 2017 was characterized by cold weather, both in May and in June, in May rainfall was also limited. In 2018, the weather was very warm in both May and June, with very limited rainfall during these months, which caused some drying of the soil surface. The years 2016 and 2019, in turn, were distinguished by fairly warm weather in May - June and a sufficiently large amount of precipitation. The second critical period was indicated in August, when the active formation of tubers was completed and their ripening was in progress. The maximum rainfall fell in 2016, exceeding more than twice the annual average. Heavy rains began on July 20, which caused the growth of weeds, but the agricultural machinery could not work, due to waterlogged soil. Weed removal was carried out manually. At the end of July, there was a massive development of late blight on all variants of the experiment, which led to large losses in the crop. A lot of precipitation fell in 2017, also. According to the results of the first year of research, we changed the type of bio fungicide, the variety of potatoes and the soil cultivation system (we began to deepen the row spacing).

Table 3. Weather conditions on experimental field I 2016–2019

Month	Weather indicators	Year				Coefficient of variation	Annual average
		2016	2017	2018	2019		
May	Temperature, C°	14.8	9.4	15.1	12.1	20.60	11.3
	Precipitation, mm	30	13	14	79.3	91.39	46
	HTK	0.67	0.45	0.64	2.11	79.37	0.97
June	Temperature, C°	16.4	13.6	16.2	18.7	12.85	15.7
	Precipitation, mm	99	69	35.2	79.3	37.80	71
	HTK	2.09	1.69	1.02	1.42	28.97	1.56
July	Temperature, C°	19	16.5	20.8	16.5	11.52	18.8
	Precipitation, mm	151	123	152	179.8	15.31	79
	HTK	2.56	2.48	2.85	3.52	16.57	2.85
August	Temperature, C°	17.2	17.4	15.7	17.0	4.56	16.9
	Precipitation, mm	190	148	60.1	94.6	46.58	83
	HTK	3.31	2.83	2.02	1.80	28.26	2.49

Note: HTK – Selyaninov hydrothermal coefficient.

The data on influence of weather conditions and doses of compost on the content of mineral forms of nitrogen in early summer soils are presented in Table 4. The low temperature in 2017 and the reduced precipitation in 2017 and 2018 led to the weak development of the mineralization processes of both soil organic matter and compost. In this regard, very low amount of mineral forms of nitrogen (nitrates and ammonium) was present in the soil, which, in turn, restrained the development of potatoes. In 2016 and 2019, there is an active development of mineralization processes due to favorable weather conditions, in connection with which the content of mineral forms of nitrogen in the soil has increased significantly, especially in the case of composting. However, in the studies of Skudra & Ruza (2019), no dependence of the content of mineral forms of nitrogen in the soil on the conditions of the year was noted.

Table 4. Influence of weather conditions and compost doses on the content of mineral nitrogen forms in soils (mg kg^{-1}) in first decade of June

Compost doze	Yeas			
	2016	2017	2018	2019
Control	40	17	13	35
40 kg N ha^{-1}	91	17	-	-
80 kg N ha^{-1}	140	21	14	48
160 kg N ha^{-1}	-	19	15	55
Equation		R		
A = -58.10+3.28B+55.86C		0.607		

Note: A – the content of mineral N forms in the soil in the first decade of June; B – doze of compost, in N ha-$^{-1}$; C – HTK.

Responsiveness of potato variety Udacha for cultivation operations was largely determined by the prevailing weather conditions. As showed above, the content of mineral forms of nitrogen in the soil, at the beginning of the formation of potato plants, was ensured by the intensity of the mineralization process. The optimal conditions for this microbiological process are determined by temperature and humidity. Apparently, the development of potato plants correlates with the intensity of the mineralization process in the soil as well as with the prevailing weather conditions.

In the period 2017–2019, the productivity of potatoes varied from year to year, however, in all years, both composting and biological preparations significantly increased the potato yield. Interesting data was obtained in 2018–2019, when we began to leave separate plots without plant protection products. The biological fungicides, in both years, gave the same increase in potatoes productivity as compost. The combined effects of biologics and composts were summarized. The maximum productivity of potatoes, which largely corresponded to the potential of the variety, was achieved in 2019. While in control 24.6 t ha^{-1} of standard potatoes tubes were obtained, the use of bio fungicides alone ensured a yield increase to 29.1–24.5 t ha^{-1} (Table 5). Compost significantly increased the yield of standard tubers. Without bio fungicides, compost provided an increase in the yield of standard tubers up to 5.9 t ha^{-1}, that is, each kilogram of compost nitrogen provided an increase of 73.7 kg ha^{-1} of standard tubers. Combination of bio fungicide and compost provided a further increase in potato yields. So, yield of 41.6 t $ha^{-1}$of standard tubes was achieved on the plots with combination of Flavobacterin + compost application. It should be noted that Flavobacterin provided a smaller proportion of small tubers in the total biomass (less than 1%) on all treated plots. The action of Kartofen, together with compost, also allowed a reliable increase. With a compost dose of 160 kg N ha^{-1}, the yield of standards tubes amounted to 40.4 t ha^{-1}.

Table 5. The influence of compost, biological plant protection products and weather conditions on the potato yield in the experiment during 2016–2019

Compost doze	Type of biological plant protection products	Yield, t ha^{-1}				
		Year	2016	2017	2018	2019
		Variety	Nevsky	Udacha	Udacha	Udacha
0	0		9.9	-	17.8	24.6
0	Extrasol		12.5			
0	Vitaplan / Kartofen		-	14.1	25.3	29.1
0	Vitaplan +Flavobacterin		-	16.6	24.0	29.5
40N kg ha^{-1}	Extrasol		8.2			
80 N kg ha^{-1}	0		-	-	24.5	30.5
80 N kg ha^{-1}	Extrasol		11.5			
80 N kg ha^{-1}	Vitaplan / Kartofen		-	18.7	29.2	32.7
80 N kg ha^{-1}	Vitaplan +Flavobacterin		-	18.4	27.6	41.6
120 N kg ha^{-1}	Extrasol		6.4			
160 N kg ha^{-1}	Vitaplan / Kartofen		-	16.4	29.3	40.4
$LSD_{0.95}$			1.9	1.64	1.8	4.0

Mathematical dependencies calculated according to the results obtained in the experiment are presented in Table 6. They confirm that the content of mineral forms of nitrogen in June predicts a possible future yield. However, in any case, it is weather conditions that have a decisive influence on productivity.

The question arises about the need to regulate the moisture content in the soil in June by agro technical methods. A rational combination of agrotechnical methods of soil cultivation contributes to the optimal moisture content in the soil during the period of active development of potatoes (June - July):

• Softening of the soil during its fall ploughing, providing moisture accumulation in the autumn and winter periods;

• Minimization of pre-planting tillage eliminating turning of furrow slice and active loosening of the arable layer (rotary tillage), ensuring minimal loss of accumulated moisture;

• Deep loosening of row-spacing in the technological process of planting potatoes, providing unhindered movement of moisture between soil horizons.

Table 6. Mathematical Regression of potato productivity against, the content of mineral N forms in the soil in June, compost doses and HTK values

Equation	R	Indicators
У2 = 73,806+6.57 A - 6.9 C – 11AC +2.24 A^2 + 8.58 C^2	0.9235	У2 – yield of potatoes, (t ha^{-1}) with compost dose 80 kg N ha^{-1}; C – HTK A – the content of mineral N forms in the soil in the first decade of June;
У = 13.02 – 0.533 A +0.504B + 0.231C	0.6382	Y – yield of potatoes, t ha^{-1} (all compost doses); B – doze of compost, in N ha^{-1}

But the most cardinal way is to organize irrigation during critical periods. This is the method used in New Zealand, which allows farmers to harvest 70–80 tons of potatoes per hectare. However, in preparing recommendations to farmers, it will be necessary to consider a wider range of factors, including social (Kubule et al., 2019).

CONCLUSIONS

1. In the results of the studies, mathematical dependences of potato productivity on the dose of compost and weather conditions were established. These dependencies allow us to proceed to the formation of models of production processes and the general model of agroecosystem crop rotation of a row crop.
2. The use of biological fungicides made it possible to activate the soil environment and potato plants, which ensured the same increase in yield as compost.
3. When using complex of variety, special tillage, compost and bio fungicides, the combination of their action a further significant increase in potato yield were ensured.
4. The conditions for preparing the soil for planting potatoes and its contents during potato growing season are formulated.

REFERENCES

Consolidated Annual Report of IFOAM - *Organics International & its Action Group. Changes for good 2018*. https://www.ifoam.bio/sites/default/files/annualreport_2018.pdf

Escobar, N., Romero, N.J. & Jaramillo, C.I. 2019. Typology of small producers in transition to agroecological Production. *Agronomy Research* **17**(6), 2242–2259. https://doi.org/10.15159/AR.19.221

Feiziene, D., Feiza, V., Povilaitis, V., Putramentaite, A., Janusauskaite, D., Vytautas Seibutis; V & Jonas Slepetys. 2016. Soil sustainability changes in organic crop rotations with diverse crop species and the share of legumes. *Acta Agriculturae Scandinavica, Section B – Soil & Plant Science* **66**(1), pp. 36–51.

Foley, J.A., Ruth DeFries, Ruth DeFries, 2005. Global Consequences of Land Use. *Science* **22 309**, Issue 5734, pp. 570–574. doi: 10.1126/science.1111772

Huhta, H. & Minin, V. 2014. *Basic principles of organic farming. Saint-Petersburg* - Saint-Petersburg - Mikkeli: AAFRDRT, 40 pp. (in Russian).

International Potato Center. 2016. Available online: http://cipotato.org/potato/ (accessed on 5 October 2016).

Kubule, A. Indzere, Z. & Muizniece, I. 2019. Modelling of the bioeconomy system using interpretive structural modeling. *Agronomy Research* **17**(4), 1665–1678. https://doi.org/10.15159/AR.19.170

Loginov, G.A., Fomin, I.M., Klein, V.F. & Varlamov, A.G.2009. Optimization of technical and technical solutions in potato growing. SPb., Pavlovsk: GNU SZNIIMESKH, 192 pp. (in Russian).

Müller-Lindenlauf, M. 2009. *Organic agriculture and carbon sequestration. Possibilities and constrains for the consideration of organic agriculture within carbon accounting systems.* Natural Resources Management and Environment Department, Food and Agriculture Organization of the United Nations, Rome, 29 pp.

Ponisio, L.C., M'Gonigle, L.K., Mace, K.C., Palomino, J., P. de Valpine & Kremen, C. 2015. *Diversification practices reduce organic to conventional yield gap. Proceedings Royal Society B* **282**, 20141396. http://dx.doi.org/10.1098/rspb.2014.1396

Popov, V.D., Minin, V.B., Maksimov, D.A. & Papushin, E.A. 2018. Justification of the intellectual system of organic production management in crop production. Technologies and technical means of mechanized production of crop and livestock products: *Theor. and scientific. - prakt. journal / IAEP*. **94**. St. Petersburg, pp. 28–41. doi 10.24411/0131-5226-2018-10086 (in Russian).

Potato industry. 2013. Ref. / Ed. Starovoitov, V.I. - ed. 2nd add. Moscow, 272 pp. (in Russian).

Raudsepp-Hearne, C, Peterson, G.D, Tengö, M., Bennett, E.M., Holland, T., Benessaiah, K., MacDonald, G.K. & Pfeifer, L. 2010. Untangling the environmentalist's paradox: why is human well-being increasing as ecosystem services degrade? *BioScience* **60**, 576–589. doi:10.1525/bio.2010.60.8.4

Reganold John P. & Wachter Jonathan M. 2016. Organic agriculture in the twenty-first century. *NATURE PLANTS* VOL. **2** | FEBRUARY. http://dx.doi.org/10.1038/nplants.2015.221

Seufert, V., Ramankutty, N. & Foley, J.A. 2012. Comparing the yields of organic and conventional agriculture. *Nature* **485**, 229–234. http://dx.doi.org/10.1038/nature11069

Shabanov, A.E., Zhevora, S.V., Anisimov, B.V., Kiselev, A.I., Dolgova, T.I., Malyuta, O.V., Zebrin, S.N. 2016. *Parameters of potential yield of potato varieties of the selection center VNIIKH*, (reference), FSBIU VNIIKH, Moscow, 13 p. (in Russian)

Skudra, I. & A. Ruza, A. 2019. Effect of nitrogen fertilization management on mineral nitrogen content in soil and winter wheat productivity. *Agronomy Research* **17**(3), 822–832. https://doi.org/10.15159/AR.19.135

Timoshina, N.A., Fedotova, L.S., Knyazeva, E.V. 2017. *Factors in the formation of yield and potato quality. Potato growing: Materials of the scientific-practical conference "Modern technologies for the production, storage and processing of potatoes"*, August 1-3, 2017 / FSBIU VNIIKH; under the editorship of S.V. Zhevory Moscow, pp. 19–26 (in Russian).

Zhuchenko, A.A. 2008. *Adaptive crop production (ecological and genetic basis). Theory and practice. In three volumes*. Moscow, Publishing house Agrorus, Volume **1**, 335 pp. (in Russian).

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https://doi.org/10.15159/AR.20.068

Design of modified movement planning system as a component of an intelligent planning system for robot manipulator

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Abstract. Different fields of industry and in-service support widely use robots, mechatronic and robotic technology systems in their activities. This is related to growing functionalities that result from using more advanced control systems the development of which is based on available achievements in the technical measures of computing. Therefore, the subject of study in this article was movement of a robot manipulator in using a fuzzy logics and neural network, and the goal of the study was to develop methods for designing combined intelligent planning and control systems for robot-manipulator movement in static dynamic environments based on the combined use of fuzzy logic apparatus and artificial neural networks to reduce the possibility of robot-manipulator's joints colliding into unknown obstacles located in its operating area. Based on this, the robot arm model has been developed after calculating in the article the missing parameters of the experimental robot manipulator in order to analyze the peculiarities of using the fuzzy logics device as well as the specifics and challenges of using neural network. As a result of the study performed in the article, significant data were obtained based on which a method was offered for an intelligent system for planning robot manipulator movement in static environment using a fuzzy blocks, which was characterized by the use of neural network corresponding each block, and localization of each solution to the task of planning robot manipulator movement in each specific situation, which enables to improve the accuracy and efficiency of movement planning.

Key words: static environment, intelligent planning system, neural network, robot manipulator, fuzzy logic.

INTRODUCTION

The method of designing an intelligent real-time planning system for robot-manipulator movement in an unknown static environment consisting of three stages of trajectory formation based on using the two fuzzy blocks of robot's each joint as well as the detailed classification model describing the locations of unknown obstacles in the operating area and the corresponding directions and types of movement in a form of multi-layer neural network perceptron allows for taking into account during each iteration the distance between the robot's joints and to the closest obstacles located on the right and on the left, and ensures the robot reaches the destination point.

In practice, use of geometric methods is unnecessary when the shortest distance is measured by the use of sensors. At the same time, unknown obstacles can have any shape, and the manipulator's operating device is equipped with a certain number of infrared distance sensor pairs separated one from another. Sensors are installed on the operating device in the places where it can have a contact with an obstacle. The density of distance sensors must ensure there are no 'blind zones' (Laurs & Priekulis, 2011; Osadcuks et al., 2014; Osadcuks & Pecka, 2016; Zhang, K. et al., 2016; Zhang, X. et al., 2016; Li, 2018; Matějka et al., 2019).

While calculating the shortest distances between manipulator's joints and the closest obstacles, only those obstacles which are within the sensory range of the distance sensors on the robot joints shall be taken into account. The shape of this range resembles a cylinder with its axis being the manipulator's joint, At the same time, the length of this range coincides with the length of the robot's joint, and the very range can be analysed as a simplified model of the environment scanned with the ultrasound distance sensors on both sides of each joint. Within the scope of computer simulations, readings of all the sensors comprise the known parameters of the function for calculating the shortest distance between each joint and the closest obstacle. It must be noted that analysis of this problem has not been included into the list of the main tasks of this dissertation research project (Sakai et al., 2002; Laurs & Priekulis, 2010; Yujie et al., 2010; Osadcuks, et al., 2014; Zhang et al., 2016).

The largest value in the range of the possible shortest distances between robot's each joint (joint number-one – d_{1max}, joint number-two – d_{2max}, joint number-three – d_{3max}, joint number-four – d_{4max}, joint number-five – d_{5max}, joint number-six – d_{6max}) and the closest obstacles can be calculated by the use of a graphical analysis with the model presented in Fig. 1. At the same time, the following must be taken into account (Laurs & Priekulis, 2008; Zou, et al., 2017; Ndawula et al., 2018; Zhou et al., 2018; Valjaots et al., 2018; Nemeikšis & Osadčuks, 2019; Obasekore et al., 2019).

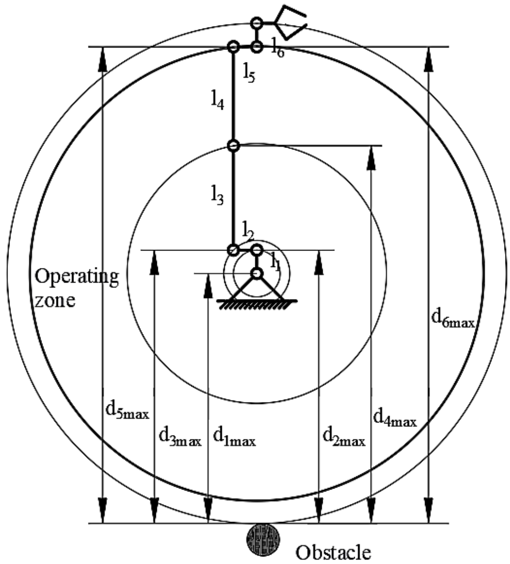

Figure 1. Graphical model for determining the largest value in the range of the possible shortest distances between the robot's joints and the closest obstacles (Yung et al., 2019).

$d_{1max} = l_1 + l_2 + l_3 + l_4 + l_5 + l_6$; $d_{4max} = 2l_1 + 2l_2 + 2l_3 + l_4 + l_5 + l_6$;

$d_{2max} = 2l_1 + l_2 + l_3 + l_4 + l_5 + l_6$; $d_{5max} = 2l_1 + 2l_2 + 2l_3 + 2l_4 + l_5 + l_6$;

$d_{3max} = 2l_1 + 2l_2 + l_3 + l_4 + l_5 + l_6$; $d_{6max} = 2l_1 + 2l_2 + 2l_3 + 2l_4 + 2l_5 + l_6$.

Note that d_{1max}, d_{2max}, d_{3max}, d_{4max}, d_{5max} and d_{6max} are used to design the diagrams of fuzzy dependency functions as the largest range (d_{nmax}) of the robot manipulator's n-link.

MATERIALS AND METHODS

A method was prepared for designing an intelligent planning system for robot manipulator real-time movement in an unknown static environment based on the process of processing information about the robot and and the surrounding static environment consisting of tree stages. The use of the method was described using a model of six-joint robot manipulator (Fig. 2).

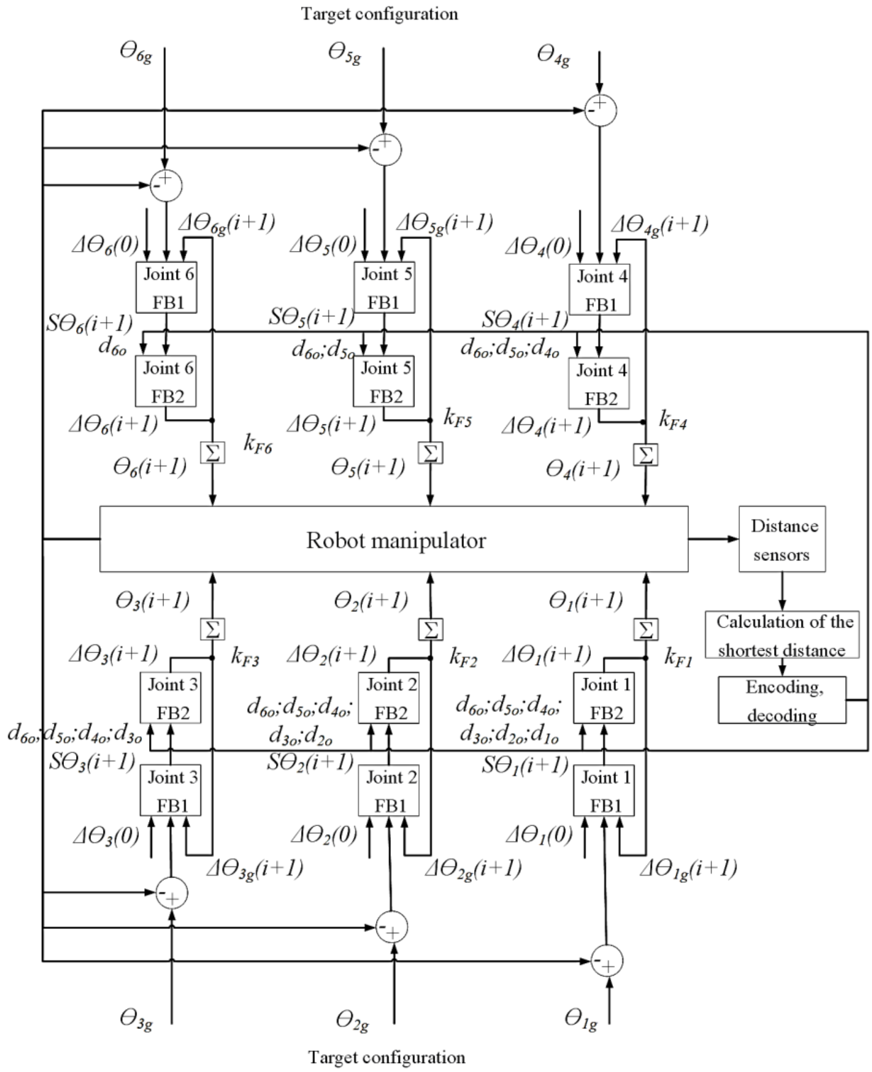

Figure 2. Intelligent planning system for six-joint robot manipulator real-time movement in an unknown static environment.

During the first stage, the final value of the distance between the robot's n-joint and an obstacle located in its operating zone (d_{no}) based on the information obtained from distance sensors, using the arrangement of the unknown obstacles in the robot's operating zone and a model of classifying the directions and movements of the respective joints as well as types of neural network multilayer (NNM).

During the second stage, the value of the initial step of the robot's n-joint movement ($S\theta_n(i+1)$which is the output of the fuzzy block number-one (FB1) with its input being the former values of changes in n-joint movement angle ($\Delta\theta_n(i)$), also the difference between the n-joint target (θ_{nd}) and current ($\theta_n(i)$) configurations ($\Delta\theta_{nd}(i+1) = \theta_{nd} - \theta_n(i)$). At the same time, the initial condition for system functioning is $\Delta\theta_n(0) = 0$.

During the third stage of the method in question, the final value of the output of the manipulator's n-joint fuzzy block number-two (FB2) - change in the movement angle - is determined based on the result parameters from the stage one and two ($\Delta\theta_n(i+1)$), when in a new iteration (i+1), the movement angle is determined as $\theta_n(i+1) = \theta_n(i) + \Delta\theta_n(i+1)$. Inputs to the FB2 are $S\theta_n(i+1)$ and d_{n0}. Internal feedback implemented in this system allows for calculation of $S\theta_n(i+1)$ depending on the $\Delta\theta_n(i)$ value, which helps to avoid robot collusion with an unknown obstacle and reach the final destination.

Designing a neural network for simulation of classifications

Designing a neural network for simulation of classifications of possible situations related to manipulator's movement starts from definition thereof. Classifications of such situations consists of systematization of unknown obstacles in the operating zone and the respective directions and types of robot's movement in each iteration.

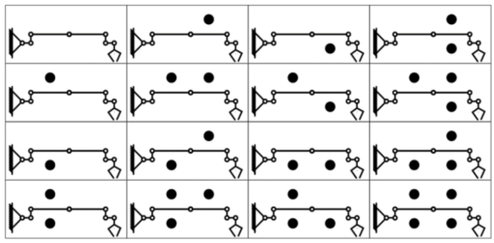

Figure 3. Classification of the closest obstacle arrangement in the operating zone of the robot manipulator.

The classification of unknown static obstacles suggested in the present thesis for six-joint manipulator consists of 16 possible situation variants with obstacles located on the right, on the left and both on the right and on the left with respect to each joint of the robot. Classification of manipulator's joint movement directions and types also has 16 possible options depending on the nature of movement: multi-step movement to the left; multi-step movement to the right; one step to the left and one step to the right.

Thus, a detailed table of classifications contains 16 items describing the positions of the possible manipulator movement in the operating zone and the related decision to be made during each iteration during the process of planning. Detailed outputs of the classification table model are the acceptable final distances between the joints of the robot and the closest obstacles on the left and on the right.

Fig. 3 presents classification of the possible arrangement of obstacles. The circles to the left and to the right of the manipulator's joint indicate obstacles located at the shortest distance, whereas a single circle can both represent a single obstacle as well as a set of obstacles.

The simulation of the classification of obstacle arrangement in the robot manipulator's operating area can be pictured as follows:

1. $D_{nR} = 0$ – no closest obstacle present to the right of the n-joint;
2. $D_{nL} = 0$ – no closest obstacle present to the left of the n-joint;
3. $D_{nR} = 1$ – an obstacle is present at the shortest distance to the right of the n-joint;
4. $D_{nL} = 1$ – an obstacle is present at the shortest distance to the left of the n-joint.

Fig. 4 shows the classification of robot manipulator's directions and types of movement, where dashed arrows mark separate steps to the right or to the left, and solid lines mark multi-step movement to the right or to the left, respectively.

Simulation of the directions and types of robot movement:

1. $D\theta_n = 0$ – n-joint multi-step movement;
2. $D\theta_n = 1$ – n-joint multi-step movement;
3. $D\theta_n = 2$ – one step of the n-joint to the left;
4. $D\theta_n = 3$ – one step of the n-joint to the right.

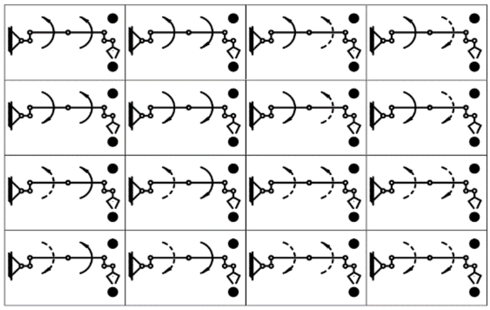

Figure 4. Classification of robot manipulator movement directions and types.

In total, the model of a detailed classification consists of 16 possible variants. Table 1 explain the functioning of a detailed classification model, where $D\theta_n$, D_{nR}, D_{nL}, D_{no} – movement directions of the manipulator's n-joint.

It should be noted that d_{nmax} is the largest value of the distance between the n-joint and an obstacle from the fuzzy range; d_{nL} – actual distance between the n-joint and the closest obstacle on the left; d_{nR} – actual distance between the j-joint and the closest obstacle on the right; OSR – one step to the right, OSL – one step to the left, that are calculated as the final difference between the target and actual configurations.

Table 1. Classification of the locations of obstacles and the directions and types of the related robot-manipulator movements in the operating area

No.	$D\Theta_n$	D_{nR}	D_{nL}	D_{no}
1.	0	0	0	d_{nmax}
2.	0	0	1	d_{nL}
3.	0	1	0	d_{nmax}
4.	0	1	1	d_{nL}
5.	1	0	0	$-d_{nmax}$
6.	1	0	1	$-d_{nmax}$
7.	1	1	0	d_{nR}
8.	1	1	1	d_{nR}
9.	2	0	0	OSL
10.	2	0	1	OSL
11.	2	1	0	OSL
12.	2	1	1	OSL
13.	3	0	0	OSR
14.	3	0	1	OSR
15.	3	1	0	OSR
16.	3	1	1	OSR

The designed structure of a neural network with the encoding and decoding blocks, where the values of the encoded outputs D_1, D_2, D_3, D_4, D_5, D_6 are used to determine the values of the final distances between the manipulator's joints and obstacles. In turn, d_1, d_2, d_3, d_4, d_5, d_6 are the wanted final distances – inputs to the fuzzy structure.

The neural network consists of two hidden layers and one output layer. The first hidden layer consists of forty neurons, the second consists of twenty five neurons, while the output layer consists of two neurons. The suggested structure of neural network was trained by backpropagation.

Detailed classification model based on neural network consists of three stages. During the first stage, manipulator's movements are transformed into codes according to the following classification variants: $D\theta_1$, $D\theta_2$, $D\theta_3$, $D\theta_4$, $D\theta_5$, $D\theta_6$, D_{R1}, D_{L1}, D_{R2},

D_{L2}, D_{R3}, D_{L3}. D_{R4}, D_{L4}, D_{R5}, D_{L5}, D_{R6}, D_{L6}. An encoding unit was developed to implement this process.

During the second stage, the encoded parameters get into the multilayer perceptron of the multilayer network. And finally, during the third stage, the outputs of the neural network are decoded (decoding unit) into the final values of the distances between robot joints and obstacles. Later, these values are used as inputs into a fuzzy structure with the decision-making process implemented within the limits of functioning thereof.

Designing a modified fuzzy movement planning system as a component of an intelligent planning system

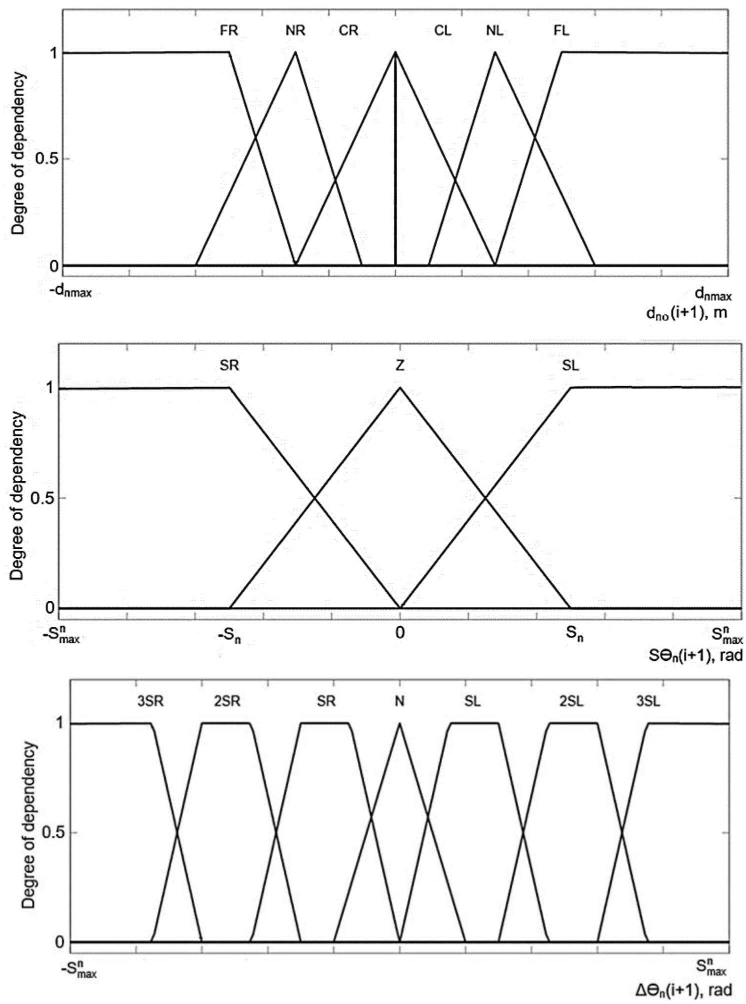

Figure 5. Dependence functions of FB2 inputs and outputs of a robot manipulator's n-joint.

The structure of a manipulator's real-time movement modified fuzzy planning system presented in the Fig. 2. In this system, the first fuzzy block (FB1) of the robot manipulator's n-joint is used to determine the $S\theta_n(i+1)$ value.

As a result of using in the planning system a detailed classification model functioning on a basis of three-layer neural network the number of inputs to FB2 for the n-joint has reduced. Therefore, the structures of FB2 for the n-joint are identical.

FB2 of the n-joint, have two inputs – movement step initial value $S\theta_n(i+1)$ and the output of the decoding unit d_{no}. Output from is $\Delta\theta_n(i+1)$. The dependence functions thereof are presented in Fig. 4.

The system of fuzzy basic rules used for FB1 and FB2 of the n-joint is presented in Table 1, which have been designed by the robot manipulator real-time movement in an unknown static environment fuzzy planning system FB2 (n-joint) (Fig. 5).

RESULTS AND DISCUSSION

N-joint robot manipulator described in Fig. 1 was also selected for computer simulation of an intelligent planning system for real-time movement in an unknown environment.

During test one, manipulator was moving from start point A configuration to target configuration point B (Fig. 6). After 2263 software iteration (set time 5.4637 s) error for θ_n was equal to 0.000°. No swinging movements in the area of target point were observed. This was obtained thanks to the functioning of the detailed classification model based on neural network.

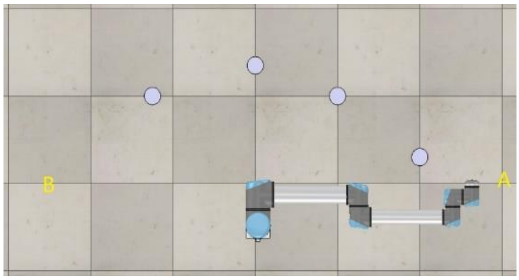

Figure 6. Results of the first test of an intelligent planning system for two-joint robot manipulator real-time movement in an unknown static environment.

Fig. 7 presents results of the test two when the robot was moving from the starting configuration point A to target configuration point B. Planning error related to the robot's joint θ_3, θ_4, θ_5 and θ_6 reaching target configuration after 2180 software iterations (set time 5.686 s), was equal to 0.000°. Moreover, no swinging movements were observed in the area of target configuration. It must be noted that there were no error of θ_1 and θ_2 as movement of the joint number one and two was successfully interrupted before potential collision to an obstacle. In this way, the manipulator reached the final configuration.

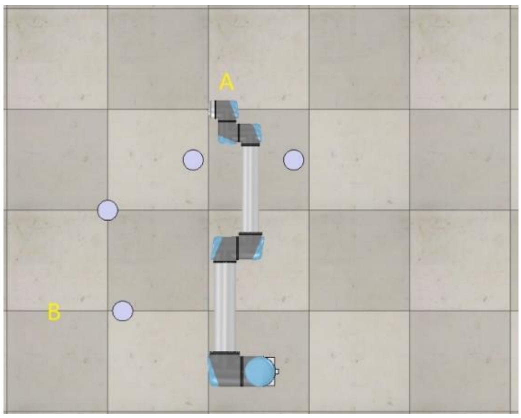

Figure 7. Results of the second test of an intelligent planning system for two-joint robot manipulator real-time movement in an unknown static environment.

Figs 8, 9, 10, 11, 12 and 13 presents the diagrams of test parameters, including D_{2o}, D_{3o}, d_{2o}, d_{3o}, $S\theta_2$, $S\theta_3$, $\Delta\theta_2$, $\Delta\theta_3$, θ_2, θ_3. It must be noted that the testing conditions provided for a situation where a robot's joint starts moving between two obstacles on the right and on the left at a short distance from one another.

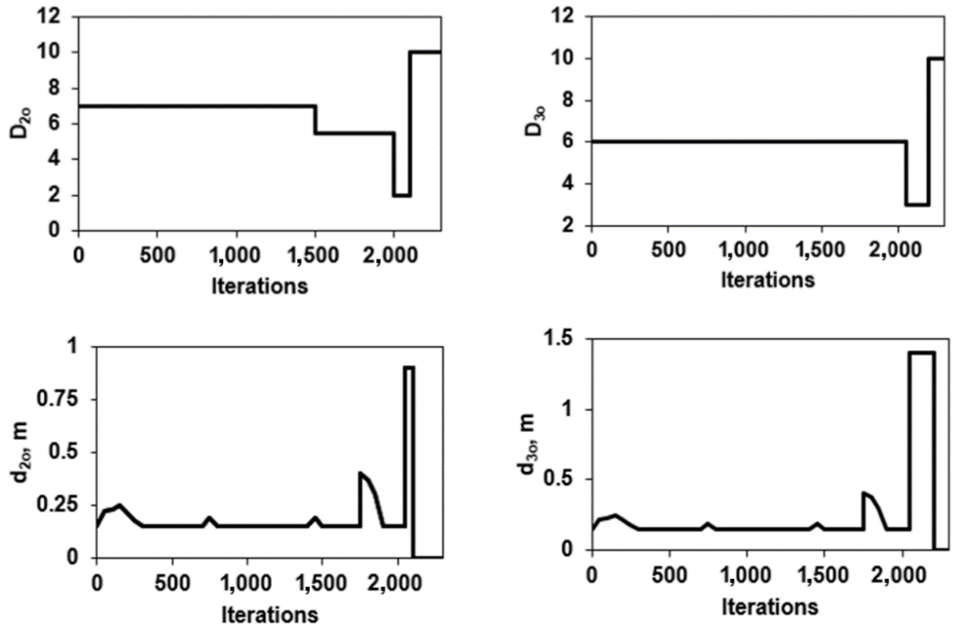

Figure 8. Parameters of the intelligent system test one results.

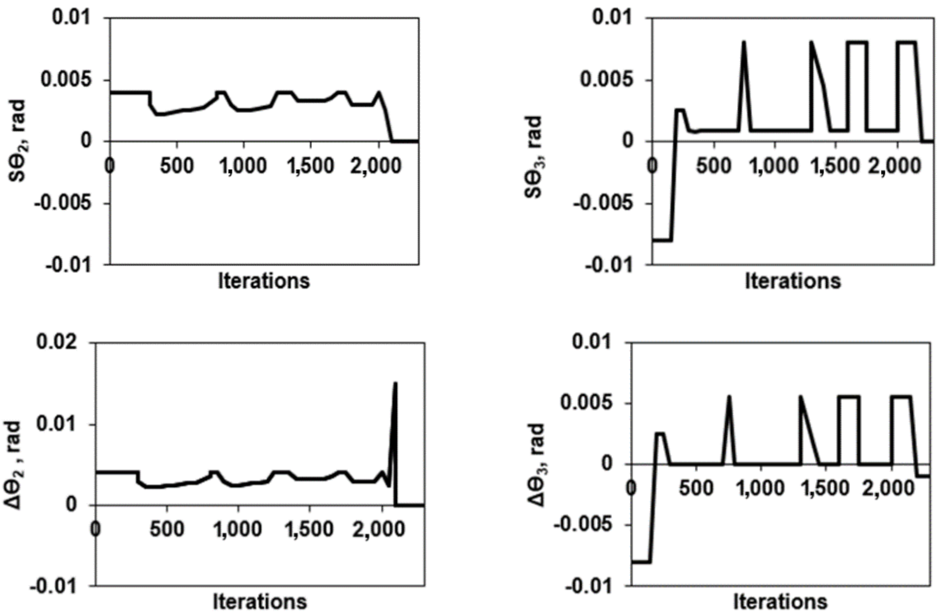

Figure 9. Parameters of the intelligent system test one results.

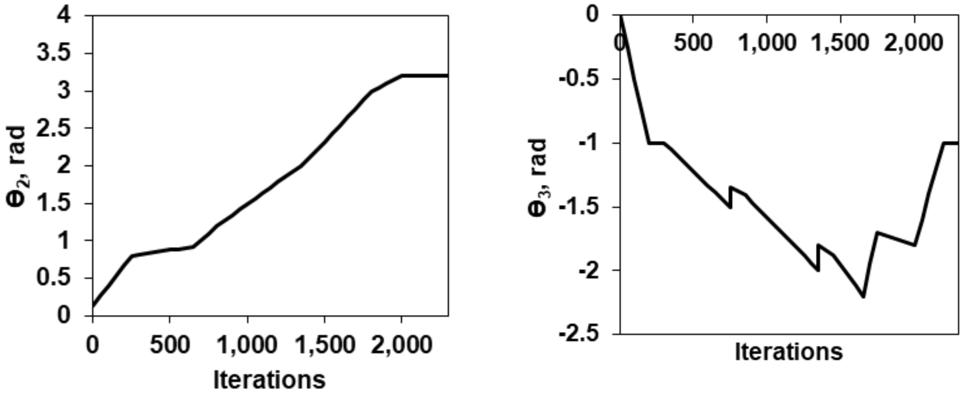

Figure 10. Parameters of the intelligent system test one results.

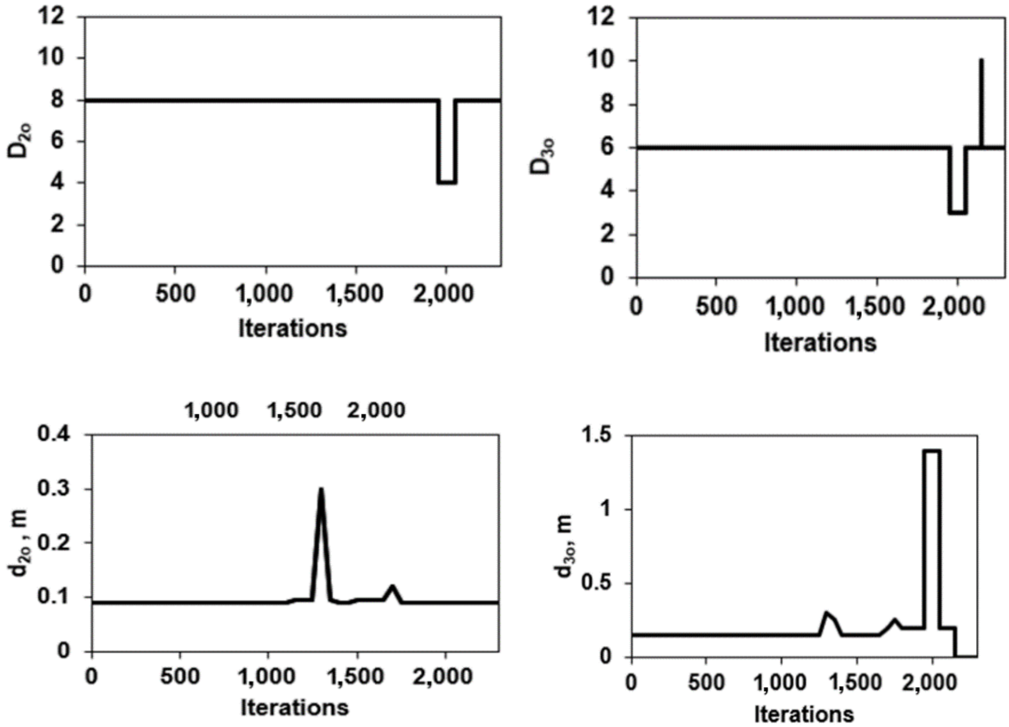

Figure 11. Parameters of the intelligent system test two results.

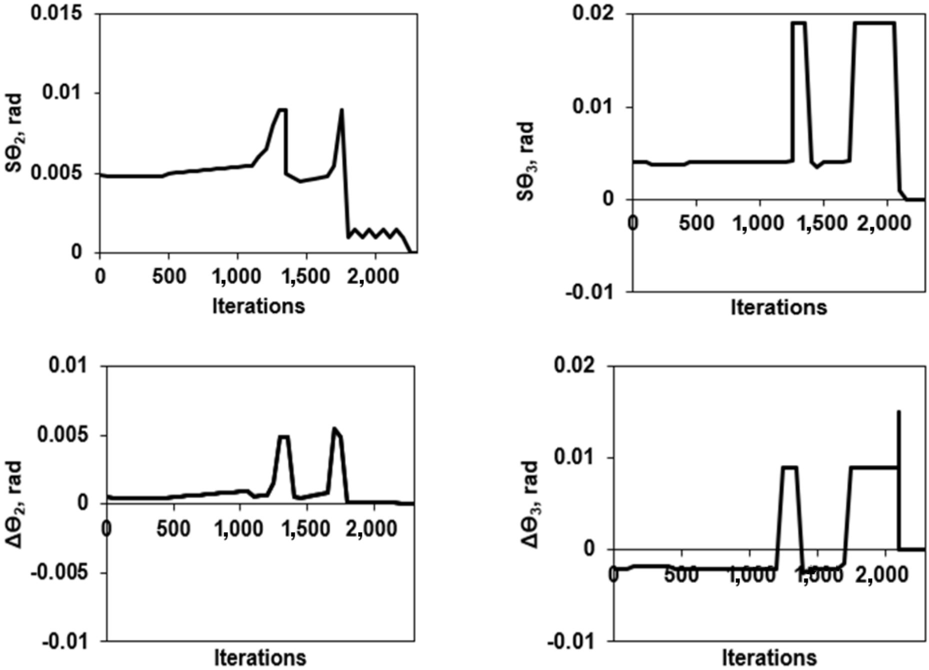

Figure 12. Parameters of the intelligent system test two results.

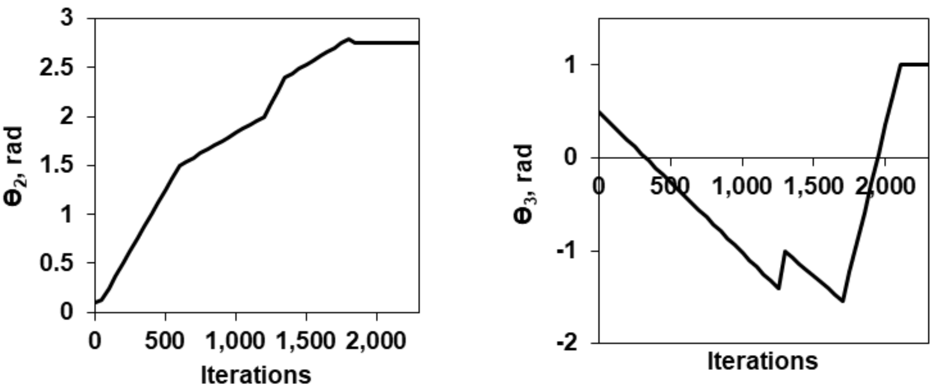

Figure 13. Parameters of the intelligent system test one results.

CONCLUSIONS

Neural network as a component of the intelligent planning system is used to simulate the 16 designed classifications of unknown obstacle arrangement in the operating zone as well as the 16 related classifications of the directions and types of movement of the two joints of the robot manipulator (the detailed classification table consists of 16 items).

A method was suggested of designing an intelligent real-time planning system for robot manipulator's movement in an unknown static environment based on the process of processing information about the robot and the surrounding environment consisting of three stages with the purpose to provide a safe trajectory. During the first stage, the final values of the distances between the manipulator's joints and the obstacles located in the operating zone are determined based on the classification model describing the arrangement of the unknown obstacles in the robot's operating zone and the respective directions and types of robot manipulator's movement. During the second stage, the values of a preliminary movement step of the robot's n-joint are calculated using the first fuzzy block of the corresponding joint. During the third stage, which is entered after completion of the first two stages, the second fuzzy block of the n-joint is used to calculate the final values of its movement angles. This method used for robot manipulators with any degree of freedom allows the following:

- taking into account during each iteration the values of the shortest distance between the robot's joints and the closest obstacles on the right and on the left;
- reducing the number of input parameters during the second stage of the fuzzy planning system;
- successfully avoid collision of the robot with the unknown static obstacles, and reach the target point without any oscillatory movements in the target point area with zero planning error.

REFERENCES

Laurs, A. & Priekulis, J. 2008. Robotic milking of dairy cows. *Agronomy Research* **6**(Special issue), 241–247.

Laurs, A. & Priekulis, J. 2010. Studies of operating parameters in milking robots with selectively guided cow traffic. *Agronomy Research* **8**(Special issue), 134–140.

Laurs, A. & Priekulis, J. 2011. Variability of milking frequency and intervals between milkings in milking robots. *Agronomy Research* **9**(Special issue), 135–141.

Li, K. & Qi, Y. 2018. Motion Planning of Robot Manipulator for Cucumber Picking. In: *2018 3rd International Conference on Robotics and Automation Engineering (ICRAE)*, Guangzhou, pp. 50–54.

Matějka, P., Kadeřábek, J. & Shapoval, V. 2019. Measurement Robotic Arm (MRA) for the evaluation of localization sensors properties. *Agronomy Research* **17**(4), 1688–1704. https://doi.org/10.15159/AR.19.140

Ndawula, I. & Assal, S.F.M. 2018. Conceptual Design and Kinematic Analysis of a Novel Open Field 3DOF Multi-Gripper Pot Seedlings Transplanting Robot. In: *2018 IEEE International Conference on Mechatronics and Automation (ICMA)*, Changchun, 2018, pp. 1458–1463.

Nemeikšis, A. & Osadčuks, V. 2019. Development of intelligent system of mobile robot movement planning in unknown dynamic environment by means of multi-agent system. *Agronomy Research* **17**(5), 1993–2004. https://doi.org/10.15159/AR.19.166

Obasekore, H., Fanni, M. & Ahmed, S.M. 2019. Insect Killing Robot for Agricultural Purposes. In: *2019 IEEE/ASME International Conference on Advanced Intelligent Mechatronics (AIM)*, Hong Kong, China, 2019, pp. 1068–1074.

Osadcuks, V. & Pecka, A. 2016. Electrical field based detection of fruits and vegetables for robotized horticulture. *Agronomy Research* **14**(S1), 1088–1098.

Osadcuks, V., Pecka, A., Lojans, A. & Kakitis, A. 2014. Experimental research of proximity sensors for application in mobile robotics in greenhouse environment. *Agronomy Research* **12**(3), 955–966.

Sakai, S., Iida, M. & Umeda, M. 2002. Heavy material handling manipulator for agricultural robot. In: *2002 IEEE International Conference on Robotics and Automation (Cat. No.02CH37292)*, Washington, DC, USA, vol. 1, pp. 1062–1068.
Zhang, X., Zhao, Z., Wei, S, Du, G., Ji, Q. & Zh, L., 2016. Study on the design and control system for wolfberry harvesting robot. In: *2016 Chinese Control and Decision Conference (CCDC)*, Yinchuan, pp. 5984–5985.
Zhang, K., Zhang, T. & Zhang, D. 2016. Synthesis design of a robot manipulator for strawberry harvesting in ridge-culture. In: *2016 Asia-Pacific Conference on Intelligent Robot Systems (ACIRS)*, Tokyo, pp. 114–117.
Zhang, K., Xu, L. & Zhang, D. 2016. Design and analysis of a 4-PPPR parallel manipulator for strawberry harvesting in the ridge cultivation model. In: *2016 2nd International Conference on Control Science and Systems Engineering (ICCSSE)*, Singapore, 2016, pp. 248–251.
Zhou, T., Zhang, D., Zhou, M., Xi, H. & Chen, X. 2018. System Design of Tomatoes Harvesting Robot Based on Binocular Vision. In: *2018 Chinese Automation Congress (CAC)*, Xi'an, China, 2018, pp. 1114–1118.
Zou, Z., Han, J. & Zhou, M. 2017. Research on the inverse kinematics solution of robot arm for watermelon picking. In: *2017 IEEE 2nd Information Technology, Networking, Electronic and Automation Control Conference (ITNEC)*, Chengdu, 2017, pp. 1399–1402.
Valjaots, E., Lehiste, V., Kiik, M. & Leemet, T. 2018. Soil sampling automation using mobile robotic platform. *Agronomy Research* **16**(3), 917–922. https://doi.org/10.15159/AR.18.138
Yujie, Cui, Jianning, Hua & Pu, Shi 2010. Kinematics simulation of an aided fruit-harvesting manipulator based on ADAMS. In: *2010 International Conference On Computer Design and Applications*, Qinhuangdao, 2010, pp. 222–226.
Yung, I., Maccarana, Y., Maroni, G. & Previdi, F. 2019. Partially Structured Robotic Picking for Automation of Tomato Transplantation. In: *2019 IEEE International Conference on Mechatronics (ICM)*, Ilmenau, Germany, 2019, pp. 640–645.

Agronomy Research **18**(S2), 1380–1387, 2020
https://doi.org/10.15159/AR.20.135

Experimental efficiency evaluation of 445 nm semiconductor laser for robotized weed control applications

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Abstract. Robotized weed control is one of perspective approaches for decreasing ecological impact of farming. Although current level of technology development allows robotized weed control to be economically reasonable only in specific applications, it is only a matter of time to introduce them in full-scale industrial farming. In general terms weed control using agricultural robots consist of two parts: recognition and spatial localization of weeds (distinguishing them from crops) and precision application of some kind of growth limiting activity. Recognition and localization is usually carried out using computer vision solutions (image filtering and transformations, artificial neural networks etc.). Growth limiting in its turn is performed by mechanical, precise chemical, thermal, cryogenic or other means. This article covers application of laser radiation for thermal destruction of unwanted plant canopies. In most cases CO_2 type lasers with 10.6 μm wavelength is used as they are affordable and they are applicable to use with plant biomass due to their spectral characteristics. Drawbacks of CO_2 lasers are low efficiency, size, weight and complex maintenance. In recent years relatively powerful short-wavelength semiconductor lasers have became broadly available on market. Light absorption of healthy green leaves is much better in blue-UV spectrum than in green, far infrared and near infrared, which is almost completely reflected by leaves. Thus an experimental study of using 12 W output 445 nm blue semiconductor laser for weed canopy cutting was carried out. The experiments were performed with direct laser radiation, the laser module was positioned using robotic manipulator with different speeds and cutting patterns.

Key words: weed, laser, efficiency, robot.

INTRODUCTION

Organic farming has increased substantially in EU during last decade. The total organic area in the EU-28 was 13.4 million hectares (ha) in 2018 compared to 10.05 million ha in 2012 (Eurostat, 2020). Weed management is considered one of the most technically challenging issues in organic agriculture, especially for delicate crops like carrots (Peruzzi et al., 2007; Peruzzi et al., 2017). New technical solutions are needed in horticulture to manage weeds with high precision and low energy consumption (Marx et al., 2012).

Thermal treatment by high intensity laser beam belongs to innovative physical methods which lacks extensive study (Pannacci et al., 2017), however along with other types of thermal treatment (Mojžiš et al., 2017) it seems to be promising approach. Visible and IR lasers cause explosive ejection, i.e. ablation of plant tissue generated by multiphoton and avalanche electron ionization (Bloembergen, 1974). Multiple research results have been published describing laser impact on plant growth (Sato et al., 2000; Mathiassen et al., 2006; Heisel et al., 2008; Marx et al., 2012). Studies typically combine different factors to determine optimal weed thermal treatment. In (Marx et al., 2012) authors evaluate the influence of 10.6 µm CO_2 laser radiation combining three laser spot diameters, three laser spot positions and six laser intensities. The treatment was applied on three growth stages of two weed species (monocotyledonous: *Echinochloa crus-galli*, dicotyledonous: *Amaranthus retroflexus*). Research additionally compares two laser guidance patterns: 1) wobbled laser beam with total diameter of 6 mm, 2) static unfocused laser beam with total diameter of 6 mm. The paper reports that lethality was greatest if high intensity treatment was carried out at early growth stages, while unfocused laser beam reduced lethality rate. In (Mathiassen et al., 2006) authors apply direct laser beam on apical meristems at the cotyledon stage of three different plant species: *Stellaria media* (common chickweed), *Tripleurospermum inodorum* (scentless mayweed) and *Brassica napus* (oilseed rape). The research reveals the biological effect by applying combination of two continuous wave diode laser types (5W 532 nm and 90W 810 nm), two spot sizes with a five different energy levels on each weed species. The green laser turned out to be more efficient and lethality was achieved at a much lower energy dose comparing to near infrared laser. In (Sato et al., 2000) authors also test two laser types (532 nm and 1,064 nm) with four emissions. The green laser with intensity between 56.6×4–144×4 GW m^{-2} and a single emission of 342GW m^{-2} affected the leaves physically, while infrared laser with intensity between 83.8×4 to 375×4 GW m^{-2} did not affect the plant at all.

A few studies have been made to simulate field conditions (Nadimi et al., 2009; Xiong et al., 2017) for laser treatment approach. The mobile robot prototype was developed equipped with two servo driven low power laser pointer lasers (Xiong et al., 2017). They simulated laser irradiation and focused mainly on traveling over the weed trays at laboratory conditions evaluating the optimum laser beam path traveling algorithm from weed to weed. Different approach was proposed by (Nadimi et al., 2009) where three conveyor belts were used to transport weed pots thus simulating mobile robot moving in the field.

All pointed studies were carried out in ideal laboratory conditions not taking into account the field environment such as soil irregularity, dust, wind, sun etc. Each of these factors can negatively impact success rate of precise laser application over apical meristems due to optical distortions (dust, sun, moisture) and non static target due to wind. According to (Marx et al., 2012) wobbled laser pattern is more efficient in case of smaller values of applied energy. Moreover, due to lower laser power (CO_2 25W), the risk of local perforation caused by intensive thermal impact reduces, thus the amount of energy not being absorbed decreases. Based on these facts in our study we focus on pattern use over weed canopy area instead of precise finding of weak spot – apical meristem. For majority of weed species, their external look change after first treatment

(Marx et al., 2012) what in field conditions will disallow to effectively find the weak spots. Therefore possible solution would be to recognize individual plant of the crop and consider as weeds all other green biomass regardless of its to species, stage of growth, anomalies in development etc.

While most of the studies regarding laser weed control rely on precise weak spot treatment or laser cutting, aim of this study was to develop robust methodology for effectiveness evaluation of weed treatment using laser patterns over green biomass that could be used in the field trials afterwards and deliver preliminary results on pattern and speed effect.

MATERIALS AND METHODS

The properties of laser treatment process evaluated in this study: treatment speed and energy required to limit or stop growth of a weed plant.

To evaluate performance of the laser in weed treatment an experimental setup was designed (see Fig. 1). Semiconductor laser module PLH 12000 with 12 W optical output power and 445 nm peak wavelength was used. The module was mounted on an industrial robot manipulator Universal Robots UR10. The robot was controlled remotely from a PC using server-client approach (Universal-Robots, 2020) over TCP protocol, thus it was possible to easily adjust laser moving speed, treatment pattern trajectory and output power. To ensure reliable real-time operation a full list of pattern trajectory coordinates are sent to robot controller before irradiating desired weed area.

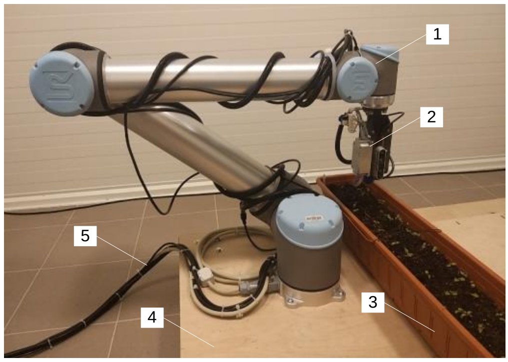

Figure 1. Experimental setup: 1 – UR 10 robot manipulator; 2 – PLH-12000 laser module; 3 – pot with weeds under treatment; 4 – moveable platform; 5 – power and communication cables to robot controller.

Area with weeds was treated individually by applying specific laser movement pattern and varying its size and movement speed. The laser was always operated at constant maximum optical output power therefore irradiation energy per square unit of area in a pattern was affected only by laser head speed. The pattern was applied to the center of a plant canopy (or pot) without taking into account plants size and individual form. Patterns were generated and sent from PC custom written software, pre-defined spiral drawing algorithm was used (Draw an Archimedes spiral, 2020).

This approach was chosen instead of precision treatment of leaves to bring the experiment conditions closer to real life situation with a mobile robot on field. In such conditions precise position of weed is complicated to determine, as well as other sporadic factors like close proximity of cultivated plants and wind effect will result in some portion of laser radiation not to reach intended target.

The following three experiment types were performed.

1. Experiment for determination effect of different treatment patterns. Two treatment patterns were used: spiral-shaped 10 mm in diameter, 8 evenly distributed loops and 'zig-zag'-shaped covering 10×10 mm square, 6 lines. Laser movement speeds were selected so that whole pattern would be drawn in approximately in the same time for both cases. One treatment per plant.

2. Experiment for determination treatment effect on multiple plants at once. Whole vegetating pot was treated using 30 mm s^{-1} or 90 mm s^{-1} speed, while all other parameters were the same. Spiral pattern was used with total diameter of 60 mm and 24 loops, all individual plants were located inside it. One treatment per pot.

3. Experiment for determination of laser energy amount on individual plants. Individual plants were treated using 30 mm s^{-1} or 90 mm s^{-1} speed, while all other parameters were the same. Spiral pattern was used with total diameter of 15 mm and 12 loops. One treatment per plant.

Table 1. Summary of experimental laser movement patterns and amount of applied energy

Exp. type	Laser movement speed, mm s^{-1}	Pattern diameter or side length	Area, mm^2	Treat-ment time, s	Total energy, J	Energy per length unit, J mm^{-1}	Energy per area unit, J mm^{-2}
1	22	10	100	6.04	72	0.60	0.725
1	20	10	79	5.88	71	0.59	0.898
2	30	60	2,827	74.50	894	0.40	0.316
2	90	60	2,827	25.28	303	0.13	0.107
3	30	15	177	9.12	109	0.38	0.619
3	90	15	177	3.38	41	0.14	0.229

Summary of experimental patterns is given in Table 1. Fig. 2 shows graphical details for each pattern. Calculations of applied energy were made for constant maximum laser output of 12W.

Figure 2. Laser movement patterns used during plant treatment: upper row – individual plant spiral, individual plant 'zig-zag', whole pot spiral; lower row – respective treatment results.

The experiments were performed on plants grown under controlled conditions in a research greenhouse at Institute for Plant Protection Research 'Agrihorts' with day temperature 21°C and night temperature 19 °C, relative humidity 60%, with natural and artificial lighting for 16h. Watering of plants was done by immersion method – every working day vegetation pots were immersed in a tap water 2 cm deep for 1 hour.

Plants with fully emerged cotyledons or emerging first true leaves were used in experiments, growth stage (GS) 11–12. Plant species for each experiment – (1) quickweed (*Galinsoga parviflora*); (2) pigweed (*Chenopodium album*); (3) cleavers (*Galium aparine*).

Evaluation of laser treatment effect on plant biomass was done after 7 days. Plants were cut just above the substrate and mass was measured on analytical balance (KERN ALJ 160-4AM).

To evaluate effectiveness of three different treatment approaches with limited samples, calculations were made with assumption that average mass of treated plants without treatment would be the same as for control group according to the following formulae.

$$\eta_w = 100 - \frac{\overline{m}}{\overline{m}_{control}} \cdot 100\% \qquad (1)$$

where η_w – relative weeding effectiveness showing decrease in average mass of individual plant in 7 days after laser treatment; $\overline{m}$ – average mass of individual plants in experimental group; $\overline{m}_{control}$ – average mass of individual plants in experimental group.

$$\eta_E = \frac{E}{\overline{m}_{control} - \overline{m}} \text{ (J m}^{-1}\text{)} \qquad (2)$$

where η_E – relative energy effectiveness showing how match optical laser energy was used to decrease mass of individual plant; E – total energy used in treatment.

RESULTS AND DISCUSSION

Results of the effect of laser treatment on changes in plant biomass are summarized in Table 2 and graphically in Fig. 3.

Although experimental data are not fit for statistical analysis due to small number of plants,there is clear tendency observed that proposed laser treatment approach limit weed growth. As expected, an increase in treatment time and thus in total treatment energy gives better result in limiting weed growth for experiments 1 and 2. Only exception is experiment (3), where slightly lower mass after 7 days of post-treatment vegetation is for group treated with lower energy. This could be explained with low number of plants and subsequent increase in fluctuations in results.

Table 2. Summary of experimental results by type of experiment: 1 – different laser treatment patterns on quickweed (*Galinsoga parviflora*); 2 – different treatment speeds, spiral pattern over multiple plants on pigweed (Chenopodium album); 3 – different treatment speeds, spiral pattern over individual plants on cleavers (*Galium aparine*)

Group	Total mass, g	Plant count	Average mass per plant, mg
1 – spiral	0.0147	9	1.6
1 – zigzag	0.0154	9	1.7
1 – control	0.2245	16	13.8
2–30 mm·s^{-1}	0.2118	34	6.0
2–90 mm·s^{-1}	0.4096	34	11.8
2 – control	0.6054	38	15.9
3–30 mm·s^{-1}	0.2499	9	27.8
3–90 mm·s^{-1}	0.2225	9	24.7
3 – control	0.7828	18	43.5

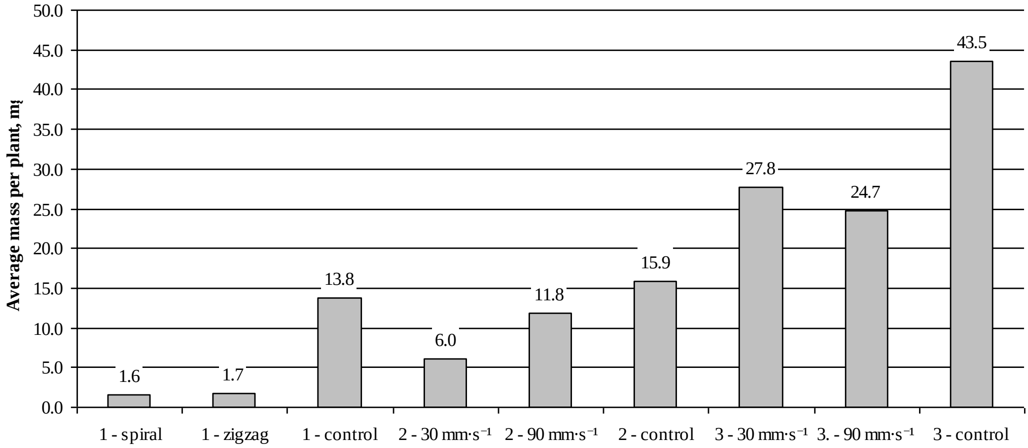

Figure 3. Average mass per plant for each experimental group after 7 days of post-treatment vegetation by type of experiment: 1 – different laser treatment patterns on quickweed (*Galinsoga parviflora*); 2 – different treatment speeds, spiral pattern over multiple plants on pigweed (*Chenopodium album*); 3 – different treatment speeds, spiral pattern over individual plants on cleavers (*Galium aparine*).

Different plant species can show different reaction to laser irradiation. This can be clearly seen on Fig. 4, where *Galium aparine* plants were able to regrow new leaves.

Table 3 shows relative performance of laser treatment in comparison to control group in each experiment according to equations (1) and (2).

If compared to two other experiments in experiment 2 total effectiveness is lower. It can be explained by the fact that distance between circles of treatment pattern was relatively large comparing with size of the leaves, which resulted in less effective coverage of all plants in the pot.

Figure 4. Experiment (3) with laser treatment of *Galium aparine*: right after treatment (top) and after 7 days of post-treatment vegetation (bottom). Yellow arrows show regrown leaves.

Energy amounts used for area treatment in our experiment are comparable to lower end values in other similar research with precision spot treatment: 0.6 to 5.9 J mm^{-2} with green 532 nm laser (Mathiassen et al., 2006) and 0.4 to 20 J mm^{-1} CO_2 10,600 nm laser (Heisel et al., 2008). Authors in these studies focused on achieving lethal outcome on plant during single laser treatment. Although there were only few lethal cases for plants after area treatment, mass reduction achieved in our experiments can serve as basis for further study with area treatment using laser patterns with presented methodology.

Table 3. Relative performance of laser treatment: 1 – different laser treatment patterns on quickweed (*Galinsoga parviflora*); 2 – different treatment speeds, spiral pattern over multiple plants on pigweed (*Chenopodium album*); 3 – different treatment speeds, spiral pattern over individual plants on cleavers (*Galium aparine*)

Group	Relative weeding effectiveness η_w, %	Relative energy effectiveness η_E, J·mg^{-1}
1 – spiral	88.2	0.221
1 – zigzag	87.6	0.179
2–30	62.0	0.128
2–90	25.8	0.105
3–30	36.2	0.039
3–90	43.2	0.012

Plant biomass measurement is a destructive (plant should be cut down) and time intensive. For next experiments alternative methods to measure treatment effectiveness are going to be considered, especially ones based on leaf area optical measurement.

Our proposed laser application method for weed control is simple to implement using current computer vision technologies and available plant datasets and does not require precision plant weak spot identification, which could be very cumbersome in real-life conditions. Moreover by simple identification of green biomass it is easy to do weeding repeatedly, even if weeds are partly damaged and visually differ from normal plants.

CONCLUSIONS

Preliminary results show, that 445 nm blue semiconductor laser can be effectively used for weed management. The best energy effectiveness is for treatment area, which is close to the size of plant canopy. Type of treatment pattern turned out not to be a significant factor in our experimental setup.

Particular plant species can regrow their leaves after laser treatment so this issue needs to be taken into account when frequency of field applications is calculated. Number of plant samples should be increased to statistically eliminate various random factors affecting both laser treatment process and plant development after it.

The following directions for further experimental studies can be formulated:

Test variable energy amount per square unit of area to find dependency curves and minimum energy necessary to limit plant development;

Search for optimum treatment pattern size by maximizing relative energy effectiveness factor;

Test treatment at different growth stages, especially for fast growing weeds.

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REFERENCES

Bloembergen, N. 1974. Laser-induced electric breakdown of solids. *IEEE Journal of Quantum Electronics QE*, **10**, 375–386.

Draw an Archimedes spiral in C# – C# Helper. *http://csharphelper.com/blog/2018/10/draw-an-archimedes-spiral-in-c/.* Accessed: 31.01.2020.

Eurostat, Organic farming statistics 2020. *https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Organic_farming_statistics#Total_organic_area.* Accessed: 31.01.2020.

Heisel, T., Jørgen, S., Christensen, S. & Andreasen, C. 2008. Cutting weeds with a CO_2 laser. *Weed Research* **41**, 19–29. 10.1046/j.1365-3180.2001.00212.x

Marx, C., Barcikowski, S., Hustedt, M., Haferkamp, H. & Rath, T. 2012. Design and application of a weed damage model for laser-based weed control. *Biosystems Engineering* **113**(2), 148–157.

Mathiassen, S.K., Bak, T., Christensen, S. & Kudsk, P. 2006. The Effect of Laser Treatment as a Weed Control Method. *Biosystems Engineering* **95**(4), 497–505. 10.1016/j.biosystemseng.2006.08.010.

Mojžiš, M., Vitázek, I. & Klúčik, J. 2017. Effect of flame weed control on various weed species at various developmental stages. *Agronomy Research* **15**(5), 2004–2011. https://doi.org/10.15159/AR.17.051

Nadimi, E., Andersson, K., Jørgensen, R., Maagaard, J., Mathiassen, S. & Christensen, S. 2009. Designing, modeling and controlling a novel autonomous laser weeding system. 10.13031/2013.29077.

Pannacci, E., Lattanzi, B. & Tei, F. 2017. Non-chemical weed management strategies in minor crops: a review. *Crop Protection* **96**, 44–58. 10.1016/j.cropro.2017.01.012.

Peruzzi, A., Ginanni, M., Fontanelli, M., Raffaelli, M. & Bàrberi, P. 2007. Innovative strategies for on-farm weed management in organic carrot. Renewable *Agriculture and Food Systems* **22**(4), 246–259. doi:10.1017/S1742170507001810

Peruzzi, A., Martelloni, L., Frasconi, C., Fontanelli, M., Pirchio, M. & Raffaelli, M. 2017. Machines for non-chemical intra-row weed control in narrow and wide-row crops: a review. *Journal of Agricultural Engineering* **48**(2), 57–70. https://doi.org/10.4081/jae.2017.583

Sato, K., Umezaki, T., Hoki, M. & Takaki, S. 2000. Fundamental Study of Laser Application for Weed and Pest Control. *Journal of the Japanese Society of Agricultural Machinery, Volume* **62**(5), 98–103. 10.11357/jsam1937.62.5_98

Universal-Robots Script Client-Server example. *http://www.zacobria.com/universal-robots-knowledge-base-tech-support-forum-hints-tips/knowledge-base/script-client-server/.* Accessed: 31.01.2020.

Xiong, Y., Ge, Y., Liang, Y. & Blackmore, S. 2017. Development of a prototype robot and fast path-planning algorithm for static laser weeding. *Computers and Electronics in Agriculture* **142**, *Part B*, 494–503. 10.1016/j.compag.2017.11.023.

Agronomy Research **18**(S2), 1388–1403, 2020
https://doi.org/10.15159/AR.20.159

Modeling the impact of weather and climatic conditions and nutrition variants on the yield of spring barley varieties (*Hordeum vulgare L.*)

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Abstract. Crop yield is a result of the interaction between plant genetic traits, soil properties, agrotechnology and climatic regimes. Low yield tend to be formed in regions where it is limited to the extent of water availability, heat stress and the short duration of the grain filling period. High temperature and drought stress are projected to reduce crop yields and threaten food security. The article presents the results of studies on the effectiveness of treatment of spring barley crops with modern growth-regulating drugs on the background of mineral fertilizers, carried out in different weather and climatic conditions in 2013–2017 yrs on the Southern chernozem in the conditions of Steppe of Ukraine. It was studied the influence of weather and climatic conditions, varietal characteristics of spring barley and nutrition variants on the formation of grain yield. It was determined that the cultivation of spring barley, the introduction of pre-sowing cultivation of mineral fertilizer at a dose of $N_{30}P_{30}$ (background) and the use of crop foliar fertilizing at the beginning of the phase of stooling and earing by the complex organo-mineral fertilizer Escort bio created favorable conditions for the growth and development of plants of the studied varieties, which in turn had a positive effect on grain yield. Thus, according to this variant of nutrition, on average, during the years of research, it was formed the yield of 3.25–3.61 t ha^{-1} grains depending on the studied variety.
Results of researches showed that weather conditions during the years of research significantly influenced on the productivity of spring barley varieties. In 2016 the amount of precipitation was the highest (174.0 mm), the temperature during vegetation of spring barley was +14.9 °C. In 2013 the amount of precipitation was the lowest (67.4 mm), the temperature was +18.5 °C. The lowest crop yield was formed in 2013, and the highest yield was formed in 2016. Studies showed that the influence of weather factors in various interfacial periods of growth and development of spring barley was significant enough for the manifestation of signs of yield and its elements and is more dependent on rainfall.

Key words: spring barley, variety, plant nutrition, weather and climatic conditions, grain yield, modeling of regularities.

INTRODUCTION

Land management for food production is a fundamental human activity, supporting the lives of nearly everyone on this planet and providing livelihoods for a large part of the population. At present, more than 1.5 billion ha – approximately 12% of the world's land area – is used for crop production (FAO 2018).

Crop yield is a result of the interaction between plant genetic traits, soil properties, agrotechnology and climatic regimes (Diacono et al., 2012; Borys & Küüt, 2016). Low yield tend to be formed in regions where it is limited to the extent of water availability, heat stress and the short duration of the grain filling period (Ewert et al., 2005). High temperature and drought stress are projected to reduce crop yields and threaten food security (Mahrookashani et al., 2017).

The impacts of climate change also have many undesirable effects on the global food supply and crop yield (Li, 2015). The average Earth surface temperature is a key indicator of climate change.

Solid evidence has shown that the global mean temperature has risen by 0.90 ±0.05 °C (95% confidence) since the 1950s, and could be rising another 1 to 3 °C by the end of this century (Hansen et al., 2010; Rohde, 2013). The Intergovernmental Panel on Climate Change (IPCC) has projected that the global warming trend from 1986–2005 to 2081–2100 will show a temperature increase of 0.3 °C to 1.7 °C based on representative concentration pathways 2.6 (RCP), 1.1 to 2.6 °C based on RCP4.5, 1.4 °C to 3.1 °C based on RCP6.0, and 2.6 to 4.8 °C based on RCP8.5 (Jonghan et al., 2019). However, temperature increases of 2–3 °C will limit the yield increases of C3 crops (such as barley, oat, and wheat) that result from elevated CO_2, and even larger temperature increases may offset CO fertilization effects altogether (Klink et al., 2014). The Intergovernmental Panel on Climate Change Fourth Assessment Report (AR4) concluded that crop yields may increase 10–15% in the mid- to high- latitudes with rising CO_2 levels and a global average temperature increase of 1–2 °C relative to 1980–1999 (Easterling et al., 2007).

High temperature and drought often occur simultaneously, but their effects on crops are usually investigated individually (Shah & Paulsen, 2003).

Drought decreased photosynthesis, stomatal conductance, viable leaf area, shoot and grain mass, and weight and soluble sugar content of kernels but increased plant water-use efficiency. High temperature hastened the decline in photosynthesis and leaf area, decreased shoot and grain mass as well as weight and sugar content of kernels, and reduced water-use efficiency. Interactions between the two stresses were pronounced, and consequences of drought on all physiological parameters were more severe at high temperature than low temperature (Shah & Paulsen, 2003).

Interpretation the mutual relations between climate and crop yield provides useful information for enhancing resilience of agricultural production systems to global climate change (Leng & Huang, 2017). Although agricultural technologies continue to improve, previous researches have shown that temperature and precipitation variations have considerable effect on crop yields, including spring barley (Lobell, 2007; Almaraz et al., 2008; Schlenker & Roberts, 2009).

Improving the technology of spring barley growth is an extremely urgent task, since under the current climatic and economic conditions cheapening of grain production and increase of its profitability is possible only in the case of application of new agrotechnical methods which do not involve high costs. Modern intensification of crop production in the conditions of acute deficiency of organic fertilizers and too high prices for mineral fertilizers involves the development of alternative measures of technology of crop cultivating. In the context of this, the study of the influence of highly effective polymer chelate fertilizers, biopreparations, growth-regulating drugs, etc. in combination with other agrotechnical elements and climate change on the formation of biometric indices of plants, productivity and quality of production becomes of increasing importance (Rozhkov & Gutyansky, 2017). There is a need for the development and implementation of resource-saving elements in plant nutrition technology, which consists of applicating of low doses of mineral fertilizers and, on their background, using of extra-root nutrition with modern drugs in the main periods of their vegetation (Gamayunova et al., 2017).

MATERIALS AND METHODS

Experimental researches were carried out during 2013–2017 yrs in the location of the educational-scientific-practical center of the Mykolaiv National Agrarian University.

The soil of experimental sites was represented by the Chernozems Calcic (CHcc). The reaction of the soil solution was neutral (pH 6.8–7.2). The content of humus in the 0–30 cm layer was 123–125 g kg^{-1}. The arable layer of soil contained moving forms of nutrients on average: nitrates (by Grandval Liagou - this method is based on interactions between nitrates and disulpfo-phenolic acid from which trinitrophenol (picric acid) is formed (Mineev et al., 2001). In alkaline environment it gives us yellow coloring due to formation of potassium trinitrophenolate (or natrium, depending from alkali used) in quantity equivalent to nitrates content) as 15–25 mg kg^{-1}, mobile phosphorus (by Machigin - this method is based on extraction of mobile phosphorus and potassium compounds from the soils with 1% ammonium carbonate solution, pH 9.0, at 25 ±2 °C) as 41–46 mg kg^{-1}, exchangeable potassium (on a flame photometer) as 389–425 mg kg^{-1} of soil (Mineev et al., 2001).

The territory of the farm locates in the third agro-climatic region and belongs to the subzone of the southern steppe of Ukraine (Panfilova et al., 2019). The climate here is temperate-continental, warm, dry, with unstable snow cover. Weather conditions by hydrothermal indices during the research years varied, which gave an opportunity to obtain objective results.

The object of research was spring barley – varieties Adapt, Stalker and Aeneas. The technology of their cultivation, with the exception of the investigated factors, was generally accepted to the existing zonal recommendations for the Southern Steppe of Ukraine.

The total area of the experimental plot (the research work was organized by the random method of choosing the plots) was 80 m^2, the basic plot was 50 m^2 (length – 21.18 m, width – 2.36 m), repetition in the experiment was done three times. Precrops was sown peas *Pisum sativum* L. The scheme of the experiment included the following options:

Factor A – variety: 1. Adapt; 2. Stalker; 3. Aeneas.

Factor B – plant nutrition: 1. Control (without fertilizers); 2. $N_{30}P_{30}$ – under pre-sowing cultivation – background (nitrogen was used in the form of ammonium nitrate (34% N), and phosphorus was in the form of double phosphorus (46% P);

3. Background + Urea K1 (1 L ha^{-1}); 4. Background + Urea K2 (1 L ha^{-1}); 5. Background + Escort-bio (0.5 L ha^{-1}); 6. Background + Urea K1 + Urea K2 (0.5 L ha^{-1}); 7. Background + Organic D2 (1 L ha^{-1}). The standard working solution was 200 L ha^{-1}. The fertilization of crops by fertilizers was carried out at the beginning of the phases of the spring barley stooling (BBCH 31) and earing (BBCH 51).

Preparations to be used for foliar application of barley crops were listed in the List of pesticides and agrochemicals authorized for use in Ukraine. Preparations of Urea K1 and Urea K2 are registered as fertilizers containing respectively N as 11–13%, P_2O_5 as 0.1–0.3%, K_2O as 0.05–0.15%, micronutrients as 0.1%, succinic acid as 0.1% and N as 9–11%, P_2O_5as 0.5–0.7%, K_2O as 0.05–0.15%, sodium humate as 3 g L^{-1}, potassium humate as 1 g L^{-1}, trace elements as 1 g L^{-1}. Organic D2 is organo-mineral fertilizer containing N as 2.0–3.0%, P_2O_5as 1.7–2.8%, K_2O as 1.3–2.0%, total calcium as 2.0–6.0%, organic matter as 65–70% (in terms of carbon). Escort-bio is a natural microbial complex that contains strains of microorganisms of genera Azotobacter, Pseudomonas, Rhizobium, Lactobacillus, Bacillus, and biologically active substances produced by them.

In the process of research, the method of the State Variety Testing of Agricultural Cultures was used (Volkodav et al., 2001). The sowing was done during the third ten-dey period of March, harvesting – the first ten-day period of July. The yield was determined by the method of continuous harvesting of each registration area (Sampo - 130 combine harvester).

Moisture content was determined by weight method. Soil samples were taken layer by layer to a depth of 100 cm before sowing barley spring and after harvesting (Kravchenko et al., 2003).

The statistical analysis (repetition was three times during 5 years of growing grain) of the research were processed using the method of multivariate disperse analysis. The obtained data were compared using analysis of variance (ANOVA). All statistical analyses were performed with Statistica 10, Agrostat New and Microsoft Excel.

To identify the dependence of yield on weather and climatic conditions (are air temperatuure , precipitiation and air humidity) during the growth and development of spring barley, linear dependence was used:

$$\hat{y}_x = a_0 + a_1x_1 + a_2x_2 + \cdots + a_nx_n \quad (1)$$

where $\hat{y}_x$ – the dependent variable, $x_1, x_2, \ldots, x_n$ – the independent indicators, $a_0, a_1, a_2, \ldots, a_n$ – the parameters of the model (Kobets & Tesyolkin, 2018).

RESULTS AND DISCUSSION

The zone of the southern Steppe of Ukraine is characterized by quite favorable agro-climatic and soil resources for growing crops. However, the limiting factor in obtaining stable yields is insufficient rainfall and their uneven distribution during the growing season of crops. Frequent droughts reduce the intensity of plant growth and development, the availability of nutrients, the yield and the product quality, and lead to soil erosion (Shevchenko et al., 2017; Panfilova, 2019; Panfilova & Mohylnytska, 2019).

Weather and climatic conditions during the growth and development of spring barley, on average over the years of research, are presented in Figs 1–3.

The analysis of meteorological indicators found that the maximum amount of precipitation, namely 83.0 mm was received in 2016 in the interfacial period earing – full ripeness of grain. The lowest amount of precipitation fell in 2013. Thus, for the full period of vegetation of spring barley, 67.4 mm of precipitation fell, which was less than in other years of research by 37.6 up to 106.6 mm or 35.8 up to 61.3%.

In general, temperature had similar patterns, but it was noted its growth in 2013, in the interphase period from earing to full ripeness of grain spring barley. The average air temperature in this period of growth and development of plants was +21.7 °C, which exceeded the indicators of 2014–2017 yrs of studies by 0.7 up to 3.7 °C or 3.2 up to 17.1%. In 2014, the air temperature increased in the interphase period of stooling – earing. The average air temperature in this period of growth and development of plants was +21.7 °C, which exceeded the indicators of 2013 yr, 2015–2017 yrs by 0.2 up to 7.0 °C or 0.9 up to 32.3%.

The relative humidity in the years of the study also changed between the phase periods of growth and development of spring barley. Thus, in 2013–2015 yrs it was the highest in the interphase period of tillering – stooling – 57 up to 75% depending on the year. In 2016 yr this figure was the highest in the interphase period of growth and development of spring barley earing – full ripeness of grain – 77%. The highest humidity in 2017 was in the period of germination – tillering – 71%.

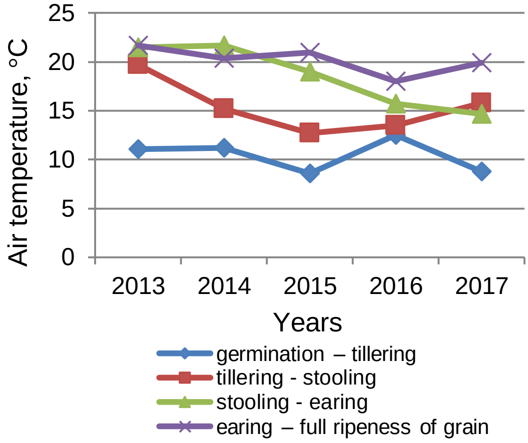

Figure 1. Air temperature, °C.

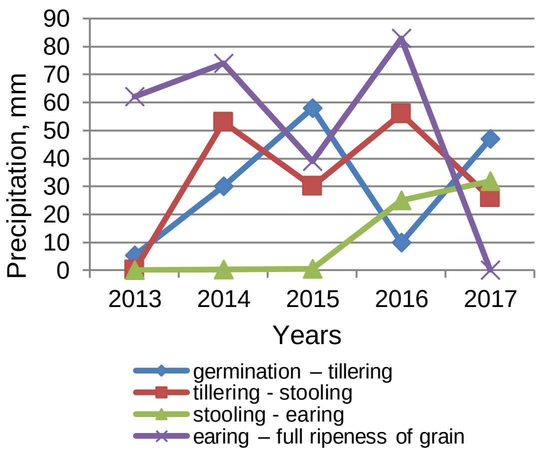

Figure 2. Precipitation, mm.

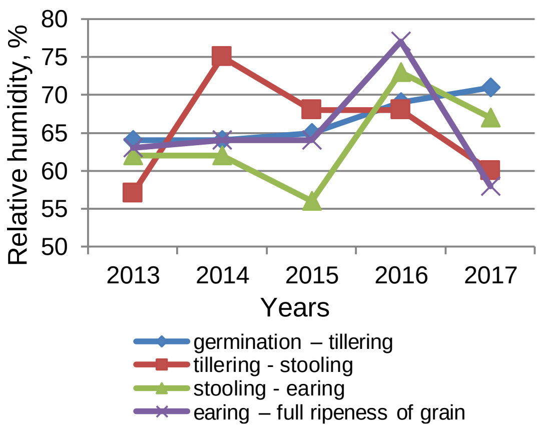

Figure 3. Relative humidity, %.

In conditions of unstable and insufficient moistening, the moisture is one of the decisive factors determining the level of crop yields. Soil moisture reserves are the major factor in the relationship between soil and plant, which is crucial for the production of strong shoots and further vegetation of plants (Kaminskyi & Gangur, 2018). Our studies determined that the water regime of the soil on the crops of spring barley had its own characteristics depending on the year of cultivation. Every year, the moisture reserves in the soil and the intensity of their spending were different, due to the amount of precipitation, temperature, humidity and so on. But the general dynamics of soil moisture on the crops of spring barley in all years of research had the same pattern. So, on average, during the years of research, the main amount of moisture in the soil accumulated in the autumn-winter period and its largest reserves reached during the sowing period, after which their amount was gradually spent by crops and decreased by the end of the growing season of the crop (Table 1). In terms of years, in 2016 yr the most moisture in the soil before sowing spring barley was 98.9 mm, in 2015 yr the slightly less moisture in the soil was 89.5 mm. Unfavorable conditions of water supply in the autumn-winter period of 2016–2017yrs provided the accumulation of the least amount of moisture in the soil as 17.5 mm. The same trend was observed after harvesting of spring barley.

It should be noted that in the variants of nutrition optimizing the total consumption of moisture during the growing season varieties of spring barley grew. So, on average, during the years of research, when applying a moderate dose of mineral fertilizers $N_{30}P_{30}$ after harvesting grain yield of Adapt variety it was remained 32.6 mm of available moisture in the meter layer of soil, after harvesting yield of Stalker it was remained 31.4 mm, and after harvesting yield of Aeneas it was remained 30.4 mm, which was less compared to the control of 3.0 up to 5.6% depending on the variety. However, the foliar fertilizing of spring barley plants during the growing season with modern growth-regulating drugs on the background of mineral fertilizers did not have a significant deterioration in the water regime of the soil and moisture reserves for the harvest period, on average, moisture reserves were 28.6 up to 31.2 mm depending on the studied variety, which was less than the control by 7.1 up to 11.2%.

On average for years of research and on the nutrition factor, it was used a few more intensively the moisture from the soil by plants of Aeneas spring barley variety. Thus, after harvesting on plots of this variety, it was remained 29.4 mm of available moisture in the soil, which was less by 1.1 up to 2.4 mm or 3.6 up to 7.5% than in other studied varieties.

According to the results of Kaminskyi & Gangur (2018) during the spring-summer vegetation period of winter wheat, it was observed the predominance of moisture consumption over its accumulation in the soil. However, during this period the productive spending of moisture dominated, thus, the soil moisture was more spent on the formation of the crop and it partly spent on physical evaporation from the soil surface. Future barley fields will decrease between 25 and 8% depending on climate projections. Barley yield will be more dependent on rainfall and extractable soil water (Cammarano et al., 2019).

Table 1. Reserves of productive moisture in the soil layer 0 up to100 cm, mm

Yrs.	Plant nutrition	Adapt Variety		Stalker Variety		Aeneas Variety	
		Terms of determination					
		before sowing	after harvesting	before sowing	after harvesting	before sowing	after harvesting
2013	Control	64.3	37.5	64.3	36.9	64.3	35.4
	$N_{30}P_{30}$ (background)	64.3	36.7	64.3	35.2	64.3	33.8
	Background + Urea K1	64.3	35.4	64.3	34.6	64.3	33.1
	Background + Urea K2	64.3	35.2	64.3	34.1	64.3	32.7
	Background + Escort-bio	64.3	33.9	64.3	32.5	64.3	31.6
	Background+UreaK1+UreaK2	64.3	34.6	64.3	33.1	64.3	32.1
	Background + Organic D2	64.3	34.3	64.3	32.8	64.3	32.0
2014	Control	70.4	38.1	70.4	37.4	70.4	36.8
	$N_{30}P_{30}$ (background)	70.4	37.5	70.4	36.2	70.4	35.5
	Background + Urea K1	70.4	36.9	70.4	35.4	70.4	34.9
	Background + Urea K2	70.4	36.5	70.4	35.0	70.4	34.6
	Background + Escort-bio	70.4	35.5	70.4	34.1	70.4	33.0
	Background+UreaK1+UreaK2	70.4	36.0	70.4	34.7	70.4	34.1
	Background + Organic D2	70.4	35.7	70.4	34.5	70.4	33.6
2015	Control	89.5	44.8	89.5	44.1	89.5	43.8
	$N_{30}P_{30}$ (background)	89.5	44.1	89.5	42.7	89.5	42.0
	Background + Urea K1	89.5	43.6	89.5	41.5	89.5	41.1
	Background + Urea K2	89.5	43.3	89.5	41.2	89.5	40.8
	Background + Escort-bio	89.5	42.2	89.5	39.8	89.5	39.1
	Background+UreaK1+UreaK2	89.5	42.7	89.5	40.4	89.5	40.0
	Background + Organic D2	89.5	42.4	89.5	40.2	89.5	39.6
2016	Control	98.9	37.6	98.9	37.0	98.9	36.5
	$N_{30}P_{30}$ (background)	98.9	36.4	98.9	35.1	98.9	33.9
	Background + Urea K1	98.9	35.9	98.9	34.4	98.9	32.1
	Background + Urea K2	98.9	35.8	98.9	34.0	98.9	31.8
	Background + Escort-bio	98.9	34.6	98.9	33.2	98.9	30.4
	Background+UreaK1+UreaK2	98.9	35.4	98.9	33.7	98.9	31.1
	Background + Organic D2	98.9	35.1	98.9	33.6	98.9	30.6
2017	Control	17.5	10.0	17.5	9.4	17.5	8.7
	$N_{30}P_{30}$ (background)	17.5	8.1	17.5	7.8	17.5	7.0
	Background + Urea K1	17.5	7.7	17.5	7.0	17.5	6.4
	Background + Urea K2	17.5	7.5	17.5	6.7	17.5	6.2
	Background + Escort-bio	17.5	6.3	17.5	5.6	17.5	5.0
	Background+UreaK1+UreaK2	17.5	7.1	17.5	6.2	17.5	5.3
	Background + Organic D2	17.5	6.5	17.5	6.0	17.5	5.3
average for 2013–2017 yrs.	Control	68.1	33.6	68.1	33.0	68,1	32.2
	$N_{30}P_{30}$ (background)	68.1	32.6	68.1	31.4	68.1	30.4
	Background + Urea K1	68.1	31.9	68.1	30.6	68.1	29.5
	Background + Urea K2	68.1	31.7	68.1	30.2	68.1	29.2
	Background + Escort-bio	68.1	30.5	68.1	29.0	68.1	27.8
	Background+UreaK1+UreaK2	68.1	31.2	68.1	29.6	68.1	28.5
	Background + Organic D2	68.1	30.8	68.1	29.4	68.1	28.2

LSD 0.5 (after harvesting)

factor A: 2013 – 0.474; 2014 – 0.375; 2015 – 0.591; 2016 – 1.042; 2017 – 0.759

factor B: 2013 – 0.709; 2014 – 0.760; 2015 – 0.844; 2016 – 0.874; 2017 – 0.774

Weather and climatic conditions of years and experience factors (variety, plant nutrition) significantly influenced on the grain yield of spring barley (Table 2).

Table 2. Yield of spring barley depending on varietal characteristics and optimization of plant nutrition, t ha^{-1}

Variety (factor A)	Plant nutrition (factor B)	Years					Average for 2013–2017 yrs.
		2013	2014	2015	2016	2017	
Adapt	Control (without fertilizers)	2.25	2.61	2.55	2.86	2.52	2.56
	$N_{30}P_{30}$ (background)	2.51	2.96	2.90	3.28	2.89	2.91
	Background + Urea K1	2.69	3.10	3.08	3.46	2.93	3.05
	Background + Urea K2	2.71	3.14	3.10	3.59	3.00	3.11
	Background + Escortbio	2.83	3.27	3.21	3.75	3.20	3.25
	Background + Urea K1 + Urea K2	2.74	3.21	3.14	3.65	3.12	3.17
	Background + Organic D2	2.79	3.24	3.18	3.71	3.18	3.22
Stalker	Control (without fertilizers)	2.34	2.69	2.62	2.88	2.64	2.63
	$N_{30}P_{30}$ (background)	2.66	3.09	3.01	3.30	3.06	3.02
	Background + Urea K1	2.79	3.20	3.18	3.65	3.15	3.19
	Background + Urea K2	2.81	3.23	3.20	3.70	3.22	3.23
	Background + Escort-bio	2.95	3.36	3.31	3.84	3.39	3.37
	Background + Urea K1 + Urea K2	2.86	3.29	3.26	3.76	3.30	3.29
	Background + Organic D2	2.91	3.32	3.29	3.80	3.35	3.33
Aeneas	Control (without fertilizers)	2.36	2.80	2.79	3.18	2.89	2.80
	$N_{30}P_{30}$ (background)	2.73	3.21	3.22	3.75	3.31	3.24
	Background + Urea K1	2.94	3.40	3.29	3.94	3.34	3.38
	Background + Urea K2	2.99	3.48	3.35	4.01	3.36	3.44
	Background + Escort-bio	3.12	3.58	3.52	4.30	3.51	3.61
	Background + Urea K1 + Urea K2	3.06	3.51	3.42	4.22	3.41	3.52
	Background + Organic D2	3.08	3.56	3.47	4.25	3.45	3.56
$LSD_{0.5}$	factor A	0.08	0.10	0.09	0.08	0.11	
	factor B	0.11	0.13	0.14	0.10	0.13	

The given data testified that plant nutrition and weather conditions during years of research significantly influenced on the productivity of spring barley varieties. In 2016 the amount of precipitation was the highest (174.0 mm), the temperature during vegetation of spring barley was +14.9 °C. In 2013 the amount of precipitation was the lowest (67.4 mm), the temperature was +18.5 °C. The lowest crop yield was formed in 2013, and the highest yield was formed in 2016.

The maximum yield of spring barley varieties in all years of our research was formed for the cultivation of culture on the background of applicating a moderate dose of mineral fertilizers and foliar nutrition of crops with Organic D2 and Escort-bio. Thus, on average, over the years of research and by factor variety, the grain yield was 3.37–3.41 t ha^{-1}, which exceeded its level in uncontrolled control by 0.71–0.75 t ha^{1} or 26.7–28.2%, and on the background of the application of mineral fertilizers in exceeded only by 0.4 t ha^{-1} or by 15.4%.

It was established by the research that application of Urea K1 and Urea K2 for foliar fertilization of plants increased the grain yield of spring barley. Thus, on average, over the years of research and by factor variety, in these experimental variants, it were formed

3.21 and 3.26 t ha^{-1}grains, which exceeded the control by 0.55–0.60 t ha^{-1} or by 20.7–22.6%, $N_{30}P_{30}$ (background) – by 0.15–0.20 t ha^{-1} or by 4.7–6.1%. But compared to the use of Organic D2 and Escort-Bio, the yield of barley was somewhat lower by 3.3–4.7 and 4.4–5.9%. The co-administration of these drugs provided the grain yield of spring barley at almost the same level as 3.33 t ha^{-1}.

Crop production per unit area (yield) is a fundamental parameter in agricultural and environmental research (Iizumi et al., 2014). The productivity as a result of functioning of agroecosystems has a complex nature and is affected by the influence of different factors. The impact of these factors can be identified through research on synchronous dynamics characteristics. The synchronous dynamics expresses itself through the forming of the correlation relationship. The correlation matrix is the basis for the principal component analysis and cluster analysis. Principal component analysis allows us to discover the main variability trends of agricultural crops' productivity (Zhukov et al., 2018). In our research we used correlation-regression analysis for study the dependence of yield on weather and climatic conditions. We see that the linear model works for the investigated varieties in the period 'tillering – stooling'.

Weather is an important factor, having an impact on the productivity and competition ability of all organisms. Because of the complexity, it is usually very difficult to find correlation between climatic conditions and stand situation in field conditions (Lillak et al., 2005).

Our studies found that for different varieties of spring barley, there is a fairly strong correlation ($0.9 \leq r \leq 0.99$) between weather and climatic conditions and the yield during the periods of 'germination-tillering', 'tillering-stooling' and 'earing – full ripeness' for the studied period (Table 3, Table 4). While the period of 'stooling-earing' is characterized by moderate and strong correlation ($0.5 \leq r \leq 0.9$).

Analyzing the obtained data we see that for establishing the dependence of grain yield on agro-climatic factors and constructing the regression equation, it is advisable to use the period of 'tillering-stooling' for Stalker and Aeneas varieties under the nutrition variant control option, since the econometric model can be considered suitable for research when the confidence probability $p \geq 0.95$.

For identifying the dependence of the yield on weather and climatic conditions during the growth and development of spring barley, we use a linear dependence. We define the variables of the econometric model: let $y -$ the yield of spring barley, t ha^{-1}; $x_1 -$ air temperature, °C; $x_2 -$ precipitation, mm; $x_3 -$ relative humidity, %.

For the Aeneas variety for the method of nutrition the control multifactorial regression has the form:

$$\hat{y} = -0.048x_1 + 0.017x_2 - 0.038x_3 + 5.45 \quad (2)$$

The regression equation shows that with an increase of air temperature x_1 (°C) by 1, the yield of spring barley decreases by 0.048 t ha^{-1}, with an increase in x_2 (precipitation, mm) it will increase by 0.017 t ha^{-1}, and with an increase in x_3 (relative humidity, %) by 1%, the yield of wheat will decrease by 0.038%.

Table 3. The statistic of multilinear regression analysis of yields impacted by weather conditions in interphase period 'germination – stooling' (r – the coefficient of multiple correlation, R^2 – coefficient of determination, p – confidence level, S_u – standard error)

Variety (factor A)	Plant nutrition (factor B)	Interphase period							
		germination – tillering				tillering - stooling			
		r	R^2	p	S_u	r	R^2	p	S_u
Adapt	Control	0.977	0.955	0.73	0.09	0.989	0.978	0.81	0.06
	$N_{30}P_{30}$ (background)	0.979	0.959	0.74	0.11	0.995	0.9896	0.87	0.06
	Background + Urea K1	0.942	0.888	0.58	0.19	0.955	0.912	0.63	0.17
	Background + Urea K2	0.948	0.899	0.60	0.20	0.957	0.916	0.64	0.18
	Background + Escort-bio	0.963	0.927	0.66	0.18	0.976	0.952	0.72	0.14
	Background+ UreaK1+UreaK2	0.720	0.518	0.19	197.38	0.955	0.912	0.63	84.47
	Background + Organic D2	0.966	0.933	0.68	0.17	0.955	0.912	0.63	84.47
Stalker	Control	0.993	0.985	0.85	0.05	0.9998	0.9997	0.98	0.01
	$N_{30}P_{30}$ (background)	0.996	0.992	0.89	0.04	0.998	0.997	0.93	0.03
	Background + Urea K1	0.955	0.913	0.63	0.18	0.978	0.956	0.73	0.13
	Background + Urea K2	0.948	0.899	0.60	0.20	0.957	0.916	0.64	0.18
	Background + Escort-bio	0.973	0.947	0.71	0.15	0.991	0.982	0.83	0.08
	Background+ UreaK1+UreaK2	0.968	0.937	0.68	0.16	0.990	0.981	0.82	0.09
	Background + Organic D2	0.969	0.938	0.69	0.16	0.991	0.981	0.83	0.09
Aeneas	Control	0.984	0.969	0.78	0.10	0.9995	0.999	0.96	0.02
	$N_{30}P_{30}$ (background)	0.970	0.941	0.69	0.18	0.998	0.996	0.92	0.05
	Background + Urea K1	0.955	0.913	0.63	0.18	0.978	0.956	0.73	0.13
	Background + Urea K2	0.971	0.943	0.698	0.18	0.969	0.939	0.69	0.18
	Background + Escort-bio	0.947	0.897	0.60	0.28	0.951	0.905	0.61	0.26
	Background+ UreaK1+UreaK2	0.9496	0.902	0.61	0.27	0.944	0.891	0.59	0.28
	Background + Organic D2	0.949	0.900	0.60	0.26	0.941	0.885	0.58	0.28

Table 4. The statistic of multilinear regression analysis of yields impacted by weather conditions in interphase period 'stooling – earing – full ripeness of grain' (r – the coefficient of multiple correlation, R^2 – coefficient of determination, p – confidence level, S_u – standard error)

Variety (factor A)	Plant nutrition (factor B)	Interphase period							
		stooling – earing				earing – full ripeness of grain			
		r	R^2	p	S_u	r	R^2	p	S_u
Adapt	Control	0.762	0.580	0.244	0.28	0.916	0.839	0.496	0.17
	$N_{30}P_{30}$ (background)	0.766	0.586	0.24	0.35	0.917	0.842	0.51	0.22
	Background + Urea K1	0.804	0.646	0.29	0.33	0.910	0.829	0.49	0.23
	Background + Urea K2	0.833	0.694	0.33	0.35	0.942	0.887	0.58	0.21
	Background + Escort-bio	0.841	0.707	0.35	0.36	0.958	0.918	0.64	0.19
	Background+ UreaK1+UreaK2	0.666	0.443	0.15	212.12	0.910	0.829	0.49	117.58
	Background + Organic D2	0.666	0.443	0.15	212.12	0.910	0.829	0.49	117.58
Stalker	Control	0.721	0.520	0.20	0.27	0.908	0.824	0.48	0.16
	$N_{30}P_{30}$ (background)	0.708	0.502	0.18	0.33	0.902	0.813	0.47	0.20
	Background + Urea K1	0.839	0.704	0.34	0.33	0.948	0.899	0.60	0.19
	Background + Urea K2	0.833	0.694	0.33	0.35	0.942	0.887	0.58	0.21
	Background + Escort-bio	0.856	0.732	0.37	0.33	0.9699	0.941	0.69	0.15
	Background+ UreaK1+UreaK2	0.842	0.708	0.35	0.34	0.957	0.916	0.64	0.19
	Background + Organic D2	0.854	0.729	0.37	0.33	0.965	0.930	0.67	0.17
Aeneas	Control	0.813	0.661	0.30	0.34	0.940	0.884	0.57	0.20
	$N_{30}P_{30}$ (background)	0.849	0.721	0.36	0.38	0.956	0.914	0.63	0.21
	Background + Urea K1	0.839	0.704	0.34	0.33	0.948	0.899	0.60	0.19
	Background + Urea K2	0.846	0.715	0.35	0.39	0.9699	0.941	0.69	0.18
	Background + Escort-bio	0.893	0.798	0.45	0.39	0.981	0.963	0.76	0.17
	Background+ UreaK1+UreaK2	0.897	0.804	0.46	0.38	0.985	0.9695	0.78	0.15
	Background + Organic D2	0.896	0.803	0.45	0.37	0.984	0.969	0.78	0.15

The coefficient of determination $R^2 = 0.999$ indicates that the variation of spring barley yield by 99.9% is determined by the variation of weather and climatic conditions.

Multiple correlation coefficient: $R = \sqrt{R^2} = 0.9995$ is a measure of the linear relationship between the dependent variable Y and the independent variables x_1, x_2, x_3. Its value shows a close linear relationship between the relevant indicators.

Analysis of variance was used to test the null hypothesis, according to which the average yield values did not consistent with each sowing scheme.

We check the hypothesis about the significance of the relationship between independent and dependent variables $F_{fact} = 339.389$ taking the table value for a given level of significance $\alpha = 0.05$ and the number of degrees of freedom $k_1 = 2$ i $k_2 = 3$: $F_{tabl} = F_{0.05;2;3} = 9.552$.

As $F_{fact} > F_{tabl}$ since the null hypothesis is rejected and with a given probability $p = 0.95$ the econometric model can be considered adequate to the actual data, i.e. the hypothesis of the significance of the relationship between the independent and dependent variables is confirmed.

Calculation of correlation coefficients for determine the relationship between the studied variables by least squares method. In this case, between the yield and air temperature there is a negative strong correlation ($r_{yx_1} = -0.797$), there is a positive strong correlation between yield and precipitation, ($r_{yx_2} = 0.845$), and there is a noticeable positive correlation between yield and relative humidity ($r_{yx_3} = 0.495$).

Over the framework of the multicollinearity study, were researched statistical sets of factors of influence (air temperature, rainfall and relative humidity) on spring barley yield. The research of multicollinearity presence between explanatory variables was done according to the Farrar-Glober algorithm. The algorithm has three types of statistical criteria. According to them the multicollinearity was checked with the whole set of independent variables (pearson's criterion (χ^2)); with each independent variable with the remaining variables (F – criteria); with each pair of independent variables (t – criteria).

In the research of multicollinearity presence for the whole group of independent variables, advanced calculations for determination of correlation matrix, included variables normalization, which distinguish influence factors in 'Tillering-plant transformation into a tube' period for the varieties - Stalker and Aeneas in power supply Control scenario. The determinant of the correlation matrix is $det\, r = 0.118076$. Pearson's criterion χ^2 was calculated by the formula:

$$\chi^2 = -\left\{n - 1 - \frac{1}{6}(2m + 5)\right\} ln(\, det\, r) \tag{3}$$

$$\chi^2 = -\left\{5 - 1 - \frac{1}{6}(2 \cdot 3 + 5)\right\} \cdot 0.118076 = -0.25583 \tag{4}$$

Calculated value of χ^2 was compared with table parameters $\chi^2_{\text{кр}} = 8.7$, with three degrees of freedom, significance $\alpha = 0.05$.

Since $\chi^2 < \chi^2_{кр}$ then multicollinearity between factors is not present.

Determination of multicollinearity fact of each independent variable with the others was made by formula: $F = (C_{kk} - 1)\left(\frac{n-m}{m-1}\right)$, where C_{kk} – is the diagonal element of C matrix (aspect of inverse matrix to correlation matrix) and compared with table parameter of F – criteria $F = 19$, significance $\alpha = 0.05$: $F_1 = 1.2567$, $F_2 = 3.6769$,

$F_3 = 2.7859$. So, based on founded values was seted that multicollinearity was not present.

Founded values of t – criteria, which was used for the determination of multicollinearity of two explanatory variables, $t_{12} = 0.7019$, $t_{13} = 0.1328$, $t_{23} = –1.4769$, were compared with table parameter $t = 4.3027$ with the significance of $\alpha = 0.05$ and assures us about multicollinearity absence.

Noteworthy fact is the result of authors team study who got high values of paired correlation coefficients of independent (factor) variables. The team explored model for multicollinearity presence as well. As far as one of the multicollinearity hallmarks is a high value determination coefficient going with the insignificance of the model coefficients, it would be appropriate to determine the significance of the each coefficient of the econometric model.

The statistical significance value of the economic model parameters (factors) was verified with the help of Student's t – statistic. Due to considering hypothesis $\mathrm{H}_0: a_{\mathrm{i}} = 0$ $(i = 0,1,2,3)$ – value of the parameter is insignificant and $\mathrm{H}_\mathrm{A}: a_i \neq 0$ $(i = 0,1,2,3)$ – value of the parameter is significant. The actual value of t – statistic had been determinated: $t_{\mathrm{a}_0} = 30.685$ $t_{\mathrm{a}_1} = 9.409$ $t_{\mathrm{a}_2} = 19.183$ $t_{\mathrm{a}_3} = 15.007$ and compared with table parameter $t = 4.3027$ with the significance of $\alpha = 0.05$. In the process of comparing actual and table values was made a conclusion about H_0 hypothesis for parameters a_0, a_1, a_2, a_3 that would be rejected in favor of an alternative. The model mentioned parameters are considered like statistically significant that have major influence on spring barley yield.

The same behavior is observed for the Stalker variety during the 'Tubing-Exit of Plants in the Tube'.

The studies allow us to conclude that the influence of weather factors in different interfacial periods is significant enough to show signs of yield and its elements and is more dependent on the amount of precipitation. This is confirmed by the calculated correlation coefficients.

It is possible that the linear relationships between temperature and precipitation changes and changes in barley yield presented here may not be representative of future crop-climate relationships: temperatures exceeding physiological thresholds, for example, can have nonlinear effects on crop yield (Schlenker & Roberts, 2009). So that the magnitude of future climate change impacts may increase from what has been observed over the past 30 years.

According to Příkopa et al. (2005), yield variability grain barley in the course of the experimental years were most affected by the weather conditions (82.3 and 76.2% share in the total variability, respectively). In our studies, climatic conditions in the period 'tillering – stooling' by 91.2–99.9% affected the productivity of spring barley.

Barley yields in cool conditions revealed modest interactions with the climate, while in warm conditions, there were stronger relationships between climate variability and barley yield (Klink et al., 2014). Warming temperatures, particularly in mid growing season, have reduced yields at nearly all sites; increased precipitation benefited yields for some time periods and locations but was detrimental to yield at others. Yield effects (as represented by adjusted r values) are stronger at climatologically warmer sites as compared with cooler sites, possibly because at the warmer sites oat and barley may be growing nearer their physiological limit. This observation echoes the findings of Lobell et al. (2011) who noted that crop yields in climatically warm countries were more sensitive to temperature increases than yields in cooler countries, and Hakala et al. (2012) found that barley cultivars from lower latitudes were the most sensitive to high temperatures.

According to Cammarano et al. (2019), there was a 9% reduction in grain yield under climate change; but the mean yield change was -27%, +4%, +8%, for the Dry, Mid, and Wet scenarios, respectively. The results of the simulations under the Wet scenario showed a higher variability of yield response. There was an interaction between the soil type, the amount of rainfall, the extractable soil water content and the maximum air temperature. Because of these relationship water-stress during the vegetative stage was experienced, affecting expansive growth. At the same time, the high number of days with *Tmax*>34 °C caused higher soil water depletion by the plant and therefore lower yields under the Wet scenario.

CONCLUSIONS

In the conditions of southern Ukraine, the application of mineral fertilizers at a dose of $N_{30}P_{30}$ under pre-sowing cultivation and the implementation of foliar nutrition of crops at the beginning of the phase of spring barley stooling and earing with the growth-regulating preparations provides the best conditions for the growth and development of plants and, as a consequence, the formation of more grain yield. In this regard, irrespective of the year of cultivation, the highest grain yield of spring barley was formed by the application of mineral fertilizers in a dose of $N_{30}P_{30}$ and nutrition of plants with the preparation Escort- bio. On average, over the years of research, in this version of the plant nutrtion, the highest level of grain productivity among the studied varieties was provided by the variety Aeneas as 3.61 t ha^{-1}.

Weather and climatic conditions in the years of research also significantly influenced on the formation of grain yield of spring barley. Thus, the lowest yield was formed in 2013 yr, and the highest yield was in 2016 yr. We used correlation-regression analysis for study the dependence of yield on weather and climatic conditions. We see that the linear model works for the investigated varieties in the period 'tillering – stooling'. It should be noted that climatic factors that affect on the level of productivity of spring barley were tested on multicolinearity, and it was constructed a multi-factor model with a level of significance $\alpha = 0.05$. For further research the possible directions would include the following: the development of a quality management decisions for effective mechanism of crop yields forecasting; taking into account the stability of spring barley yield levels for the southern Steppe of Ukraine.

REFERENCES

Almaraz, J., Mabood, F., Zhou, X., Gregorich, E. & Smith, D. 2008. Climate change, weather variability and corn yield at a higher latitude locale: Southwestern Quebec. *Climatic Change* **88**, 187–197.

Borys, N. & Küüt, A. 2016. The influence of basic soil tillage methods and weather conditions on the yield of spring barley in forest-steppe conditions. *Agronomy Research* **14**(2), 317–326.

Cammarano, D., Ceccarelli, S., Grando, S., Romagosa, I., Benbelkacem, A., Akar, T., Al-Yassin, A., Pecchioni, N., Francia, E. & Ronga, D. 2019. The impact of climate change on barley yield in the Mediterranean basin. *European Journal of Agronomy* **106**, 1–11. doi: 10.1016/j.eja.2019.03.002

Diacono, M., Castrignanò, A., Troccoli, A., De Benedetto, D., Basso, B. & Rubino, P. 2012. Spatial and temporal variability of wheat grain yield and quality in a Mediterranean environment: A multivariate geostatistical approach. *Field Crops Research* **131**, 49–62. doi: 10.1016/j.fcr.2012.03.004

Easterling, W.E., Aggarwal, P.K., Batima, P., Brander, K.M., Erda, L., Howden, S.M., Kirilenko, A., Morton, J., Soussana, J.F., Schmidhuber, J. & Tubiello, F.N. 2007. Food, fibre and forest products. Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental. Panel on Climate Change. Parry ML, Canziani OF, Palutikof JP, van der Linden PJ, Hanson CE (eds.), 273–313.

Ewert, F., Rounsevell, M.D.A., Reginster, I., Metzger, M.J. & Leemans, R. 2005. Future scenarios of European agricultural land use. I. Estimating changes in crop productivity. *Agric Ecosyst Environ* **107**, 101−116.

FAO statistical yearbook 2018. *World Food and Agriculture - Statistical Pocketbook* 2018, Rome. 255 pp.

Gamayunova, V.V., Dvoretsky, V.F., Sydakin, O.V. & Glushko, T.V. 2017. Formation of the overweight of spring wheat and triticale under the influence of optimization of their nutrition in the south of Ukraine. *Bulletin of ZhNAEU* **2**(61), 20–28.

Hakala, K., Jauhiainen, L., Himanen, S.J., Rötter, R., Salo, T. & Kahiluoto, H. 2012. Sensitivity of barley varieties to weather in Finland. J. *Agric.Sci* **150**(2), 145–160. doi: 10.1017/S0021859611000694

Hansen, J., Ruedy, R., Sato, M. & Lo, K. 2010. Global surface temperature change. *Reviews of Geophysics* **48**(4), 4004. doi: 10.1029/2010RG000345

Jonghan, K., Ng, C.T., Jeong, S., Kim, J.-H., Lee, B. & Kim, H-Y. 2019. Impacts of regional climate change on barley yield and its geographical variation in South Korea. *International Agrophysics* **33**(1), 81–96. doi: 10.31545/intagr/104398

Kaminskyi, V.F. & Gangur, V.V. 2018. Dynamics of productive moisture in the soil for the cultivation of winter wheat in the crop rotations of the Left-Bank Forest-Steppe of Ukraine. *Bulletin of the Poltava State Agrarian Academy* **3**, 11–14. doi:10.31210/visnyk2018.03.01

Klink, K., Wiersma, J.J., Crawford, C.J. & Stuthman, D.D. 2014. Impacts of temperature and precipitation variability in the Northern Plains of the United States and Canada on the productivity of spring barley and oat. *International Journal of Climatology* **34**(8), 2805–2818. doi: 10.1002/joc.3877

Kobets, S.P. & Tesyolkin, A.I. 2018. Approach to forecasting the yield of winter wheat considering the influence of the main meteorological factors. *Global and national problems of Economics* **23**, 701–705.

Kravchenko, M.S., Tsarenko, O.M., Mishchenko, Y.G. & Tomashivsky, Z.M. 2003. *Practicum on agriculture*. Meta, Kyiv, 320 pp.

Leng, G. & Huang, M. 2017. Crop yield response to climate change varies with crop spatial distribution pattern. *Scientific Reports* **7**, 1463.

Li, L.H.C. 2015. Assessing the spatiotemporal dynamics of crop yields and exploring the factors affecting yield synchrony. McMaster University, Hamilton, Ontario, 142 pp.

Lillak, R., Linke, A., Viiralt, R. & Laidna, T. 2005. Invasion of broad-leaved weeds into alfalfa stand during time of utilisation of alfalfa stands in low-input farming system. *Agronomy Research* **3**(1), 65–72.

Iizumi, T., Yokozawa, M., Sakurai, G., Travasso, M. I., Romanenkov, V., Oettli, P., Newby, T., Ishigooka, Y. & Furuya, J. 2014. Historical changes in global yields: major cereal and legume crops from 1982 to 2006. *Global Ecology and Biogeography* **23**, 346–357. doi: 10.1111/geb.12120

Lobell, D.B. 2007. Changes in diurnal temperature range and national cereal yields. *Agricultural and Forest Meteorology* **145**, 229–238.

Lobell, D.B., Schlenker, W. & Costa-Roberts, J. 2011. Climate Trends and Global Crop Production Since 1980. *American Association for the Advancement of Science* **333**(6042), 616–620. doi: 10.1126/science.1204531

Mahrookashani, A., Siebert, S., Hüging, H. & Ewert, F. 2017. Independent and combined effects of high temperature and drought stress around anthesis on wheat. *Journal of Agronomy and Crop Science* **203**(6), 453–463. doi: 10.1111/jac.12218

Mineev, V.G., Sychev, V.G., Amelyanchik, O.A., Bolysheva, T.N., Gomonova, N.F., Durynina, E.P., Egorov, B.C., Egorova, E.V., Eden, N.L., Karpova, E.A., Prizhukova, V.G. 2001. *Practice in Agrochemistry*: a textbook, Moscow State University Publishing House, Moscow, 689 pp.

Panfilova, A. 2019. The estimation of influence of weather and climatic conditions on the productivity of spring barley. The impact of climate change on spatial development of Earth’s territories: implications and solutions: *Proceedings of the 2nd International Scientific and Practical Conferenc*e (pp. 140–142), Kherson, UA: Kherson State Agricultural University.

Panfilova, A., Korkhova, M., Gamayunova, V., Fedorchuk, M., Drobitko, A., Nikonchuk, N. & Kovalenko, O. 2019. Formation of photosynthetic and grain yield of spring barley (*Hordeum vulgare* L.) depend on varietal characteristics and plant growth regulators. *Agronomy Research* **17**(2), 608–620. doi: 10.15159/AR.19.099

Panfilova, A. & Mohylnytska, A. 2019. The impact of nutrition optimization on crop yield of winter wheat varieties (*Triticum aestivum* L.) and modeling of regularities of its dependence on structure indicators. *Agriculture & Forestry* **65**(3), 157–171. doi: 10.17707/AgricultForest.65.3.13

Příkopa, M., Richter, R., Zimolka, J. & Cerkal, R. 2005. The influence of the year, fore-crops and fertilisation on yield and content of crude protein in spring barley. *Plant soil environ* **51**(3), 144–150.

Rohde, R., Muller, R.A., Jacobsen, R., Muller, E., Perlmutter, S., Rosenfeld, A., Wurtele, J., Groom, D. & Wickham, C. 2013. A new estimate of the average earth surface land temperature spanning 1753 to 2011. *Geoinformatics and Geostatistics: An Overview* **1**, 1. doi:10.4172/2327-4581.1000101

Rozhkov, A.O. & Gutyansky, R.A. 2017. The dynamics of the leafarea formation of spring barley crops depending on the effect of seeding norm and extra-rootnutrition. *Poltava State Agrarian Academy Newsletter* **4**, 32–37.

Shah, N.H. & Paulsen, G.M. 2003. Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. *Plant and Soil* **257**(1), 219–226.

Shevchenko, M.S., Desyatnik, L.M., Lorinez, F.V. & Shevchenko, S.M. 2017. Agrosystems methods of regulation of water use in agrocenoses. *Grain crop* **1**(1), 119–124.

Schlenker, W. & Roberts, M.J. 2009. Nonlinear temperature effects indicate severe damages to U.S. crop yields under climate change. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 15594–15598. doi: 10.1073/pnas.0906865106

Zhukov, O.V., Pelina, T.O., Demchuk, O.M., Demchuk, N.I. & Koberniuk, S.O. 2018. Agroecological and agroeconomic aspects of the grain and grain legumes (pulses) yield dynamic within the Dnipropetrovsk region (period 1966–2016). *Biosystems Diversity* **26**(2), 170–176. doi: 10.15421/011826

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The impact of herd health on the efficiency of dairy farms

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Abstract. Maximization of profit is one of the main interests of any farmer. Profit depends on managerial decisions and many economic factors, but also on the health of the herd. Thus, it is important to study how different factors related to herd health impact farms' economic performance. The objective of this paper is to determine how herd health influences farm technical efficiency by comparing Estonian farm data from two periods, the years 2012 and 2017. Typically, the major herd health issues are related to udder problems, followed by reproduction issues and limb disorders. We used the FADN (Farm Accounting Data Network) database and data from Estonian Livestock Performance Recording Ltd. The two-stage mathematical approach was chosen as the research method. In the first stage the DEA (Data Envelopment Analysis) was used to estimate farms' technical efficiency. The output-oriented VRS (Variable Returns to Scale) approach was applied to the data of 64 farms. In the second stage, we used the FRM (Fractional Regression Model) to define which the technical efficiency drivers were among herd health and economic factors. The study revealed that major changes have occurred between the two periods analysed. The main herd health factors influencing farms' technical efficiency are the somatic cell count (SCC) and age at first calving.

Key words: dairy farms technical efficiency, herd health, DEA, FRM.

INTRODUCTION

Impact of diseases on milk production

The success of modern dairy farming lies in the production of high-quality milk. To produce and supply safe and valuable products to consumers, dairy farmers have to ensure a healthy herd, balanced feeding, appropriate housing conditions, qualified labour, as well as successful management, which is a key tool for increasing the efficiency and profitability of a dairy farm (Noordhuizen & Cannas da Silva, 2009). Cow health has a major impact on the quantity and quality of milk. Diseases constitute an important economic issue. They result in a decline in milk production, which in turn causes loss of income and dairy products, increased costs for farmers and loss of food value as estimated by consumers (Fig. 1).

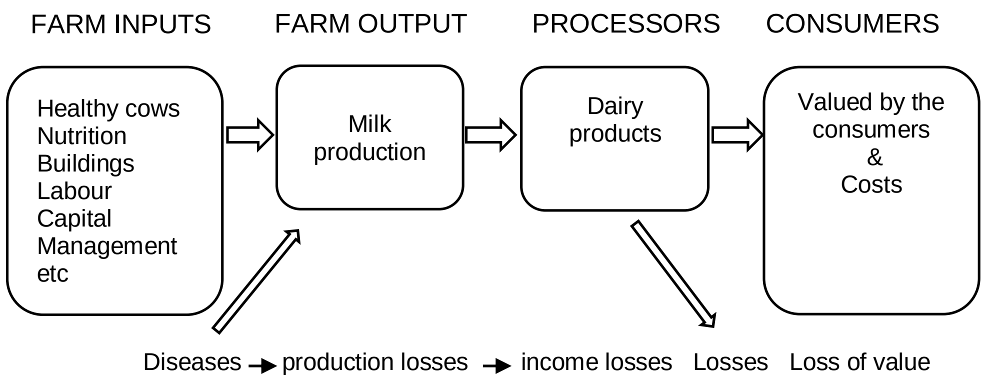

Figure 1. An illustration referring to the impact of diseases as an economic issue at farm and consumer level (adapted from Noordhuizen & Cannas da Silva, 2009).

Changes in dairy farming

The dairy sector has undergone major changes, caused by economic pressures, technological innovation, customer expectations and different regulations for the organisation of production (Barkema et al., 2015). Improvements in breeding, feeding, housing and management have supported the increase in milk production per cow (Kimura & Sauer, 2015). It is found that improved cow comfort and welfare is associated with increased herd productivity and profitability on free-stall farms (Villettaz Robichaud et al., 2019). Cattle housing conditions and housing type play an important role in ensuring the longevity of cows and preventing udder diseases (Ruud et al., 2010; Leso et al., 2019). Due to the shortage of qualified labour, modern technology is the most important helping tool for the farmer. Increasingly advanced technologies and machines help dairy farmers to monitor and enhance the welfare of farm animals and prevent diseases in dairy herds in ways that is difficult to achieve by human effort alone (Barkema et al., 2015; Gargiulo et al., 2017).

The EU accession in 2004 brought a significant change to the dairy sector in Estonia. Estonian dairy cattle are mostly kept in large intensive production farms which use innovative technological solutions and achieve high average milk production per cow. The dairy sector was modernised, switched to a free-stall barn system, new innovative feeding and milking technologies were introduced. (Luik & Viira, 2016; Gaworski et al., 2018; Luik-Lindsaar et al., 2019) Studies show that investments into modern dairy technologies can assure the increased milk yield and therefore sustainability of businesses (Kiiman et al., 2013; Luik & Viira, 2016; Cielava et al., 2017).

Herd health problems

Several researchers have studied dairy herd health problems from different aspects. High milk production means high incomes, but it can contribute to poorer cow health and fertility, resulting in a higher culling rate, most often caused by udder diseases (especially mastitis) and limb diseases (Horvath et al., 2017; Gussmann et al., 2019; Krpálková et al., 2019).

One of the most important characteristics of udder health, milk quality and composition is the number of somatic cells (SCC) in raw milk (Cinar et al., 2015). High levels of somatic cells are associated with mastitis (Özkan Gülzari et al., 2018). Mastitis is a disease that causes economic loss in the form of lost milk, loss of milk quality, premature culling of dairy cows, and treatment costs (Horvath et al., 2017). Özkan Gülzari et al. (2018) found that milk loss increased with an increase in SCC, indicating the effect of disease on production. Geary et al. (2013) pointed out that higher SCC has a considerable negative impact on farm, dairy processor and whole industry profitability. The acquisition of new milking technologies and better milking hygiene ensure higher milk quality, which results in a lower somatic cell count in milk (Sant'Anna & Paranhos da Costa, 2011). The high quality milk with low SCC is crucial in the dairy industry to produce high-quality products (e.g. cheese production), the raw milk quality affects the products shelf-life and better organoleptic properties for the consumer. As a result, producers, processors and consumers have lower food losses and food waste (Østerås & Sánchez Mainar, 2019).

Study conducted by Archer et al. (2013) showed that SCC is negatively associated with lifetime milk production. The key factor to increase revenue is to improving udder health early in the first lactation. Eastham et al. (2018) found that lower calving age is associated with a lower SCC, increased lifetime daily milk yield, improved reproductive performance, and improved udder health.

The number of somatic cells is one component of the price of milk to be sold. Therefore, determining somatic cell counts is important for farm management as the quality of milk is directly linked to the income of the cattle owner (Hadrich et al., 2018). Having healthy cows with healthy udders is a prerequisite for producing quality milk.

Cow health is a complex issue because it is difficult to draw a line between udder, fertility and limb diseases. Furthermore, these diseases are frequently interrelated (Koeck et al., 2014). One disease can easily lead to another, e.g. lameness might cause problems with standing and walking, because the cow lies down more. The desire to lie down frequently may in turn cause udder diseases, including milk fever (higher SCC), and decreased food intake, which can lead to a decreased milk yield (Potter et al., 2018). On the other hand, milk fever is also the biggest source of milk losses (Hadrich et al., 2018).

Increased milk production has a direct impact on cow health. Average milk yield in Estonia has increased from 3,968 kg in 1991 to 9,326 kg in 2018 (Statistics Estonia, 2020). Nor et al. (2014) confirm in their study that the culling rate of cows has grown, which is affirmed by Estonian data. While in 2002, the average herd life of cows in Estonia was 3.0 lactations, it had fallen to 2.4 by 2017 (Annual report 2002, 2017). In 2012, which was the first year of the analysed period, the main culling reasons of cows were udder diseases (21.1%), fertility issues (20.2%), and limb diseases (15.5%) (Results…, 2013). In 2017, the largest number of cows were culled due to udder diseases (20.4%), fertility issues (19.0%), and limb diseases (17.9%) according to Estonian Livestock Performance Recording Ltd. (Results…, 2018).

In the 1990s, herd health and production management programmes (HHMP) were developed in the Netherlands. The aim of HHMP programmes is to improve herd health through routine monitoring, problem analysis and preventive actions (Noordhuizen & Wentink, 2001; Duval et al., 2018; Svensson et al., 2018). The results of HHMP programmes are expected to improve herd health significantly, it has been recommended by veterinarians to Estonian dairy farmers.

The aim of this study is to determine the impact that animal health and economic indicators have on technical efficiency in the sample of 64 Estonian dairy farms in 2012 and 2017.

MATERIALS AND METHODS

The Estonian National level FADN dataset and data from Estonian Livestock Performance Recording Ltd. was used for analysing the same 64 dairy farms in 2012 and 2017. Data from both years was analysed using DEA and FRM, and then compared.

The output and input variables for the DEA analysis were chosen to characterise dairy production (Table 1). In the DEA model, there is one output and five inputs. The output variable is the sales revenue, and inputs are the number of cows, the amount of land, labour, capital, and production costs. These variables are common in technical efficiency analyses (Sipiläinen et al., 2009; Latruffe et al., 2012; Allendorf & Wettemann, 2015). The output and input variables are from FADN. The total sales revenue includes sales revenue from milk and other sales revenue from agricultural products. Sales revenue from animals is not taken into account as it can be considered as sales revenue from assets. The number of cows represents the annual average number of cows in the farm. The land variable is measured in hectares and includes all arable land. The labour variable is measured in hours and includes all working hours, both paid and unpaid. The capital expenditure is equalized to the annual depreciation. Intermediate consumption has been included in this work as production costs.

Output and input variables are in different units to represent the actual use of resources, e.g. labour is measured in hours to reflect the real labour input instead of labour costs, which are sometimes underestimated; the land variable is measured in hectares, which allows to compare farms more fairly than using the value of land or rent taxes.

Comparing the variables in 2012 and 2017 from the first stage analysis, it can be noted that some changes have occurred at farm level. The production output and input variables on average have grown from 2012 to 2017, except the agricultural area, which has decreased slightly (Table 1).

On average, milk yield increased by 1.13 times and sales revenue increased by 1.41 times in the sample farms during the analysed period. The increased sales revenue is a result of increased production volume rather than an increased milk price. The increase of milk price was 9% in 2017 compared to 2012 (Statistics Estonia, 2020). Some authors have found that higher milk yield influences technical efficiency positively (Sipiläinen et al., 2009). Therefore, it is crucial to consider milk yield as the factor of determining technical efficiency.

Capital and intermediate consumption increased by 1.26 and 1.22 times respectively. Prices of goods and services currently consumed in agriculture decreased by 1.45% and prices of agricultural investments increased by 3.63% from the year 2012 to 2017 (Statistics Estonia, 2020). The fact that input variables increased more than input prices suggests that the actual use of inputs has grown from the year 2012 to 2017.

In our sample the average number of cows per farm has increased from 302 to 348 from the year 2012 to 2017. In comparison, in 2017the national average herd size was 150 cows (Results…, 2018). The total number of cows was 19,308 in 2012 and 22,295 in 2017 in our sample. According to the total number of dairy cows in Estonia, approximately 25% cows are represented in our analysis.

Table 1. Descriptive statistics of the variables for Data Envelopment Analysis and fractional regression model

Variables		2012					2017				
	Unit	Min	Max	Mean	St. Dev	Median	Min	Max	Mean	St. Dev.	Median
Output and inputs in DEA											
total sales revenue	thousand euros	25.9	9,970.3	935.7	1,500.0	380.9	32.8	12,607.1	1,322.6	2,060.4	422.8
dairy cows	number	19	1,638	302	366	154	16	1,840	348	438	140
agricultural area	ha	37	5,729	922	1,156	447	39	5,612	912	1,143	366
labour	h	2,150	254,376	38,424	48,902	14,078	2,100	368,925	38,789	57,096	13,150
capital expenditure	thousand euros	3.0	735.4	133.7	159.1	63.3	3.1	792.7	168.1	200.6	64.5
intermediate consumption	thousand euros	29.3	9,510.8	969.8	1,477.0	376.9	40.2	11,317.4	1,185.3	1,783.4	437.4
Variables in FRM											
technical efficiency	score	0.394	1.000	0.803	0.154	0.806	0.514	1.000	0.835	0.135	0.837
milk yield per year	kg cow^{-1}	4,987	9,953	7,667	1,346	7,653	5,426	12,814	8,682	1,728	8,801
milk fat content	%	3.32	4.77	4.11	0.25	4.10	3.42	4.73	4.06	0.26	4.02
milk protein content	%	3.08	3.62	3.36	0.09	3.36	3.19	3.58	3.35	0.08	3.35
somatic cell count	10^3 ml^{-1}	129	609	343	121	325	67	1,070	266	139	267
age at first calving	days	735	1,305	875	124	843	704	1,249	835	107	814
productive period	days	844	2,247	1,317	340	1,210	881	2,817	1,212	315	1,130
age at culling	days	1,613	3,233	2,193	381	2,131	1,644	3,807	2,076	368	1,980
culling rate (udder)	%	0.0	57.1	27.8	15.1	28.6	0.0	50.0	24.0	11.2	22.8
share of EHF	%	0.6	100.0	76.5	32.6	93.4	0.0	100.0	78.4	33.9	99.8
share of own feed	%	18.4	100.0	59.7	19.7	57.8	4.7	95.0	56.3	17.3	54.8
feed costs per milk kg	euro kg^{-1}	0.075	0.292	0.174	0.049	0.171	0.085	0.310	0.171	0.042	0.164

Comparing the variables from the second stage analysis, some important changes can be observed between 2012 and 2017 (Table 1). The technical efficiency score which is a dependent variable in fractional regression increased. The increase in technical efficiency is in line with the economic theory, the farms must be efficient and become more efficient in order to be competitive (Cooper et al., 2007). The SCC decreased by 22.45% from 2012 to 2017. The change is significant and is presumably reflected in decreased treatment costs and increased revenue. The rate of culling due to udder diseases also decreased by 3.8 percentage points, and together with a decreased SCC it indicates improved udder health. Due to the increased milk yield, milk fat and protein content decreased slightly.

The age at first calving decreased by 40 days on average, from 875 to 835 days between 2012 and 2017. Earlier age at first calving should increase the productive period, but due to intensive production, the productive period decreased as well as the age at culling (longevity).

The share of Estonian Holstein breed (EHF) increased, which has been a trend for years. The share of EHF increased by 1.9 percentage points between 2012 and 2017. The EHF has a higher milk yield and lower milk fat and protein content compared to the Estonian Red (Results…, 2019). The above-mentioned decreased milk fat and protein content could be associated with the increased share of EHF.

The share of own feed describes the share of home-grown feed cost out of total feed costs. The share of home-grown own feed decreased by 3.4 percentage points, which means that the share of purchased feed increased. The latter is mainly concentrated feed, which enables to produce a higher milk yield.

Intermediate consumption, which includes feed cost also increased, at the same time feed costs per milk kg decreased slightly. The reason for decreased unit costs could be associated with increased milk yield, which grew more prosperously than feed costs.

We used the two-stage approach to analyse herd health and economic indicators that are potentially connected with farm technical efficiency. Farm performance was evaluated in the first stage using the Data Envelopment Analysis and the result of the analysis is considered as a variable that describes management capabilities. In the second stage, we looked for variables that could affect farm performance.

The output-oriented variable returns to scale model (VRS) was used in the Data Envelopment Analysis to calculate the technical efficiency of dairy farms. The DEA refers to dairy farms as decision making units (DMUs). Production outputs (sales revenue) and inputs (cows, land, labour, capital, production costs) are variables in the DEA model. The DEA compares the output-input ratios of different DMUs on a relative scale and constructs a best practice frontier comparing the best DMUs to others. Farms that determine the best practice frontier were defined as technically efficient, whilst others as technically inefficient, and their efficiency was calculated in comparison to the most efficient farms on the relative scale. Technical efficiency scores were calculated for each farm.

We used the output-oriented model where outputs were maximised and inputs were kept at their current levels (Eq. 1):

$\theta^* = \max \theta$

subject to $\sum_{j=1}^{n} \lambda_j x_{ij} \leq \theta x_{i0} \quad i = 1, 2, \ldots, m;$

$$\sum_{j=1}^{n} \lambda_j y_{rj} \geq y_{r0} \quad r = 1, 2, \ldots, s; \tag{1}$$

$\sum_{j=1}^{n} \lambda_j = 1 \; ; \lambda_j \geq 0; \; j = 1, 2, \ldots, n.$

where *DMUo* represents one of the *n* DMUs under evaluation, and x_{io} and y_{ro} are the i^{th} input and r^{th} output for *DMUo*, respectively. If $\theta^* = 1$, the current input levels cannot be reduced (proportionally), indicating that *DMUo* is on the frontier. Otherwise, if $\theta^* < 1$, *DMUo* is dominated by the frontier (Zhu, 2009).

In the second stage the fractional regression model (FRM) was used. The technical efficiency score from the DEA was the dependent variable and factors that potentially affect technical efficiency are independent variables. The technical efficiency score is within the range of 0 to 1. A model that is suitable for analysing the dependent variable in the DEA framework is the fractional regression model (Ramalho et al., 2010; Ramalho & Ramalho, 2011). We used the one-part cauchit model. The one-part cauchit model was chosen as the proportion of efficient farms is small. The advantage of the second part of the two-part model appears when there is a considerable amount of efficient farms.

The technical efficiency score from the first stage of the analysis was regressed in the second stage fractional regression model to find out the variables that affect technical efficiency.

RESULTS AND DISCUSSION

Results of the DEA analysis

The average technical efficiency has grown between the years 2012 and 2017. The average technical efficiency was 0.803 (80.3%) in 2012 and 0.835 (83.5%) in 2017. The technical efficiency score shows the actual amount of production compared to the potential level of outputs. The results suggest that farmers could have produced 19.7% more outputs in 2012 and 16.5% more outputs in 2017, whilst keeping the inputs at the same level. The number of technical efficient farms was 13 (20.3%) in 2012 and 16 (25%) in 2017.

On average, technical efficiency has increased, but this is not the case for all farmers. We divided the farms into three groups according to their technical efficiency change from 2012 to 2017 (Table 2). Farms whose technical efficiency change was above 0.05 points are in the group with positive change in technical efficiency (PosCh). Farms whose technical efficiency change was in the range of -0.05–0.05 points are in the group with neutral change in technical efficiency (NeutCh). If the farm's technical efficiency decreased by more than 0.05 points, the farm is in the group with a negative change in technical efficiency (NegCh).

Some significant differences emerged from group comparisons. Above all, the group of farmers with positive technical efficiency change had the lowest average technical efficiency score (0.699) in 2012, and the highest (0.882) in 2017. This group hence had the highest positive change and they are the new frontrunners in 2017.

Vasiliev et al. (2011) reached a similar result analysing Estonian dairy producers between 2000–2006. They found that innovators had the lowest initial technical efficiency score and the highest score in 2006.

Table 2. Changes in variables in three technical efficiency change groups between 2012 and 2017

Variables	Unit	Variables increase/decrease (%)		
		PosCh	NeutCh	NegCh
total sales revenue	thousand euros	+621 (+72.9)	+341 (+31.8)	+168 (+20.2)
dairy cows	number	+94 (+29.0)	+32 (+10.9)	+12 (+4.0)
agricultural area	ha	-61 (-5.9)	-27 (-2.8)	+80 (+10.9)
Labour	h	-78 (-0.2)	+3,931 (+11.4)	-4,541 (-10.7)
capital expenditure	thousand euros	+36 (+23.7)	+43 (+37.1)	+20 (+14.3)
intermediate consumption	thousand euros	+276 (+28.6)	+205 (+19.2)	+156 (+19.0)
milk yield per year	kg cow^{-1}	+1,110 (+14.8)	+1,159 (+15.4)	+679 (+8.3)
milk fat content	%	-0.017 (-0.41)	-0.072 (-1.75)	-0.045 (-1.09)
milk protein content	%	+0.018 (+0.53)	-0.024 (-0.71)	-0.009 (-0.26)
somatic cell count	10^3 ml^{-1}	-112.0 (-31.2)	-75.5 (-24.9)	-35.1 (-9.2)
age at first calving	days	-54.7 (-6.3)	-33.3 (-3.8)	-29.3 (-3.4)
productive period	days	-181.3 (-12.5)	-79.0 (-6.2)	-48.4 (-4.0)
age at culling	days	-211.1 (-9.0)	-60.4 (-2.8)	-86.6 (-4.1)
culling rate (udder)	%	+5.4 (+27.1)	-12.0 (-36.2)	-2.6 (-8.7)
share of EHF	%	+2.0 (+2.6)	-0.7 (-0.9)	+5.8 (+8.0)
share of own feed	%	-6.6 (-10.9)	-3.9 (-6.7)	+1.3 (+2.1)
feed costs per milk kg	euro kg^{-1}	-0.022 (-11.8)	+0.011 (+6.9)	+0.002 (+0.9)
number of farms	number	21	26	17

The group with positive technical efficiency change had the biggest growth in sales revenue (+72.9%) and in the number of dairy cows (+29%), whilst their agricultural area decreased, which refers to the intensification of production.

The growth of intermediate consumption and capital expenditure was the fastest in positive and neutral change technical efficiency groups. The changes in intermediate consumption and capital expenditures are associated with better feeding (e.g. total mixed feed ratios) and better housing conditions (new or renovated barns). As a result, milk yield has increased and SCC has decreased by +14.8% and -31.2% respectively, in the group with positive technical efficiency change.

In the farms where the technical efficiency change was negative, milk yield increased by 8.3% and SCC decreased by 9.2%. Additionally, they had smaller agricultural areas (814 ha) and fewer dairy cows (306 cows) compared to the farms with positive technical efficiency change (982 ha and 416 cows).

Farms with negative technical efficiency change had the highest labour use per cow – 144 hours in 2012 and 124 hours in 2017 – which could lead to the lower technical efficiency. At the same time, farmers in the group with positive technical efficiency change had the lowest labour use per cow – 124 hours in 2012 and 96 hours in 2017. Stokes et al. (2007) found similar results analysing labour use. They found that too much labour with too little milk and butterfat production characterize inefficient dairy producers.

The results indicate that high technical efficiency and intensive production led to some negative aspects: the productive period and longevity decreased the most in the group with positive technical efficiency change. Nevertheless, these farms had the highest productive period (1,272 days) and age at culling (2,128 days), as opposed to the farms with negative technical efficiency change where the productive period was 1,169 days and age at culling 2007 days in 2017.

The decrease in the share of home-grown feed was the most pronounced in the group with positive technical efficiency change, yet those farmers' feed costs per kg milk decreased the most (-11.8%). Additionally, in 2012 they had the highest feed cost per milk kg (0.186 eur kg^{-1}), but the lowest in 2017 (0.164 eur kg^{-1}), which can be linked to the scale effect.

Results of the fractional regression analysis

The SCC had a statistically significant negative impact on technical efficiency in 2012. The average partial effect shows that if the SCC increased by 100 x 10^3 mL^{-1}, the technical efficiency would decrease by 0.02 points (Table 3). SCC is an indicator of potential mastitis, and is associated with reduced animal health (Telldahl et al., 2019). Mastitis is one of the most frequent diseases and causes of loss of income and milk, and increased costs (Horvath et al., 2017; Hogeveen et al., 2019). Cinar et al. (2015) found that high SCC has a negative effect not only on milk yield but also on milk composition and quality. Halasa et al. (2009) found that fat and protein production were also affected negatively by a new case of subclinical mastitis. Technical efficiency studies have found that higher SCC predicts inefficiency or has a negative impact on technical efficiency (Allendorf & Wettemann, 2015; Luik-Lindsaar et al., 2018; Luik-Lindsaar et al., 2019). Thus, all kind of preventions of mastitis (Barkema et al., 2015; Gargiulo et al., 2017) together with better housing conditions (Ruud et al., 2010; Villettaz Robichaud et al., 2019) are important factors to increase income and reduce costs, which in turn leads to increased technical efficiency. The SCC had no significant impact on technical efficiency in 2017.

Feed costs per kg milk (FeedEuroKg) had a significant negative impact on technical efficiency in 2012. According to the average partial effect, if the feed costs increased by 0.01 euro per kg milk, the technical efficiency would decrease by 0.0123 points (Table 3). The value of feed costs per kg milk contains information on both the cost and milk production: the higher the milk production, the lower the cost per unit of milk. A healthier herd has a better dry matter and nutrient intake therefore every euro spent on feed produces more milk and revenue in healthier herds. Feed cost per kg milk decreased slightly (-1.72%), but average milk yield increased markedly (+13.2%) in 2017 compared to the year 2012. Considering the increase in milk yield, it would have been reasonable to expect a greater decline in feed costs per kg milk. One of the reasons why the latter was not the case was the increased share of purchased feed, which is mainly concentrated feed at a higher price. The share of home-grown feed (ShoOFeed) had a significant negative impact on technical efficiency in 2012 and 2017, which means that a higher share of purchased feed (concentrated feed) helps to achieve higher technical efficiency through higher milk yield. Therefore, it is important to achieve lower production costs through focusing more on having a healthier herd with better food intake and higher milk yield.

Table 3. Factors affecting technical efficiency of dairy farms according to the results of the one-part cauchit model in 2012 and 2017

Variables	2012					2017				
	estimate	Std. Err	*t*-value	Pr(>|*t*|)	Average partial effect	estimate	Std. Err	*t*-value	Pr(>|*t*|)	Average partial effect
Intercept	-6.451	6.827	-0.945	0.345		7.947	6.007	1.323	0.186	
Milk	0.000	0.000	0.955	0.340	0.0000	0.000	0.000	0.179	0.858	0.0000
Fat	1.313	0.964	1.362	0.173	0.1412	0.981	0.745	1.317	0.188	0.0820
Protein	2.128	1.258	1.692	0.091 *	0.2288	-1.255	1.894	-0.662	0.508	-0.1049
SCC	-0.002	0.001	-1.830	0.067 *	-0.0002	0.000	0.001	-0.038	0.969	0.0000
AgeFirstCalv	-0.001	0.001	-1.199	0.231	-0.0001	-0.005	0.002	-2.298	0.022 **	-0.0004
ProdPer	0.000	0.002	-0.119	0.906	0.0000	0.001	0.002	0.573	0.567	0.0001
AgeCull	-0.001	0.002	-0.402	0.688	-0.0001	-0.001	0.002	-0.383	0.702	-0.0001
ShoCullUdd	0.005	0.008	0.674	0.500	0.0006	-0.030	0.019	-1.612	0.107	-0.0025
EHF	0.008	0.003	2.323	0.020 **	0.0008	0.010	0.005	2.221	0.026 **	0.0008
ShoOFeed	-0.017	0.007	-2.370	0.018 **	-0.0018	-0.028	0.011	-2.629	0.009 ***	-0.0023
FeedEuroKg	-11.479	2.707	-4.240	0.000 ***	-1.2342	-3.004	2.800	-1.073	0.283	-0.2510
Number of observations				64					64	
R^2				0.534					0.476	

***; ** and * denote coefficients which are significant at 1, 5 or 10%, respectively-

The share of EHF had a significant positive effect on technical efficiency in 2012 and 2017. The average partial effect showed that if the share of EHF increased by 1%, the technical efficiency would increase by 0.0008 points.

One of the factors that determines a dairy herd profitability is the productive period, which depends on the age at first calving. The age at first calving (AgeFirstCalv) had a significant negative impact on technical efficiency in 2017. The age at first calving decreased by 40 days from 2012 to 2017. The average age at first calving was 27.4 months in 2017 in our sample, which is slightly higher than the Estonian average (25.8 months) (Results…, 2018). According to Froidmont et al. (2013), the optimal age at first calving is in the range of 22–26 months. Reducing the age at first calving can lead to an increase in technical efficiency. The decrease in age at first calving by 1 month increases technical efficiency by 0.0122 points.

The decreased SCC and age at first calving are positive changes in Estonian dairy herds according to our sample farms, whereas the productive period (ProdPer) and longevity (AgeCull) are factors that need to improve. The share of culling caused by udder problems (ShoCullUdd) has no significant impact on technical efficiency.

The dairy cattle information system Vissuke is a good tool for recording and analysing herd health at farm level for Estonian dairy farmers (Lillik, 2015). The NGO Piimaklaster, in cooperation with the Estonian University of Life Sciences, has carried out an HHMP project (2017–2019) whose results show that systematic work on livestock health improves animal health and productivity, as well as economic profitability of production through this (Mõtus et al., 2019). Dairy farmers have to pay attention to cow health in order to remain competitive and ensure profitability.

CONCLUSIONS

Herd health is an important issue for any farmer as it influences the farm's revenue, costs and technical efficiency. Increased consumer awareness of healthy food and animal welfare requires farmers to produce high quality raw milk. Therefore, today it is not only crucial to focus on quantities but to also have an increased focus on the high quality of raw milk.

One indicator of udder health is the number of somatic cells in raw milk. High level of SCC characterizes herd health and is associated with losses in both the quantity and quality of milk. The present study showed that a high SCC has a negative impact on farm technical efficiency. High SCC increases costs and decreases revenue, therefore it directly influences farms' economic performance. It emerged that decreasing the age at first calving increases technical efficiency. Therefore, reducing the SCC and age at first calving are the key factors to increasing technical efficiency.

To ensure healthier herds, farms' technical efficiency, sustainability of production and catering to consumers' expectations, it is essential to manage farms consciously and include herd health programmes into the farm management process.

REFERENCES

Allendorf, J.J. & Wettemann, P.J.C. 2015. Does animal welfare influence dairy farm efficiency? A two-stage approach. *Journal of Dairy Science* **98**, 7730–7740.

Annual Report. 2002. https://www.epj.ee/jkk/piimaveised/statistika/aastaaruanded (in Estonian).

Annual Report. 2013. https://www.epj.ee/jkk/piimaveised/statistika/aastaaruanded (in Estonian).
Annual Report. 2017. https://www.epj.ee/jkk/piimaveised/statistika/aastaaruanded (in Estonian).
Archer, S.C., Mc Coy, F., Wapenaar, W. & Green, M.J. 2013. Association between somatic cell count early in the first lactation and the lifetime milk yield of cows in Irish dairy herds. *Journal of Dairy Science* **96**, 2951–2959.
Barkema, H.W., von Keyserlingk, M.A.G., Kastelic, J.P., Lam, T.J.G.M., Luby, C., Roy, J.-P., LeBlanc, S.J., Keefe, G.P. & Kelton, D.F. 2015. Invited review: Changes in the dairy industry affecting dairy cattle health and welfare. *Journal of Dairy Science* **98**, 7426–7445.
Cielava, L., Jonkus, D. & Paura, L. 2017. Lifetime milk productivity and quality in farms with different housing and feeding systems. *Agronomy Research* **15**(2), 369–375,
Cinar, M., Serbester, U., Ceyhan, A. & Gorgulu, M. 2015. Effect of Somatic Cell Count on Milk Yield and Composition of First and Second Lactation Dairy Cows. *Italian Journal of Animal Science* **14**, 105–108.
Cooper, W.W., Seiford, L.M. & Zhu, J. 2007. *Handbook on Data Envelopment Analysis*. Springer. 608 pp.
Duval, J.E., Bareille, N., Madouasse, A., de Joybert, M., Sjöström, K., Emanuelson, U., Bonnet-Beaugrand, F. & Fourichon, C. 2018. Evaluation of the impact of a Herd Health and Production Management programme in organic dairy cattle farms: a process evaluation approach. *Animal* **12**(7), 1475–1483.
Eastham, N.T., Coates, A., Cripps, P., Richardson, H., Smith, R. & Oikonomou, G. 2018. Associations between age at first calving and subsequent lactation performance in UK Holstein and Holstein-Friesian dairy cows. *Plos ONE* **13**(6), e0197764.
Froidmont, E., Mayeres, P., Picron, P., Turlot, A., Planchon, V. & Stilmant, D. 2013. Association between age at first calving, year and season of first calving and milk production in Holstein cows. *Animal* **7**, 665–672.
Gargiulo, J.I., Eastwood, C.R., Garcia, S.C. & Lyon, N.A. 2017. Dairy farmers with larger herd sizes adopt more precision dairy technologies. *Journal of Dairy Science* **101**, 5466–5473.
Gaworski, M., Leola, A., Kiiman, H., Sada, O., Kic, P. & Priekulis, J. 2018. Assessment of dairy cow herd indices associated with different milking systems. *Agronomy Research* **16**, 83–93. https://doi.org/10.15159/AR.17.075
Geary, U., Lopez-Villalobos, N., O'Brien, B., Garrick, D.J. & Shalloo, L. 2013. Examining the impact of mastitis on the profitability of the Irish dairy industry. *Irish Journal of Agricultural and Food Research* **52**, 135–149.
Gussmann, M., Denwood, M., Kirkeby, C., Farre, M. & Halasa, T. 2019. Associations between udder health and culling in dairy cows. *Preventive Veterinary Medicine* **171**, 104751.
Hadrich, J.C., Wolf, C.A., Lombard, J. & Dolak, T.M. 2018. Estimating milk yield and value losses from increased somatic cell count on US dairy farms. *Journal of Dairy Science* **4**, 3588–3596.
Halasa, T., Nielen, M., De Roos, A.P.W., Van Hoorne, R., de Jong, G., Lam, T.J.G.M. van Werven, T. & Hogeveen, H. 2009. Production loss due to new subclinical mastitis in Dutch dairy cows estimated with a test-day model. *Journal of Dairy Science* **92**, 599–606.
Hogeveen, H., Steeneveld, W. & Wolf, C.A. 2019. Production Diseases Reduce the Efficiency of Dairy Production: A Review of the Results, Methods, and Approaches Regarding the Economics of Mastitis. *Annual Review of Resource Economics* **11**, 289–312.
Horvath, J., Toth, Z. & Miko, E. 2017. The analysis of production and culling rate with regard to the profitability in a dairy herd. *Advanced Research in Life Sciences* **1**, 48–52.
Kiiman, H., Tänavots, A. & Kaart, T. 2013. The yield and quality of milk on the farms using twice a day conventional milking in comparison with the farms using three times a day conventional and automatic milking systems (Lehmade piimatoodang ja kvaliteet kahekordsel platsilüpsil võrreldes kolmekordse platsilüpsi ning automaatlüpsiga). *Agraarteadus: Journal of Agricultural Science* **XXIV**(2), 55–64 (in Estonian).

Kimura, S. & Sauer, J. 2015. Dynamics of dairy farm productivity growth: Cross-country comparison. *OECD Food, Agriculture and Fisheries Papers*, No. **87**, OECD Publishing, Paris. http://dx.doi.org/10.1787/5jrw8ffbzf7l-en.
Koeck, A., Loker, S., Miglior, F., Kelton, D.F., Jamrozik, J. & Schenkel, F.S. 2014. Genetic relationships of clinical mastitis, cystic ovaries, and lameness with milk yield and somatic cell score in first-lactation Canadian Holsteins. 2014. *Journal of Dairy Science* **97**, 5806–5813.
Krpálková, L., Cabrera, V.E., Zavadilová, L. & Štípková, M. 2019. The importance of hoof health in dairy production. *Czech Journal of Animal Science* **64**, 107–117.
Latruffe, L., Fogarasi, J. & Desjeux, Y. 2012. Efficiency, productivity and technology comparison for farms in Central and Western Europe: The case of field crop and dairy farming in Hungary and France. *Economic Systems* **36**, 264–278.
Leso, L., Pellegrini, P. & Barbari, M. 2019. Effect of housing systems on performance and longevity of dairy cows in Northen Italy. *Agronomy Research* **17**, 574–581. https://doi.org/10.15159/AR.19.107
Lillik, M. 2015. Registered health records - a key factor in decision making and planning (Registreeritud terviseandmed – võtmetegur otsuste ja plaanide tegemisel). Estonian Livestock Performance Recording Ltd. *www.epj.ee* (in Estonian).
Luik, H. & Viira, A.-H. 2016. Feeding, milking and manure systems in Estonian dairy barns. *Agraarteadus: Journal of Agricultural Science* **XXVII**(2), 92–107 (in Estonian).
Luik-Lindsaar, H., Põldaru, R. & Roots, J. 2019. Estonian dairy farms' technical efficiency and factors predicting it. *Agronomy Research* **16**, 806–820. https://doi.org/10.15159/AR.18.125
Luik-Lindsaar, H., Viira, A.-H., Viinalass, H., Kaart, T. & Värnik, R. 2018. How do herd's genetic level and milk quality affect performance of dairy farms? *Czech Journal of Animal Science* **63**, 379–388.
Mõtus, K., Viira, A.-H., Kalmus, P., Kalmus, K., Kavak, A. & Luik-Lindsaar, H. 2019. Herd health management program in Estonian dairy farms - effects on herd health, production and farm economic performance (Karjatervise programmi rakendamine Eesti piimaveisekarjades – mõju karja tervisele ning ettevõtte majandusnäitajatele). *Terve loom ja tervislik toit*, pp. 131–141 (in Estonian).
Noordhuizen, J.P.T.M. & Wentink, G.H. 2001. Developments in veterinary herd health programmes on dairy farms: A review. *Veterinary Quarterly* **23**, 162–169.
Noordhuizen, J.P. & Cannas da Silva, J. 2009. Animal Hygiene and Animal Health in Dairy Cattle Operations. *The Open Veterinary Science Journal* **3**, 17–21.
Nor, N.M., Steeneveld, W. & Hogeveen, H. 2014. The average culling rate of Dutch dairy herds over the years 2007 to 2010 and its association with herd reproduction, performance and health. *Journal of Dairy Research* **81**, 1–8.
Østerås, O. & Sánchez Mainar, M. 2019. Mastitis Prevention and Therapy for Sustainable Dairy Production. In: *IDF Mastitis Conference*, May 14–16, 2019, Copenhagen, Denmark.
Potter, T.L., Arndt, C. & Hristov, A.N. 2018. Short communication: Increased somatic cell count is associated with milk loss and reduced feed efficiency in lactating dairy cows. *Journal of Dairy Science* **101**, 9510–9515.
Ramalho, E.A., Ramalho, J.J.S. & Henriques, P.D. 2010. Fractional regression models for second stage DEA efficiency analyses. *Journal of Productivity Analysis* **34**, 239–255.
Ramalho, E.A. & Ramalho, J.J.S. 2011. Alternative estimating and testing empirical strategies for fractional regression models. *Journal of Economic Surveys* **25**, 19–68.
Results of Animal Recording in Estonia 2012. 2013. Estonian Animal Recording Centre Tartu, Estonia, 52 pp.
Results of Animal Recording in Estonia 2017. 2018. Estonian Livestock Performance Recording Ltd, Tartu, Estonia, 52 pp.

Results of Animal Recording in Estonia 2018. 2019. Estonian Livestock Performance Recording Ltd, Tartu, Estonia, 52 pp.

Ruud, L.E., Bøe, K.E. & Østerås, O. 2010. Associations of soft flooring materials in free stalls with milk yield, clinical mastitis, teat lesions, and removal of dairy cows. *Journal of Dairy Science* **93**, 1578–1586.

Sant'Anna, A.C. & Paranhos da Costa, M.J.R. 2011. The relationship between dairy cow hygiene and somatic cell count in milk. *Journal of Dairy Science* **94**, 3835–3844.

Sipilainen, T., Kortelainen, M., Ovaska, S. & Ryhanen, M. 2009. Performance of Finnish dairy farms and its determinants: A comparison of parametric, semiparametric, and nonparametric methods. *Acta Agriculturae Scandinavica, Section C – Food Economics* **6**, 173–184.

Statistics Estonia. 2020. *www.stat.ee*. Accessed 20.01.2020.

Stokes, J.R., Tozer, P.R. & Hyde, J. 2007. Identifying Efficient Dairy Producers Using Data Envelopment Analysis. *Journal of Dairy Science* **90**, 2555–2562.

Svensson, C., A,lvasen, K., Eldh, A.C., Frossling, J. & Lomander, H. 2018. Veterinary herd health management **-** Experience among farmers and farm managers in Swedish dairy production. *Preventive Veterinary Medicine* **155**, 45–52.

Telldahl, C., Hansson, H. & Emanuelson, U. 2019. Modelling animal health as a production factor in dairy production **-** a case of low somatic cell counts in Swedish dairy agriculture. *Livestock Science* **230**. Publishing.

Vasiliev, N., Suuster, E., Luik, H., Varnik, R., Matveev, E. & Astover, A. 2011. Productivity of Estonian dairy farms decline after accession to the European Union. *Agricultural Economics – Czech* **57**, 457–463.

Villettaz Robichaud, M., Rushen, J., de Passillé, AM., Vasseur, E., Orsel, K. & Pellerin, D. 2019. Associations between on-farm animal welfare indicators and productivity and profitability on Canadian dairies: I. On freestall farms. *Journal of Dairy Science* **102**, 4341–4351.

Özkan Gülzari, S., Vosough Ahmadi, B. & Stott, A.W. 2018. Impact of subclinical mastitis on greenhouse gas emissions intensity and profitability of dairy cows in Norway. *Preventive Veterinary Medicine* **150**, 19–29.

Zhu, J. 2009. *Quantitative Model for Performance Evaluating and Benchmarking*. Springer, 327 pp.

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Rational selection and usage of rotary type milking equipment

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Abstract. The advisable time of operation of the rotary type milking equipment per day depending on the specific exploitation costs has been investigated. It has been stated that these costs can be reduced, if the rotary type milking equipment load is increased. Therefore, it is advisable to organise the work of milkers in two shifts, so that the milking equipment is operated even up to 16 h per day. For this reason the herd of cows should be accordingly large. For instance, milking twice with the equipment with 50 milking places the cow herd can reach having even 2,500 cows, but at milking three times – up to 1,400 cows.

Key words: milking, rotary type equipment, milking time, exploitation costs.

INTRODUCTION

Rotary type milking equipment is widely spread in Latvia. The theoretical productivity of the equipment can reach 434 cows per hour (Mangalis & Priekulis, 2018), and therefore this equipment is suitable for large cow herds. Nevertheless, the productivity of the equipment depends on the number of milking places that are installed on the rotary platform (Kirsanov et al., 2016). The more the milking places, correspondingly increase the theoretical labour productivity of the rotary equipment. Therefore, in the largest farms at present rotary platforms with 50 and 80 milking places are used. In this case the total milking time does not exceed 6–8 hours per day. However, the productivity of the rotary platform depends also on the speed at which the animals enter and leave the rotary platform. If the number of milking places is increased, it has to be done in shorter time as the time of platform rotation is a set value (Edwards et al., 2012; Kic, 2015). To make the cow movement faster now rotary equipment with radial location of animals on the rotary platform and orientation to the central part of the rotary equipment are used. Though, our previous research shows (Mangalis & Priekulis, 2018) that such approach cannot completely solve the problem. Besides, the increased number of milking places also considerably increases the size and production costs of the milking equipment (Wagner et al., 2001).

There is another solution of this problem that is used in the large farms of the USA and Canada (Edwards et al., 2012; Edwards et al., 2013). For milking cows less productive rotary type equipment is used, considering that the total milking time should reach 18–20 h per day. This approach essentially increases the load of the milking equipment and reduces the payback time. Besides, such rotary type milking equipment is cheaper and the problem that cows are late for milking when entering and leaving the rotary platform is also reduced. However, the milking time increases accordingly and also fatigue of people participating in milking should be taken into account.

Therefore, the aim of the present research is to state the economic efficiency of milking cows using rotary type milking equipment with reduced labour productivity in order to reach the total milking time 15–20 hours per day.

MATERIALS AND METHODS

For the research three farms in Latvia were selected, where rotary type milking equipment with 20, 50 and 80 milking places, produced by the GEA company, is used. At all of these farms the milk yield was approximately the same, i.e. ranged from 9,000 to 9,930 kg cow^{-1} year^{-1} (mean 9,477 kg cow^{-1} year^{-1}), and during milking cow mechanical movers were used, but the number of people participating in milking and the main exploitation indicators were different (Table 1).

Table 1. Description of the dairy farms includet in the research

Indicators	Milk farms		
	A	B	C
Number of milk cows	300	600	2,000
Average milk yield, kg cow^{-1} year^{-1}	9,930	9,000	9,500
Number of milking places	20	50	80
Time of one platform revolution, min revolution^{-1}	7	8	10
Preparation and finishing time before and after milking, h day^{-1}	1.0	1.5	1.67
Number of milkers in a shift	2	3	4
Number of movers in a shift	1	1	1
Wages for milkers, € month^{-1}	700	700	700
Wages for movers, € month^{-1}	500	500	500
Rotary equipment cost, €	220,000	400,000	700,000
Rotary equipment repair costs, € month^{-1}	1,500	2,000	2,500
Electric drive power, kW	8	13.5	25
Consumption of teat disinfectants, l day^{-1} cow^{-1}	0.02	0.02	0.02
Consumption of milking equipment washing detergents, l day^{-1}	3	6	10

Consumption of cold water for cow teat washing was assumed according to the recommendations (Priekulis, 2012) 0.8 L per one cow, but for milking machines – 12 L for one milking of the herd. Besides, approximately 50% of this amount is hot water with temperature 50–60 °C. In turn, for washing the floors of the milking parlour in all versions of the research it was assumed 3.5 L calculating per one cow for one milking of the herd.

Also the prices of all exploitation means (cold water, teat disinfection and milking equipment washing costs) in all cases were assumed to be equal considering the current situation on 01.11.2019.

The total milking time per day was calculated according to formula

$$t_{sl} = \frac{z_g \cdot t_c \cdot n_{sl}}{60 \cdot z_v} + n_{sl} \cdot t_{sag} \quad (1)$$

where t_{sl} – milking time, h day^{-1}; t_c – time of one rotary platform revolution, min; t_{sag} – time for preparation before milking and finishing time, h.; n_{sl} – number of milking the herd per day; z_g – total number of milk cows; z_v – number of milking places on the rotary platform.

All data necessary for the calculation were obtained in the dairy farms mentioned in Table 1, based on the results of inspecting the milking equipment and our timekeeping.

The economic efficiency of milking cows was determined according to the calculated exploitation costs (Gaworski & Leola, 2014; Gaworski et al., 2017). For this reason it was assumed that milking of cows should be done two as well as three times per day, but the size of the herd can be 250, 500, 750, 1,000, 1,250, 1,500, 1,750 and 2,000 cow correspondingly.

The calculations of the economic efficiency of milking cows included the wages for people participating in milking, milking equipment machine costs and the costs of exploitation means (consumed water, chemicals). For calculation two computer programs were prepared: one – for stating the time of milking, the other – for calculation of the exploitation costs.

RESULTS AND DISCUSSION

The milking time using the milking equipment given in Table 1 and milking two as well as three times per day is summarised in the Fig. 1.

The data presented in the Fig. 1, shows that the milking time is influenced not only by the number of cows in the herd, but also by the number of milking places on the rotary platform and the number of milkings per day. If, for instance, there are 1,000 cows in the herd and they are milked twice, then operating the rotary type equipment with 80 milking places the milking time is 7.5 h, with 50 milking places – 8.3 h, but with 20 milking places – 13.7 h per day. According to the research performed in Estonia (Reppo et al., 2007; Sada et al., 2016) the work of milkers is not to be considered in the category of physically hard work. Therefore, the shift of milkers can last

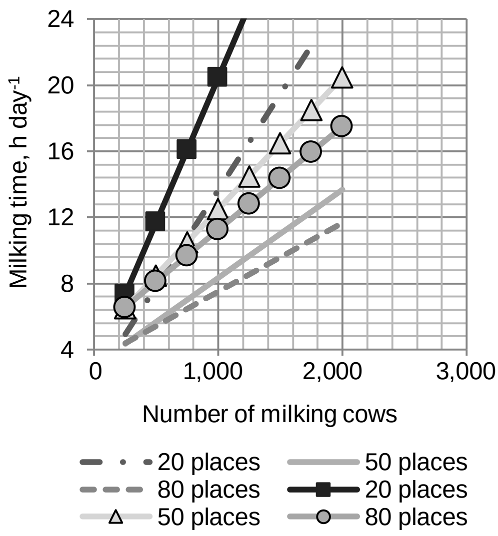

Figure 1. Milking time, h day^{-1}, depending on the number of cows, number of milking places on the rotary platform and number of milking per day (unmarked lines show milking two times per day, marked lines - milking three times per day).

for 8 h per day. It means that introducing the work in two shifts the total milking time can last for 16 h per day. Therefore, at milking twice with the equipment with 20 milking places a herd with up to 1,200 cows can be milked, but using the equipment with 50 places – approximately a herd of 2,500 cows.

If, in turn, milking is performed three times and milkers are working in two shifts, then with the rotary equipment with 20 places approximately a herd of 700 cows can be milked, with 50 places – 1,400 cows, but with 80 places – 1,750 cows correspondingly. It means that introducing the work of milkers in two shifts rotary type milking equipment with considerably reduced number of milking places can be used.

The exploitation costs of different types of rotary milking equipment depending on the number of milking times per day and the size of the herd are shown in Figs 2, 3.

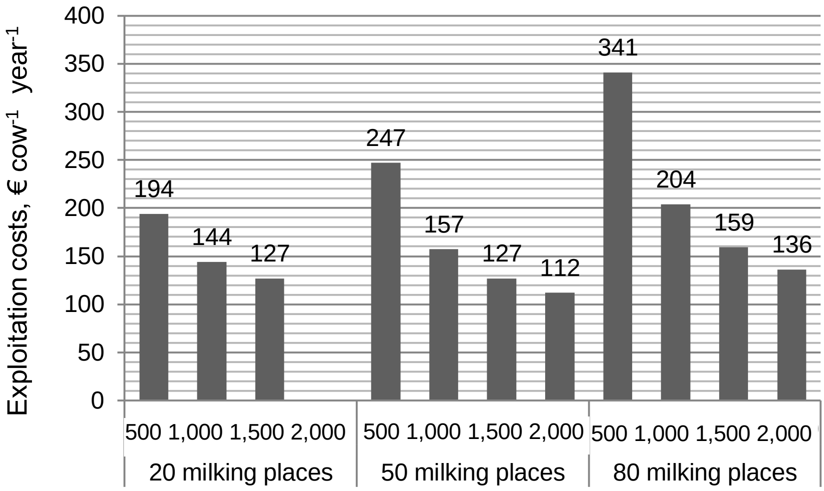

Figure 2. Exploitation costs milking twice, in € cow^{-1} year^{-1}, depending on the size of the herd (500, 1,000, 1,500 or 2,000 cows) and the number of milking places.

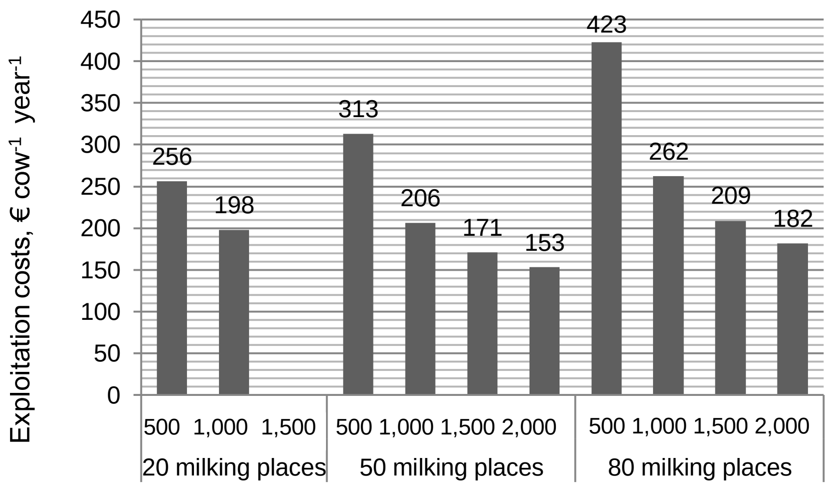

Figure 3. Exploitation costs milking three times, in € cow^{-1} year^{-1}, depending on the size of the herd (500, 1,000, 1,500 or 2,000 cows) and the number of milking places.

The data in Fig. 2 show that the exploitation costs are influenced by the size of the rotary equipment (number of milking places) as well as by the size of the herd. If, for instance, there are 1,000 cows in the herd, then milking twice and operating the rotary type equipment with 20 milking places the exploitation costs will sum up to 144 € cow^{-1} year^{-1}, operating the equipment with 50 milking places – 157 € cow^{-1} year^{-1}, but with 80 milking places already 204 € cow^{-1} year^{-1}. Besides, it is essential to use the milking equipment for milking a possibly larger herd. The research results shown in the Fig. 2 demonstrate that increasing the herd by a step corresponding to 500 animals the exploitation costs reduce by 40–12%. Therefore, it is advisable to choose the milking equipment that can ensure operation of a possibly large herd without exceeding the limited working time of the milkers (8 h).

A similar situation can be seen also in Fig. 3. Though, in this case the milking time of the herds has increased (by 1.5 times per day), and therefore the corresponding exploitation costs become higher. Besides, the equipment with 20 milking places cannot be used for herds with 1,500 cows as in this case the working time for milkers exceeds 24 h per day.

The relationship between the specific costs of milking and the total milking time is shown in Fig. 4. According to the formula (1) it is possible to calculate that in the farm B, where there are 600 cows and they are milked twice, the total milking time is 6.2 h per day. In turn, (Fig. 4) shows that in this case the specific exploitation costs are about 200 € cow^{-1} year^{-1}. If the size of the herd is increased up to 2,250 cows, the total milking time increases up to 15 h per day, but the specific costs reduce up to 107 € cow^{-1} year^{-1}. It is almost two times difference.

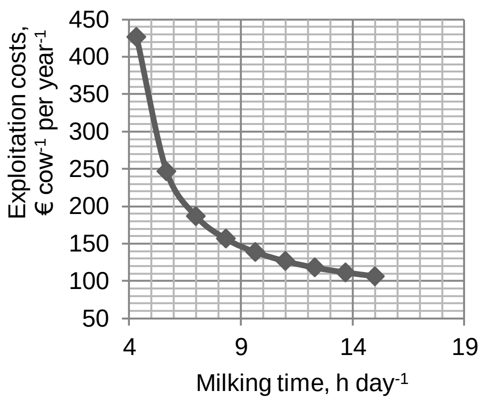

Figure 4. Variations of milking specific exploitation costs depending on the total milking time, if rotary equipment with 50 milking places is operated and cows are milked twice a day.

CONCLUSIONS

It is rational to introduce two working shifts for milkers and to ensure that the rotary type equipment is operated even up to 16 h per day. If the cows are milked twice per day, then at the above mentioned preconditions the rotary equipment with 20 milking places can serve a herd up to 1,200 cows, but with 50 milking places – a herd with approximately 2,150 cows. If, in turn, the cows are milked three times, then the rotary type milking equipment with 20 milking places is suitable for milking up to 700 cows, with 50 milking places – for a herd with up to 1,400 cows, but with 80 milking places – for a herd with up to 1,750 cows.

The specific costs of rotary type milking equipment in € per cow per year, are essentially dependent on the size of the cow herd. When bigger number of the cows to be milked, it is possible to expect the smaller costs correspondingly. If, for instance, in the result of increasing the number of cows the total milking time changes from 6 to 16 hours per day, the specific exploitation costs for milking reduce in double amount.

REFERENCES

Edwards, J.P., Lopez-Villalobos, N. & Jago, J.G. 2012. Increasing platform speed and the percentage of cows completing a second rotation improves throughput in rotary dairies. *Animal Production Science* **52**, 969–973.

Edwards, J.P., Jago, J.G. & Lopez-Villalobos, N. 2013. Large rotary dairies achieve high cow throughput but are not more labour efficient than medium-sized rotaries. *Animal Production Science* **53**(6) 573–579.

Gaworski, M. & Leola, A. 2014. Effect of technical and biological potential on dairy production development. *Agronomy Research* **12**(1), 215–222.

Gaworski, P., Kaminska, N. & Kic, P. 2017. Evaluation and optimization of milking in some Polish dairy farms differed in milking parlours. *Agronomy Research* **15**(1), 112–122.

Kirsanov, V.V., Tareeva, O.A., Andreev, V.I. & Vasilieva, I.A. 2016. The Influence of Factors on the Rhythm of Conveyor Milking Installation operation. *Journal of NSEEU* **6**(61), pp. 30–37 (in Russian).

Kic, P. 2015. Criteria for optimization of milking parlor on dairy farm. /*14th International Scientific Conference "Engineering for rural development"*. Proceedings, Volume 14, Jelgava, May 20–22, pp. 40–43.

Mangalis, M. & Priekulis, J. 2018. Productivity of rotary parlours. /*17th International Scientific Conference "Engineering for rural development"*. Proceedings, Volume **17**, Jelgava, May 23–25, pp. 72–76.

Priekulis, J. 2012. *Modern Milk Production Farm: Technology, Machinery, Operation*./Ed.J. Priekulis Jelgava: LLU, 240 pp. (In Latvian).

Reppo, B., Mikson, E., Toropov, S. & Nurm, N. 2007. Working environment of rotary milking parlour.35. Symposium "Actual Tasks on Agricultural Engineering", Opatija, Croatia, pp. 310–319.

Sada, O., Leola, A. & Kic, P. 2016.Choosing and evaluation of milking parlours for dairy Farms in Estonia. *Agronomy Research* **14**(5), 1694–1701.

Wagner, A., Palmer, R.W., Bewley, J. &. Jackson-Smith, D.B. 2001. Producer satisfaction, efficiency, and investment cost factors of different milking systems. *J. Dairy Sci*. **84**, 1890–1898.

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The use of DNA markers for the evaluation of maize lines and hybrids based on cytoplasmic male sterility

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Abstract. The use of cytoplasmic male sterility (CMS) is very important for the production of maize hybrids. The new inbred lines and hybrids of maize of Ukrainian breeding were studied. In the field, four pairs of sterile analogue of line RL106S were obtained during four backcrossing (17M, 19M, 23M, 27M and 29M) and maintainer lines of RL106fS (18M, 20M, 24M, 28M and 30M) for S type cytoplasm and RL108C (195C, 201C, 205C, 207C, 209C) i RL108fC (196C, 202C, 206C, 208C, 210C) for C type respectively. For S type, the following combinations were obtained: RL23S×RL106fS, RL107S×RL106fS, RL98S×RL106fS, RL105S×RL106fS, RL113S×RL106fS and for C type: RL109C×RL108fC, RL110C×RL108fC, RL112C×RL108fC, RL114C×RL108fC, RL115C×RL108fC. The obtained hybrid combinations were planted the following year in a control nursery for field trials. According to the results of the field assessment, all the hybrids were sterile. The types of sterility of the studied lines and hybrids were determined using polymerase chain reaction (PCR) with specific primers for C and S types of cytoplasms. The presence of specific amplicons 398 and 799 bp was determined in sterile lines with C and S types of cytoplasm, respectively. Amplicons 398 and 799 bp were identified in simple-cross and simple reconstituted hybrids on a sterile basis, and can be used to determine the type of hybrid and its maternal component at the stages of selection and examination of new hybrids.

Key words: *Zea mays* L., DNA markers, cytoplasmic sterility.

INTRODUCTION

The traditional crop for biogas production and alternative energy source in the world is maize on silage (Križan et al., 2017; Križan et al., 2018). In comparison to sunflower and straw, maize has better fusibility, lower content of sulfur and chlorine (Lisowski et al., 2019). Many factors and specific features are taken into account during the growing of maize on silage: selection of hybrids of different maturity group, time and methods of sowing, density of planting, harvesting time. For each soil and climatic zone, hybrids of different biotypes are selected, which are capable of forming not only a high green herbage yield, but also a considerable part of the grain of middle dough stage. Parent components are important in the production of hybrids (Gayosso-Barragán et al., 2020). The use of cytoplasmic male sterility (CMS) enables to reduce the cost for cutting of tassels of maternity plant significantly (Allen et al., 2007; Chekanova, 2013). Three types of maize CMS are most widely: T type (Texas), S type (USDA) and C type (Charrua). The use of T type has been greatly reduced because it confers susceptibility to *Helminthosporium maydis*, thus types S and C are prevalent in breeding and in the maize seed industry. CMS is expressed when a sterility factor is present in the cytoplasm and recessive alleles of the restoration of fertility (Rf) genes are located in the nucleus (Laughnan & Gabay-Laughnan, 1983; Konovalov et al., 1990; Krivosheev & Ignatev, 2019). Tester lines containing nuclear Rf genes are traditionally used to determine and classify the CMS types (Liu et al., 2002; Anisimova & Gavrilova, 2012). However, the test-crossing procedure is a laborious process which requires a lot of time and material resources.

The determination of the sterility of maize lines is a part of the qualification examination of plant varieties for distinctness, uniformity and stability (DUS). The guide of DUS includes the determination time of anthesis and anthocyanin colouration at base of glume of tassel, which can be used to find distinctness between lines and their sterile analogues. However, it is known that the ability to form anthers of maize may depend on environmental factors, as well as the time of observation. These reasons are connected with additional difficulties for an objective assessment of lines during qualification examination. Thus, it is relevant to use fast and reliable methods for identifying the main types of sterility in seeds and in vegetative plants. The knowledge of molecular structures and mechanisms that underlie of CMS has increased significantly with the development of molecular genetic analysis methods. It is known that mutations responsible for CMS reside in mitochondrial DNA (mtDNA) in many plant species (Liu et al., 2002; Palilova et al., 2005). The results of a study of the mitochondrial genome structure and gene expression have enabled to use them for determination the major types of maize cytoplasm.

The polymorphism of mtDNA can be assessed by molecular genetic markers (Manson et al., 1986; Stevanovic et al., 2016). The application of RFLP (Restriction Fragment Length Polymorphism) analysis and methods based on polymerase chain reaction (PCR) are described. Specific primers were developed by Liu et al. (2002) to identify the three major types of CMS based on the mtDNA sequence. The studies were conducted to evaluate the molecular genetic polymorphism of mtDNA in maize and to determine new subtypes of S sterility in maize using DNA markers (Slischuk et al, 2011; Vancetovic et al., 2013). Thus, the aim of this research is to identify the C and S types of sterility in maize by DNA markers for using in DUS examination of varieties.

MATERIALS AND METHODS

Plant material

The lines and hybrids of maize were provided by Research Institute of Agrarian Business (Dnipro, Ukraine). Plant material of maize associated with S and C sterility types included: 5 pairs of sterile analogues of lines and their maintainers, 5 sterile analogues of lines, 5 sterility based single-cross hybrids and 2 restored hybrids.

The sterile analogues of lines were obtained by the backcrossing method (Mustyatsa & Mistrets, 2007) for 5–7 cycles. The main requirements to sterile analogues were the complete sterility of plants and selection within the line for the ability to maintain sterility. As it is known, almost all lines of heterotic plasma Iodent have ability to restore C type of CMS that is why they are used as maternal forms of hybrids which is possible based only on S type of CMS. To study the C type of CMS, the Lancaster heterotic group lines were used (Table 1).

Table 1. Characteristics of S and C type of maize CMS lines

Line	S type	Heterotic group	Line	C type	Heterotic group
RL106S	sterile	Iodent	RL108C	sterile	Lancaster
RL106fS	fertile	Iodent	RL108fC	fertile	Lancaster
RL23S	sterile	Iodent	RL109C	sterile	Lancaster
RL107S	sterile	Iodent	RL110C	sterile	Lancaster
RL98S	sterile	Iodent	RL112C	sterile	Lancaster
RL105S	sterile	Iodent	RL114C	sterile	Lancaster
RL113S	sterile	Iodent	RL115C	sterile	Lancaster

The level of tassels sterility during flowering was determined by examining them in the breeding nursery. According to the results of the evaluation, the studied samples were grouped by sterility classes (Gontarovskiy, 1971): class 0 – complete sterility, all or almost all sterile anthers are in closed spikelets; class 1 – complete sterility, a significant amount of sterile anthers emerge; class 2 – incomplete sterility, the number of fertile anthers does not exceed 25%; class 3 – partial fertility, the number of fertile anthers is 25–75%; class 4 – incomplete fertility, the number of fertile anthers > 75%, sterile anthers are rare; class 5 – complete fertility.

Field studies were carried out in pilot plots of Research Institute of Agrarian Business (Vesele village, Dnipropetrovsk region, Ukraine), laboratory studies were conducted in Laboratory Molecular Genetic Analysis of Ukrainian Institute for Plant Variety Examination (Kyiv, Ukraine) during 2018–2019.

DNA extraction and PCR

DNA was isolated from 50 mg of green maize leaves in five repetitions (leaves from five separate plants of each sample). The extraction approach includes the use of CTAB as a lysing solution, double purification the mixture with chloroform and dissolution DNA in TE buffer (solution with Tris and EDTA) (Velikov, 2013; Aukenov et al., 2014; Gupta, 2019; Prysiazhniuk et al., 2019). Two pairs of primers to mitochondrial genes were used in the study according to two main types of sterility (Liu et al., 2002). The sequences and characteristics of the primers are shown in Table 2.

Table 2. Primer characteristics

Type of cms	Sequences 5’→3’	Amplicons size, bp	Sequence GenBank
CMS C	F – ATGCTAATGGTGTTCCGATTCC R – AGCATCATCCACATTCGCTAG	398	S81074
CMS S	F – CAACTTATTACGAGGCTGATGC R – AGTTCGTCCCATATACCCGTAC	799	AF008647

The reaction mixture (10 μL) contained 1×DreamTaq^{TM} Green buffer, 1 u DreamTaq^{TM} polymerase (ThermoScientific), 200 μM deoxynucleoside triphosphates mix (dNTPs), 10 ng DNA sample, 0.2 μM each primer. The PCR was performed using T-Cy IQ5 (CreaCon, The Netherlands). The amplification parameters were: initial denaturation (96 °C) 2 min, 30 cycles: denaturation (94 °C) 45 s; annealing (55 °C) 30 s; elongation (72 °C) 1 min; final elongation (72 °C) 2 min. The products of the amplification reaction were visualized by electrophoresis in a 3% agarose gel in

0.5×TBE (tris-borate buffer solution). DNA electrophoresis has been carried out for 1.5 hour at an electric field intensity of 5 V cm^{-1} (Abramova, 2006; Prysiazhniuk et al., 2019). The size of amplicons was determined using TotalLab v2.01 software (trial version).

To test the non-specific PCR products which are obtained as a result of amplification with the studied markers, PCR in silico was carried out on maize mitochondrion DNA sequences of two fertile cytotypes NA and NB (GenBank: DQ490952.1 and AY506529.1) using the SnapGene software (trial version) (Kalendar et al., 2017).

RESULTS AND DISCUSSION

Field studies

Four pairs of sterile analogues of lines were obtained during four backcrossing RL106S (17M, 19M, 23M, 27M та 29M) and maintainers RL106fS (18M, 20M, 24M, 28M and 30M) for S type of CMS and RL108C (195C, 201C, 205C, 207C, 209C) and RL108fC (196C, 202C, 206C, 208C, 210C) for C type of CMS respectively. Sterility control of groups of each backcrossing cycle was conducted in a breeding nursery to exclude cases of late emergence of fertile anthers on lateral branches during the drying of silk on ears.

The evaluation of the sterility maintain was carried out on hybrid combinations, which had as a parent component a sterile inbred line of maize, and as pollinators, inbred maintainer of line of the corresponding type. For S type, the following combinations were obtained: RL23S×RL106fS, RL107S×RL106fS, RL98S×RL106fS, RL105S×RL106fS, RL113S×RL106fS and for C type: RL109C×RL108fC, RL110C×RL108fC, RL112C×RL108fC, RL114C×RL108fC, RL115C×RL108fC. The obtained hybrid combinations were planted the following year in a control nursery for field assessment. According to the results of the field assessment, all the obtained hybrids were sterile.

The possibility of fertility restoration was studied on hybrid combinations RL23S×RL106fS and RL105S×RL106fS by pollination of S type inbred restorer of line RL34SB and RL109C×RL108fC and RL110C×RL108fC – by C type inbred restorer of line RL77CB. The used lines which were growing in the control nursery were identified as fertile.

Laboratory studies

As a result of the analysis of maize lines, which showed sterility in the field, amplicons of the expected size 799 bp were obtained using primers to S type of sterility (Fig. 1 and Fig. 2).

As shown in Fig. 1, maize lines that have a sterile cytoplasm of S type showed the presence of 799 bp DNA fragment. Amplicon 799 bp was identified in sterile analogues of lines: 17M, 19M, 23M, 27M and 29M. In fertile lines which are maintainers, from 2 to 3 amplicons have been identified. DNA fragments of size 632, 855, and 1,088 bp were identified in lines 18zM, 28zM, and 30zM; two amplicons, 855 and 1,145 bp were detected in lines 20zM.

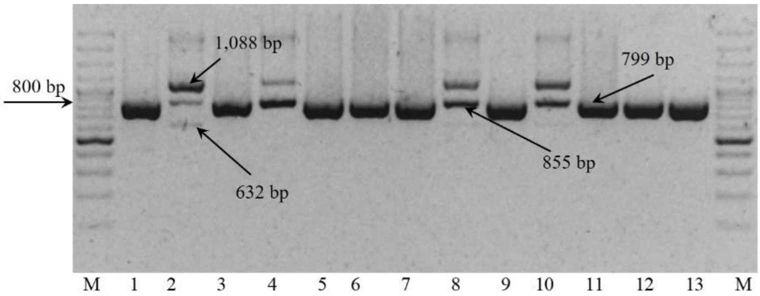

Figure 1. Results of PCR of maize lines with S type of sterility: M – 100 bp DNA Ladder O'GeneRuler (Thermo Scientific); 1, 3, 5, 6, 7, 8 – sterile analogues of lines of S type; 2, 4, 8, 10 – fertile lines of S type; 11, 12, 13 – sterile lines of S type.

In all sterile lines with S type of cytoplasm (RL23S, RL107S, RL98S, RL105S and RL113S), amplicons of the expected size 799 bp were identified (Fig. 2). Sterility based single-cross hybrids and restored hybrids of S type have been shown the presence of typical amplicon 799 bp as well.

It was noted that the 24zM line, which was selected as fertile in the field, turned out to be sterile by PCR with 799 bp amplicons. According to the classification of maize fertility levels according to Gontarovsky (1971), lines are completely sterile when all or almost all of the anthers are completely or partially in spikelets (0 and 1 class). There is a class 'incomplete fertility', in this case the number of fertile anthers does not exceed 25%. It is also known that environmental factors significantly affect the expression of sterility in S type lines. Whereas, the 24zM line was determined to be fertile in the field, but showed a sterile cytoplasm of S type by PCR.

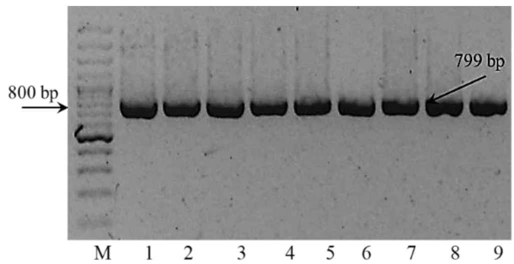

Figure 2. Results of PCR of maize sample with S type of sterility: M – 100 bp DNA Ladder O'GeneRuler (Thermo Scientific); 1, 2 – sterile lines of S type; 3–7 – sterility based single-cross hybrids (S type); 8–9 – restored hybrids (S type).

According to the results of PCR analysis of lines and hybrids with C type of sterility, the obtained amplicons were 398 bp (Fig. 3 and Fig. 4).

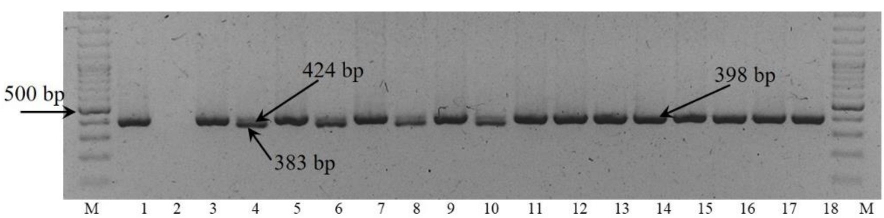

Figure 3. Results of PCR of maize sample with C type of sterility: M – 100 bp DNA Ladder O'GeneRuler (Thermo Scientific); 1, 3, 5, 6, 7, 8 – sterile analogues of lines of C type; 2, 4, 8 – fertile lines of C type; 11–15 – sterile lines of C type; 16–18 – sterility based single-cross hybrids (C type).

According to obtained data, in all sterile analogues of lines of C type (195C, 201C, 205c, 207c, 209C), an amplicon of expected size 398 bp was detected. In fertile lines 202zC, 206zC, 208zC and 210zC, two amplicons of sizes 383 and 424 bp were identified. In the fertile line 196 zC, in contrast to others, by markers to C type of sterility, any amplification product was not found. The presence of amplicon 398 bp was also identified in sterile lines with C type of sterility (RL109C, RL110C, RL112C, RL114C and RL115C). In sterility based single-cross hybrids of C type (RL109C× RL108fC, RL110C×RL108fC and RL112C×RL108fC), the amplicon of size 398 bp was detected (Fig. 3 and Fig. 4).

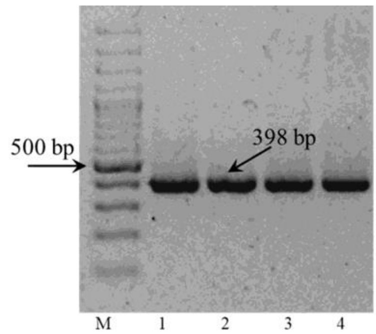

Figure 4. Results of PCR of maize sample with C type of sterility: M – 100 bp DNA Ladder O'GeneRuler (Thermo Scientific); 1–2 –sterility based single-cross hybrids (C type); 3–4 – restored hybrids (C type).

In Fig. 4, the presence of a amplicons of size 398 bp in sterility based single-cross hybrids RL114C×RL108fC and RL115C×RL108fC, as well as in restored hybrids (RL109C×RL108fC)× RL77CB та (RL110C×RL108fC)× RL77CB are shown. Therefore, the presence of amplicons of the expected size in sterility based single-cross hybrids and restored hybrids indicates the possibility of using DNA markers to evaluate hybrids on the method of breeding and determination of the type of sterility of lines (S and C), which formed the basis of a particular hybrid.

According to results of the studies, it was found that in the maintainers of lines of C type of sterility with relevant primers, two amplicons were identified which had the same size for all samples. Similar results were also obtained with primers to S type of sterility, however, the sizes of amplicons in that type of sterility in the maintainers of lines vary. According to the results obtained by Liu et al. (2002), in fertile lines, no amplicons were found by markers to S and C types of sterility. Also, the absence of any amplification products in fertile lines was noted by Slischuk et al. (2011). They were investigating 88 lines of maize, which included fertile lines, sterile analogues of lines, maintainers and fertility restorers of lines of Ukrainian and USA breeding. In our study, no amplicon was identified in one fertile line only with a marker to C type of sterility.

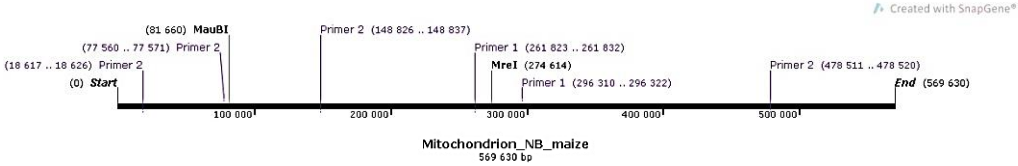

Figure 5. Results of PCR in silico of mitochondrion DNA sequence to NB cytotype with primers to C type of sterility.

To test the hypothesis whether there is a certain pattern concerning identified amplicons in fertile lines by markers to C and S types of sterility, PCR in silico was carried out on maize mitochondrion DNA sequences of two fertile cytotypes NA and

NB. Figs 5 and 6 show the results of PCR in silico with primers to cytotype NB as more common type among commercial lines and hybrids (Clifton et al., 2004).

According to obtained data, 2 to 4 binding sites were detected by testing primers to C type of sterility on the mitochondrion DNA sequence of NB cytotype. From 2 to 12 binding sites were determined by primer to S types of sterility (Fig. 6).

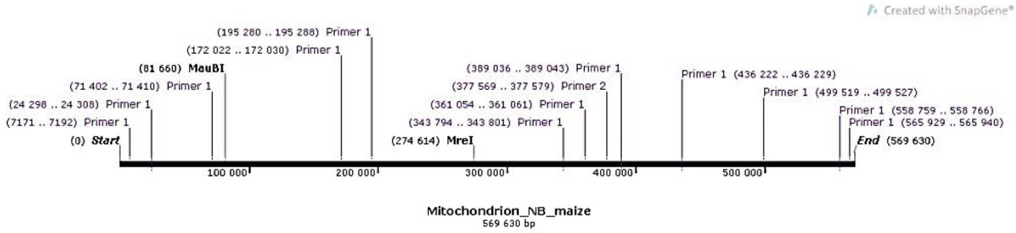

Figure 6. Results of PCR in silico of mitochondrion DNA sequence to NB cytotype with primers to S type of sterility.

It should be noted that an amplification product of size less than 50 kb (11,500 bp) was obtained only as a result of PCR in silico with primers to S type of sterility. According to the results of PCR in silico of mitochondrion DNA sequence to NA cytotype with primers to C and S types of sterility, from 2 to 4 binding sites were obtained and no amplification products of size less than 50 kb were detected.

Vancetovic et al. (2013) studied maize lines with S type of sterility in order to search for new sources of sterility. As a result of analysis of 24 different sources, additional amplicons were identified by authors, which differ from size of typical amplicons of the S type of sterility (799 bp). The authors suggested that the obtained profile is a consequence of the duplication of a smaller part of the mitochondrial genome of S type of CMS (Dewey et al., 1991; Darracq et al., 2010; Vancetovic et al., 2013).

Thus, as described above, there are about 20 sources of S type of sterility (Levings & Sederoff, 1983; Vancetovic et al., 2013) and Beckett (1971) also classified two types of C type of sterility, it can be assumed that the presence of nonspecific amplification products in fertile lines is explained by the presence of certain mitochondrion DNA sequences which are associated with various sources of sterility, which may have been included in the breeding process of the studied lines (Beckett, 1971; Krivosheev, 2018). Therefore, the presence of amplification products of any size which differs from products indicated the type of sterility is uninformative and cannot be used as a marker characteristic.

CONCLUSIONS

As a result of the studies, it was determined that DNA markers to C and S types of sterility are a quick and reliable approach for identifying sterile maize lines, in contrast to field studies, which have a number of limitations (temperature, humidity, sowing dates, daylight hours). Furthermore, it was found that the studied markers are equally efficient for the analysis of sterility based single-cross hybrids and restored hybrids. The use of DNA markers for determination of sterility is especially useful as part of a qualification examination of DUS. This approach makes it possible to quickly determine the difference between lines which are morphologically identical and differ only by the type of cytoplasm, and also to determine the type of CMS based hybrid.

REFERENCES

Abramova, Z.I. 2006. *Protein and Nucleic Acid Research: A Study Guide*. Kazan, 157 pp. (in Russian).

Allen, J.O., Fauron, C.M., Minx, P., Roark, L., Oddiraju, S., Lin, G.N., ... & Du, F. 2007. Comparisons among two fertile and three male-sterile mitochondrial genomes of maize. Genetics **2**, 1173–1192. https://doi.org/10.1534/genetics.107.073312

Anisimova, I.N. & Gavrilova, V.A. 2012. Structural and functional diversity of the genes that suppress phenotype of cytoplasmic male sterility in plants. *Proceedings on applied botany, genetics and breeding* **170**, 3–16 (in Russian).

Aukenov, N.E., Masabaeva, M.R. & Hasanova, U.U. 2014. Isolation and purification of nucleic acids. State of the problem at the present stage. *Science and healthcare* **1**, 51–53 (in Russian).

Beckett, J.B. 1971. Classification of Male-Sterile Cytoplasms in Maize (Zea mays L.) 1. *Crop Science* **5**, 724–727.

Chekanova, A. 2013. Reaction of maize lines with erect placing of leaves on CMS of M and C-types. *Journal of Uman National University of Horticulture* **83**,122–127 (in Ukrainian).

Clifton, S.W., Minx, P., Fauron, C.M.R., Gibson, M., Allen, J.O., Sun, H., ... & Meyer, L. 2004. Sequence and comparative analysis of the maize NB mitochondrial genome. *Plant physiology* **3**, 3486–3503.

Darracq, A., Varré, J.S. & Touzet, P. 2010. A scenario of mitochondrial genome evolution in maize based on rearrangement events. *BMC genomics* **1**, 233.

Dewey, R.E., Timothy, D.H. & Levings, C. 1991. Chimeric mitochondrial genes expressed in the C male-sterile cytoplasm of maize. *Current genetics* **6**, 475–482. https://doi.org/10.1007/BF00334775

Gayosso-Barragán, O., Rodríguez-Herrera, S.A., Petroli, C.D., Antuna-Grijalva, O., López-Benítez, A., Mancera-Rico, A., Luévanos-Escareño, M.P. & Lozano-del Río, A.J. 2020. Genetic components for fodder yield and agronomic characters in maize lines. *Agronomy Research* **18**. https://doi.org/10.15159/AR.20.001

Gontarovskiy, V.A. 1971. Genetic classification of sources of cytoplasmic male sterility in maize. *Genetics* **9**, 22–30 (in Russian).

Guidelines for the conduct of tests for distinctness, uniformity and stability 2009. https://www.upov.int/edocs/tgdocs/en/tg002.pdf

Gupta, N. 2019. DNA Extraction and Polymerase Chain Reaction. *Journal of Cytology* **2**, 116–117. https://doi.org/10.4103/JOC.JOC_110_18

Kalendar, R., Khassenov, B., Ramankulov, Y., Samuilova, O. & Ivanov, K.I. 2017. Fast PCR: An in silico tool for fast primer and probe design and advanced sequence analysis. *Genomics* **3–4**, 312–319. https://doi.org/10.1016/j.ygeno.2017.05.005

Konovalov, Yu.B., Dolgodvorova, L.I. & Stepanova, L.V. 1990. *Particular breeding of field crops*. Moscow, Agropromizdat, 542 pp. (in Russian).

Krivosheev, G.Ya. 2018. Classification of maize lines according to the content of alleles of 'C' type fertility-restorer genes. *Grain Economy of Russia* **1**, 81–88 (in Russian).

Krivosheev, G.Ya. & Ignatev, A.S. 2019. Reconstructive and stabilizing ability of the maize lines in sterile cytoplasm "M" and "C" types of CMS. *Grain Economy of Russia* **2**, 38–41. https://doi.org/10.31367/2079-8725-2019-62-2-38-41 (in Russian)

Križan, M., Krištof, K., Angelovič, M. & Jobbágy, J. 2017. The use of maize stalks for energy purposes and emissions measurement during their combustion. *Agronomy Research* **2**, 456–467.

Križan, M., Krištof, K., Angelovič, M., Jobbágy, J. & Urbanovičová, O. 2018. Energy potential of densified biomass from maize straw in form of pellets and briquettes. *Agronomy Research* **2**, 474–482. https://doi.org/10.15159/AR

Laughnan, J. R. & Gabay-Laughnan, S. 1983. Cytoplasmic male sterility in maize. *Annual review of genetics* **1**, 27–48. https://doi.org/10.1146/annurev.ge.17.120183.000331

Levings, C.S. & Sederoff, R.R. 1983. Nucleotide sequence of the S-2 mitochondrial DNA from the S cytoplasm of maize. *Proceedings of the National Academy of Sciences* **13**, 4055–4059.

Lisowski, A., Świętochowski, A. & Dąbrowska, M. 2019. Physicochemical properties and agglomeration parameters of biogas digestate with addition of calcium carbonate. *Agronomy Research* **4**, 1568–1576. https://doi.org/10.15159/AR.19.161

Liu, Z., Peter, S.O., Long, M., Weingartner, U., Stamp, P. & Kaeser, O. 2002. A PCR assay for rapid discrimination of sterile cytoplasm types in maize. *Crop science* **2**, 566–569.

Manson, J.C., Liddell, A.D., Leaver, C.J. & Murray, K. 1986. A protein specific to mitochondria from S-type male-sterile cytoplasm of maize is encoded by an episomal DNA. *The EMBO journal* **11**, 2775–2780.

Mustyatsa, S.I. & Mistrets, S.I. 2007. Use of the germplasm of heterotic groups BSSS and Reid Ayodent in the selection of early ripen corn. *Corn and sorghum* **6**, 8–12 (in Russian).

Palilova, A.N., Orlov, P.A. & Voluevich, E.A. 2005. Basic and applied problems of nuclear-cytoplasmic genetic systems interactions in plants. *Vavilov Journal of Genetics and Breeding* **4**, 499–504 (in Russian).

Prysiazhniuk, L., Honcharov, Yi., Melnyk, S. & Dikhtiar, I. 2019. Application of DNA markers for the assessment of allele state of the key genes of carotenogenesis in maize (Zea mays L.) seeds. *The Journal of Microbiology, Biotechnology and Food Sciences* **5**, 1141–1144.

Slischuk, G.I., Kozhukhova N.E. & Sivolap, Yu.M. 2011. Molecular-genetic analysis of maize mitochondrion regions associated with CMS. *Cytology and Genetics* **3**, 15–19 (in Ukrainian).

Stevanovic, M., Camdzija, Z., Pavlov, J., Markovic, K., Vancetovic, J., Drinic, S.M., ... & Markovic, K. 2016. The application of protein markers in conversion of maize inbred lines to the cytoplasmic male sterility basis. *Genetika* **2**, 691–698. https://doi.org/10.2298/GENSR1602691S

Vancetovic, J., Ignjatovic-Micic, D., Nikolic, A., Bozinovic, S., Markovic, K., Andelkovic, V. & Markovic, K. 2013. Potentially a new subtype of the cytoplasmic male sterility S-type in maize. *Genetika* 1, 145–151. https://doi.org/0.2298/GENSR1301145V

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https://doi.org/10.15159/AR.20.157

Isolation of streptomycetes causing common scab from 3-years old potato samples from South America

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Abstract. In this paper, we aimed at assessing the best conditions for the isolation of actinobacteria from old potato samples. A set of media and pretreatments were tested. The optimal were chosen for the isolation of actinobacteria from potatoes from Peru, Chile and Argentina. Isolates were tested on the presence of thaxtomin phytotoxin by amplification of the respective gene. Phylogenetic position of strains was compared with their geographical origin, pathogenic potential and existence of common scab (CS) symptoms on potato sample. We demonstrated that RNAlater can be successfully used for the long-term preservation of potato peel for subsequent isolation of actinobacteria on R2A medium. Many streptomycetes were thaxtomin-positive, though they are distantly-related to described pathogens causing CS. Genus *Nocardia* was first reported to be thaxtomin-positive. Potentially pathogenic strains were isolated not only from infected potato but also from those lacking CS symptoms. Some strains from scabby potatoes were thaxtomin-negative.

Key words: common scab, streptomycetes, thaxtomin, potato disease.

INTRODUCTION

Common scab (CS) is one of the most widespread potato diseases caused by several species of streptomycetes, a genus of the phylum Actinobacteria, able to produce phytotoxins thaxtomins (Loria et al., 2006). Thaxtomin is the only known pathogenicity determinant in *Streptomyces*, and the presence or absence of an operon encoding thaxtomin synthetase *txtAB* is 100% correlated with pathogenicity in more than 100 isolates for which pathogenicity assays have been carried out (Wanner, 2009).

The disease is characterized by shallow, raised or deep corky lesions not only on potato (*Solanum tuberosum L.*) tubers, but also on roots of other crops, such as beet (*Beta* *vulgaris L.*), carrot (Daucus carota L.), and turnip (*Brassica rapa L.*). The most widespread causal agent *Streptomyces scabies* also inhibits the growth of seedlings of radish (*Raphanus sativus L.*), alfalfa (*Medicago sativa L.*), cauliflower (*Brassica oleracea L.*), colza (*Brassica napus L.*), and turnip (Goyer et al., 2000).

There are different measures to control potato diseases, such as crop rotation (Simson et al., 2017) or selection of the resistant potato varieties (Razukas et al., 2009). However, isolation of the phytopathogenic microorganisms and the study of their resistance and ecological optima is of great importance for disease management. The most common method for the isolation of *Streptomyces scabiei* is plating a freshly collected homogenate from infected tissue or soil suspension from the infected field on water agar or semi-selective media for streptomycetes (Meng et al., 2011; Dees et al., 2013). However, when we used the classical conditions (such as adding nalidixic acid and cycloheximide for the elimination of gram-negative bacteria and fungi) and media for isolation of pathogenic streptomycetes from three-years-old samples of infected potato from South America, we encountered a problem. In most cases at different dilutions Actinobacteria on Petri dishes were over-competed by fast-growing non-filamentous bacteria and could not be isolated.

There are several reported conditions favoring actinobacteria, which can be used for selective isolation. For instance, spores of actinomycetes are more resistant to desiccation as compared to gram-negative bacteria (Kumar & Jadeja, 2016). Streptomycetes can also survive low water potential attributed to dry conditions or to a high concentration of substances in solution (Zviagintsev et al., 2007). Calcium may simulate the aerial mycelium formation and spore germination of streptomycetes including *S. scabiei* (Lambert & Manzer, 1991; Natsume et al., 2001). Adjusting of selective media or soil sample pretreatment with calcium carbonate facilitates the isolation of Actinobacteria (Alferova & Terekhova, 1988; Fang et al., 2017).

In this paper, we aim at assessing the optimal conditions for the isolation of streptomycetes causing common scab of potato from 3-years old potato samples from Peru, Chile and Argentina. The objectives: 1) to choose the optimal medium and pretreatments for selective isolation of actinobacteria and elimination of non-targeted bacteria from old potato samples; 2) to assess isolates in terms of the ability to produce thaxtomin.

MATERIALS AND METHODS

Isolation of Actinobacteria

Potato samples with and without common scab lesions from localities in Chili, Peru, and Argentina (Fig. 1) were collected in 2016 and stored as air-dried potato peel or 5 mm sliced potato-peel in RNAlater Stabilization Solution (Invitrogen) in a -70°°C freezer until the processing for isolation. No surface sterilization was applied to the samples. The set of pretreatments and inoculation procedures for the isolation of Actinobacteria is listed in Table 1.

To choose medium and pretreatment the most suitable for isolation of actinobacteria, dried potato peel (0.1 g) from one selected sample site was ground with mortar and pestle, serially diluted in sterile distilled water and aliquots of 100 µl of the diluted suspension was plated on freshly prepared agar media (in triplicates). The following isolation media were used: Gauze's agar 1 (Gauze et al., 1983); R2A agar

(Reasoner & Geldreich, 1985); Oatmeal agar (Gauze et al., 1983), and Water agar (WA) containing 20 g of agar per liter of water. To test the effect of 0.1% calcium carbonate and 3% agar, R2A and WA medium were supplemented accordingly. Further, the media will be referred to as Rc, R3, Wc, W3 respectively. Each of these media was supplemented with cycloheximide (300 mg L^{-1}) and nalidixic acid (20 mg L^{-1}) to prevent the growth of fungi and gram-negative bacteria.

For assessment of potato preservation form best suited for isolation of actinobacteria, R2A and WA medium were chosen for inoculation. Dried potato peel was serially diluted as described previously or 1 cm diameter piece was used for imprinting into the agar and then streaked out by inoculation loop. Potato peel preserved in RNAlater solution was plated by the serial dilution method.

Following 1-week incubation at 28 °C (2 weeks for WA medium), actinomycete- like and other non-actinobacterial colonies developed on the isolation media were counted.

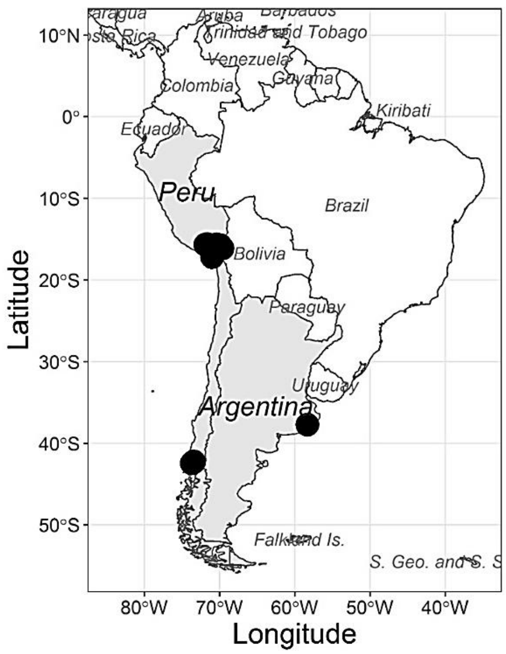

Figure 1. The geographic location of sampling sites in Chile, Peru, and Argentina.

Table 1. Selected conditions for the assessment of isolation of Actinobacteria from 3-years old potato samples

Tested condition	
Media	(a) Gauze-1 (b) Oatmeal Agar (c) R2A (d) Water Agar
Pretreatment and media adjustment	(a) Supplement medium with 0.1% CaCO3 (b) Higher agar content in medium (3%)
Choice of sample form for plating	(a) Dilution of suspension from dried potato peel (b) Imprint of dried potato peel (c) Dilution of sliced potato peel in RNA later solution

The best medium, sample form and pretreatment procedure was chosen for the inoculation of potato samples from 11 sites in Chile, 26 in Peru, and 3 in Argentina. The criteria for selection (in descendent by priority order): 1) ability to isolate actinobacterial strains from separated non-overcompeted colonies; 2) morphological diversity of actinobacteria colonies; 3) lower time of sample processing before the inoculation; 4) shorter incubation time.

Morphologically distinct colonies were selected and purified on the R2A medium. The purified cultures were preserved as glycerol suspensions (20%,v/v) at -70 °C.

Graphical and statistical analysis was performed in Excel and R software (R Core Team, 2019).

Phylogeny and pathogenicity

DNA from pure strains was extracted using a commercial kit according to the manufacturer (DNeasy PowerLyzer Microbial Kit, Qiagen). 16S rRNA gene was amplified with primers *16Seu27f* and *PH* (Bruce et al., 1992) and sequenced by the capillary Sanger sequencing (Macrogen Europe Inc., Amsterdam, the Netherlands). The resulted sequences were amplified with primers 16Seu27f and pH (Bruce et al., 1992) and aligned using SILVA Incremental Aligner v.1.6.0. (Quast et al., 2013) with GenBank sequences of 7 type strains of known-to-date pathogenic streptomycetes causing CS of potato. The best-fit model of nucleotide substitution was selected using jModelTest v.2.1. (Darriba et al., 2012). A phylogeny was inferred using maximum-likelihood analysis in FastTree 2.1.10. (Price et al., 2009). The phylograms were finalized using iTOL (Letunic & Bork, 2016).

Thaxtomin production ability was inferred as a positive PCR reaction targeting a fragment of the *txtAB* (*txtA* and *txtB*) gene using primers *stx1a* and *stx1b* (Flores-González et al., 2008). The thaxtomin-positive isolates later are referred to as *stx*-positive to emphasize primer used.

RESULTS AND DISCUSSION

Media and pretreatments

The results showing the bacterial colonies developed on different media is presented in Fig. 2. A non-parametric *Kruskal-Wallis test* showed that there was a statistically significant difference in ratios of Actinobacterial colonies to other Bacteria colonies (A:B) between the different media used for inoculation (*Chi-square* = 9.31, *p-value* = 0.025, *df* = 3), with a mean rank of A:B ratio score of 6.0 for Gauze, 4.5 for OA, 4.5 for WA and 11.0 for R2A medium. No actinomycete was observed on the OA medium and only 1-2 non-actinobacterial colonies. Although the OA medium is known to stimulate thaxtomin production and support the good growth of *S.scabiei* (Loria et al., 2006), it did not prove to be a good selective medium for actinobacterial isolation from potato.

The highest number of actinobacterial colonies with a relatively small number of non-mycelial bacteria were developed on R2A agar. In comparison with WA and mineral Gauze medium, R2A agar is 'nutrient-rich', but nutrients are diluted to mimic oligotrophic environment, as the medium was originally developed for the isolation of bacteria from the potable water (Reasoner & Geldreich, 1985). Surprisingly, Gauze 1 medium, traditional for the selective isolation of streptomycetes from the soil (Gauze et al., 1983), was not suitable for the development of actinobacteria, as plates were over-competed by non-actinomycetes. The soil streptomycetes are known to be oligotrophic and slow-growers which feed on recalcitrant carbon source (e.g. starch as in Gauze medium) (Zviagintsev & Zenova, 2001). It is possible that plant-associated actinobacteria, in contrast to soil inhabitants, require more nutrients because the inner and outer plant environment is rich in plant exudates. While Gauze is a defined medium with starch as the only carbon source, the R2A medium contains small quantities of starch, yeast extract, peptone, casamino acids and pyruvate, which may be present in potato tuberosphere. There was a minimal number of colonies both of actinobacteria and other bacteria developed on WA in comparison with R2A, though actinobacterial colonies were not over competed by fast-growing bacteria and could be isolated.

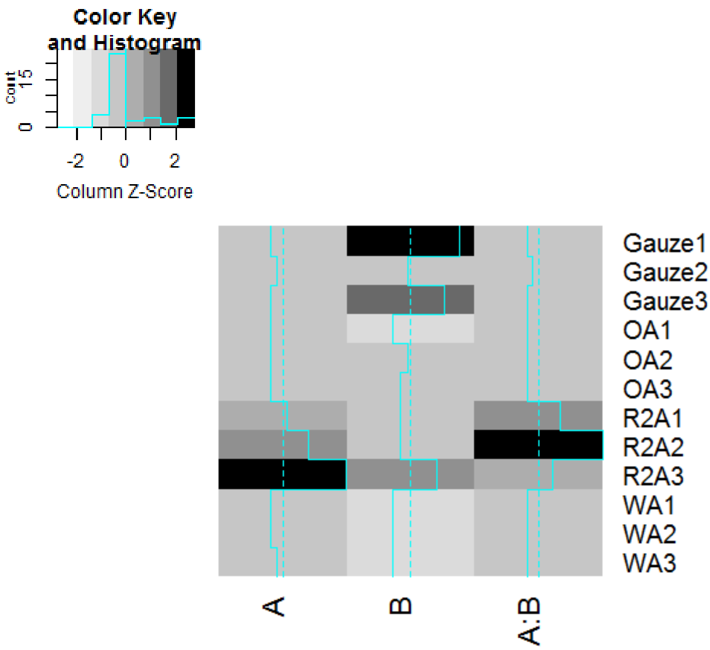

Figure 2. Heatmap of colony numbers of Actinobacteria (A), other bacteria (B), and their ratio (A:B) after inoculation of potato sample suspension from the same dilution on different media (Gauze, OA, R2A, and WA) in triplicates (numbers 1, 2, 3 near the respective medium).

A comparison of inoculation source, medium and medium modifications is presented in Fig. 3. We expected the best sample pretreatment to be homogenization of dried potato peel in a mortar, as streptomycetes spores are usually well preserved in air-dried soil samples (Doroshenko et al., 2005). Indeed, as we see in a heatmap (Fig. 3), the highest actinobacterial counts with low non-targeted bacteria colonies were registered from the inoculated suspension of the dried peel on R2A agar and R2A medium supplemented with 0.1% CaCO3 (Rc).

However, R2A medium with higher agar content (referred to as R3) stimulated the growth of fast-growing bacteria from the same suspension used for the inoculation. The higher agar content equals to lower water potential in a medium (Buah et al., 1999), so we expected streptomycetes to have the advantage to develop in such an environment, as their spores are resistant to desiccation (Doroshenko et al., 2005). The competing fast-growing non-actinomycete bacteria that appeared on R3 plates are possibly more resistant to a higher water potential than streptomycetes. Moreover, actinobacteria produce exospores which are less resistant to many environmental factors in comparison with endospores of other bacteria (Abel-Santos, 2015).

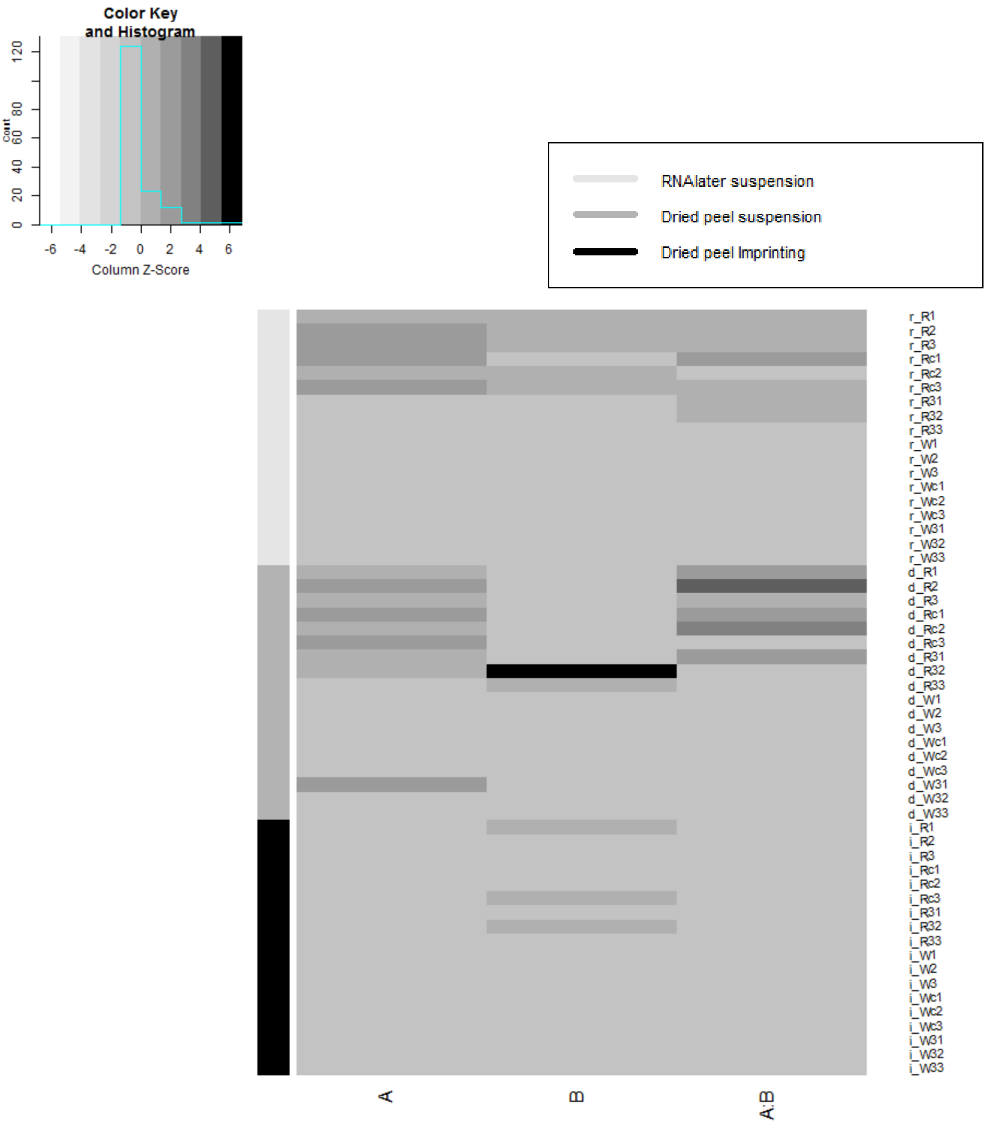

Figure 3. Comparison of different sample forms for plating (left bar) and media adjustments (rows labels). R stands for R2A agar; Rc – R2A with 0.1% CaCO3; R3 – R2A with 3% agar; W – WA. Numbers 1,2,3 near the respective medium indicates triplicates. Heatmap reflects colony numbers of Actinobacteria (A), other bacteria (B), and their ratio (A:B).

Imprinting of dried potato peel was the poorest method for streptomycetes isolation, as only non-actinomycetes grew on all tested media. It is possible that actinomycetes are not on the surface of the dried peel and need to be desorbed from inside by sample homogenization. Inoculation from RNAlater suspensions on R2A and Rc promoted the growth of actinobacteria more prominent, then other bacteria, though not so effectively as from dried peel suspension.

A *Kruskal-Wallis rank sum test* detected the significant difference in A:B ratios between three sample inoculation treatments (*Chi-squared* = 8.7794, *df = 2*, *p-value* = 0.0124). However, pairwise comparisons using *Wilcoxon rank sum test* as a *post-hoc test* with *False Discovery Rate p-value* adjustment (Benjamini & Hochberg, 1995) indicated the significant differences only between A:B ratios on plates inoculated from the Dry peel Imprints and the Dry peel Suspension ($p = 0.011$), and between plates inoculated from the Dry peel Imprints and the RNAlater Suspension ($p = 0.011$). Although we observed the differences between media adjustments on a heatmap, no significant differences were supported by the *Kruskal-Wallis rank sum test* between A:B ratios detected on control plates, and plates with 3% agar content or with CaCO3 supply (*Chi-squared* = 1.29, *df* = 2, *p-value* = 0.5246).

Water agar plating was inefficient for the isolation of streptomycetes from old potato samples in all cases, except one plate inoculated with the dried peel suspension, but not in replicates. A *Wilcoxon rank sum test* with continuity correction showed that there was a significant difference ($p < 0.001$) between scores given for the ratio of Actinobacteria over other Bacteria that appeared on an R2A medium compared to the Water Agar medium ($W = 306$, *p-value* = 3.036e-07). The median score for the R2A medium was 27.0 compared to 10.5 for WA. The effect size is approximately 0.854 which is very large according to Cohen's (1988) classification of effect sizes which is 0.1 (small effect), 0.3 (moderate effect) and 0.5 and above (large effect). It is possible that after such prolonged sample storage, to develop viable colonies bacteria need more nutrients, like in the R2A medium. Moreover, actinobacterial colonies developed on WA appeared to be morphologically identical, while R2A allowed actinobacterial differentiation (different forms and colors of colonies), which is useful in actinobacterial screening. WA requires 2 weeks for colonies to develop, while only 1 week of incubation is sufficient for the R2A medium. Thus, we believe that the WA medium is not the best choice for the isolation of pathogenic streptomycetes from potato samples, as it was commonly used so far.

Storage the peel in RNAlater solution were comparably efficient for the streptomycete isolation, while at the same time facilitating the manipulation with sample and greatly reducing the time for plating. This is the first report that RNAlater solution may be used for the long-term preservation of plant samples for subsequent isolation of actinobacteria.

As R2A medium and R2A medium supplemented with 0.1% CaCO3 were similarly efficient, we have chosen to use usual R2A without any supplement for subsequent isolation of actinomycetes from 3-years old potato samples from 40 different localities of South America. We used serial dilution from RNAlater solution for inoculation.

Actinobacterial isolates from South American potato samples

A total collection of 123 actinobacteria was isolated from 40 localities in Peru, Chile, and Argentina. The phylogenetic position and pathogenicity potential of strains are presented in Fig. 4. Most of the actinomycetes belong to the genus *Streptomyces*, while 19 to the genus *Nocardia*.

As could be expected, closely related strains come from the same regions. Surprisingly, many *stx*-positive strains were not closely related to known pathogens causing CS. Moreover, thaxtomin genes were never described in the genus *Nocardia* before. What is interesting, *stx*-positive nocardias were isolated exclusively from healthy plants. On the one hand, it may be attributed to the horizontal gene transfer (HGT) of the pathogenicity island which bares genes for the thaxtomin production (Loria et al., 2006). On the other hand, little is known about the actinobacterial community on potato samples coming from these regions, probably the origin of the potato itself.

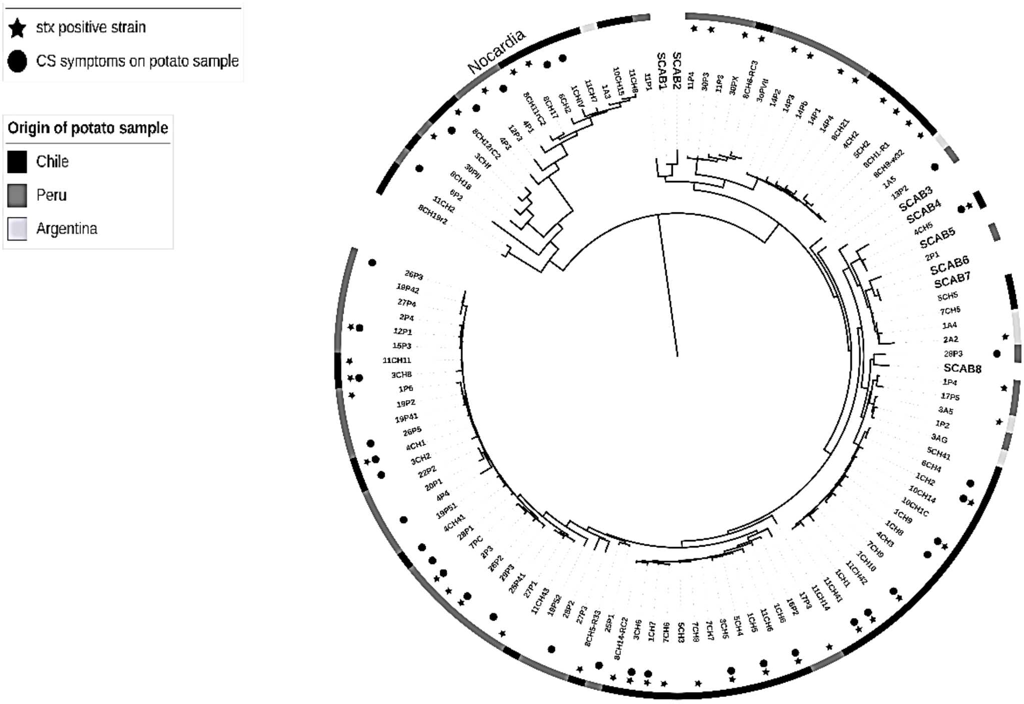

Figure 4. ML Phylogenetic tree based on the 16S rRNA gene of actinobacterial strains (belonging to the genus *Streptomyces* and *Nocardia*) isolated from 3-years old potato samples from different localities in South America. SCAB1-8 is designation of type strains of pathogenic streptomycetes causing common scab of potato (CS): 1 – *Streptomyces scabiei* ATCC 49173T (NR_025865.2); 2 – *S. niveiscabiei* KACC 20254T (NR_037095.1); 3 – *S. europaeiscabiei* KACC 20186T (NR_042790.1); 4 – *S. puniciscabiei* LMG 21391T (NR_025156.1); 5 – *S .reticuliscabiei* CFBP 4531T (NR_025293.1); 6 – *S. stelliscabiei* CFBP 4521T (NR_025294.1); 7 – *S. acidiscabies* ATCC 49003T (D63865.1); 8 – *S. turgidiscabies* ATCC 700248T (NR_040828.2).

Several thaxtomin-positive strains were isolated from potato with CS symptoms. However, many of them came from healthy potato, while many non-pathogenic strains were isolated from infected potato. The presence of scab causing streptomycetes in the tuberosphere of the healthy plant may be connected with suppressivity of soil, which has special bacterial communities protecting the plant (Kopecky et al., 2018). The absence of the pathogenic streptomycetes on infected potato may be due to poor sampling or due to another organism causing the infection.

It was reported previously that all of the known disease-causing *Streptomyces* species also have non-pathogenic members (Dees et al., 2013). In this study, we detected the presence of the pathogenicity determinant in the strains distantly-related to the so-far described pathogens and in actinobacteria coming from the "healthy" plant environment. It is unclear if these observations may be attributed to the HGT and how it may affect disease epidemiology and plant protection. As we tested only the presence of the *txtAB* operon of the thaxtomin gene cluster, we do not know for sure that *stx*-positive strains are virulent. Future work should focus on the *in vitro* and *in vivo* virulence assays on a broad range of hosts to confirm the pathogenic potential of *Nocardia*, streptomycetes distantly-related to known pathogens and strains coming from the 'healthy' source. It is also challenging to compare pathogenicity gene pool of the 'healthy' and 'diseased' plant environments not only in terms of thaxtomin genes present but also other independent virulence factors (such as *nec1*, *tomA*, *fas* operon), which may be present on the same pathogenicity island and affect the disease.

CONCLUSIONS

Isolation of phytopathogenic actinobacteria from old plant samples may require different conditions than it is traditionally used for the selective isolation of actinomycetes from soils or freshly collected environmental samples. RNAlater solution may be effectively used for long-term storage of potato peel for subsequent isolation of actinobacteria. The optimal medium for the isolation may be R2A agar, which was first developed for bacterial isolation from potable water.

South America, the mother of the potato plant, is an interesting object to study bacterial communities in tuberosphere, their role in plant protection, and the origin of plant diseases. We analyzed actinobacteria coming from infected and healthy potato samples from different locations in Peru, Chile, and Argentina. Regardless of the presence of the CS symptoms, samples differed in a share of pathogenic actinobacteria: while from some samples all isolates were thaxtomin-positive, others contained only thaxtomin-negative isolates, and third both *stx*-positive and *stx*-negative actinobacteria. Genes for the thaxtomin production were detected in *Nocardia,* which have never been described to have genes for the thaxtomin production. It is obvious that complex actinobacterial community populates tuberosphere and affects the potato plant in different ways. On the other hand, there are many other potato diseases with common symptoms but different causes. Finally, horizontal gene transfer may play a role in pathogenesis.

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REFERENCES

Abel-Santos, E. 2015. Chapter 9-Endospores, Sporulation and Germination. In Y.-W. Tang, M. Sussman, D. Liu, I. Poxton, & J. Schwartzman (Ed.), *Molecular Medical Microbiology* (Second Edition) pp. 163–178. Academic Press. https://doi.org/10.1016/B978-0-12-397169-2.00009-3

Alferova, I. & Terekhova, L. 1988. Use of the method of enriching of soil samples with calcium carbonate for isolation of Actinomyces. *Antibiot Khimioter* **33**, 888–890.

Benjamini, Y. & Hochberg, Y. 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society*. Series B (Methodological), **57**(1), 289–300. JSTOR.

Bruce, K.D., Hiorns, W.D., Hobman, J.L., Osborn, A.M., Strike, P. & Ritchie, D.A. 1992. Amplification of DNA from native populations of soil bacteria by using the polymerase chain reaction. *Appl. Environ. Microbiol.* **58**, 3413–3416.

Buah, J.N., Kawamitsu, Y., Sato, S. & Murayama, S. 1999. Effects of Different Types and Concentrations of Gelling Agents on the Physical and Chemical Properties of Media and the Growth of Banana (Musa spp.) in Vitro. *Plant Production Science* **2**(2), 138–145. https://doi.org/10.1626/pps.2.138

Cohen, J. 1988. Statistical power analysis for the behavioral sciences (2nd ed.). Hillsdale, NJ: Erlbaum. https://doi.org/10.4324/9780203771587

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* **9**, 772–772.

Dees, M.W., Sletten, A. & Hermansen, A. 2013. Isolation and characterization of Streptomyces species from potato common scab lesions in Norway. *Plant Pathology* **62**, 217–225.

Doroshenko, E.A., Zenova, G.M., Zvyagintsev, D.G. & Sudnitsyn, I.I. 2005. Spore Germination and Mycelial Growth of Streptomycetes at Different Humidity Levels. *Microbiology* **74**(6), 690–694. https://doi.org/10.1007/s11021-005-0125-5

Fang, B.-Z., Salam, N., Han, M.-X., Jiao, J.-Y., Cheng, J., Wei, D.-Q., Xiao, M. & Li, W.-J. 2017. Insights on the Effects of Heat Pretreatment, pH, and Calcium Salts on Isolation of Rare Actinobacteria from Karstic Caves. *Front. Microbiol.* **8**.

Flores-González, R., Velasco, I. & Montes, F. 2008. Detection and characterization of Streptomyces causing potato common scab in Western Europe. *Plant Pathology* **57**, 162–169.

Gauze, G., Preobrazhenskaya, T., Sveshnikova, M., Terekhova, L. & Maximova, T. 1983. A guide for the determination of actinomycetes. Genera Streptomyces, Streptoverticillium and Chainia. Nauka, Moscow. 248 pp.

Goyer, C., Charest, P.-M., Toussaint, V. & Beaulieu, C. 2000. Ultrastructural effects of thaxtomin A produced by *Streptomyces scabies* on mature potato tuber tissues. *Can. J. Bot.* **78**, 374–380.

Kopecky, J., Samkova, Z., Sarikhani, E., Kyselková, M., Omelka, M., Kristufek, V., Divis, J., Grundmann, G.G., Moenne-Loccoz, Y. & Sagova-Mareckova, M. 2018. The effect of susceptible and resistant potato cultivars on bacterial communities in the tuberosphere of potato in soil suppressive or conducive to common scab disease. *bioRxiv* 340257.

Kumar, R.R. & Jadeja, V.J. 2016. Isolation of Actinomycetes: A Complete Approach. *Int. J. Curr. Microbiol. App. Sci* **5**, 606–618.

Letunic, I. & Bork, P. 2016. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res* **44**, W242–W245.

Loria, R., Kers, J. & Joshi, M. 2006. Evolution of Plant Pathogenicity in Streptomyces. *Annual Review of Phytopathology* **44**, 469–487.

Meng, Q., Yin, J., Rosenzweig, N., Douches, D. & Hao, J.J. 2011. Culture-Based Assessment of Microbial Communities in Soil Suppressive to Potato Common Scab. *Plant Disease* **96**, 712–717.

Price, M.N., Dehal, P.S. & Arkin, A.P. 2009. FastTree: Computing Large Minimum Evolution Trees with Profiles instead of a Distance Matrix. *Mol. Biol. Evol.* **26**, 1641–1650.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. & Glöckner, F. O. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res* **41**, D590–D596.

Razukas, A., Jankauskiene, Z., Jundulas, J. & Asakaviciute, R. 2009. Research of technical crops (potato and flax) genetic resources in Lithuania. *Agronomy Research* **7**, 59–72.

R Core Team. 2019. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. URL https://www.R-project.org/

Reasoner, D.J. & Geldreich, E.E. 1985. A new medium for the enumeration and subculture of bacteria from potable water. *Applied and Environmental Microbiology* **49**, 1–7.

Simson, R., Tartlan, L., Loit, E. & Eremeev, V. 2017. The effect of different pre-crops on Rhizoctonia solani complex in potato. *Agronomy Research* **15**, 877–885.

Wanner, L.A. 2009. A Patchwork of Streptomyces Species Isolated from Potato Common Scab Lesions in North America. *American Journal of Potato Research* **86**(4), 247–264. https://doi.org/10.1007/s12230-009-9078-y

Zviagintsev, D.G., Zenova, G.M., Doroshenko, E.A., Griadunova, A.A., Gracheva, T.A. & Sudnitsyn, I. I. 2007. Actinomycete growth in conditions of low moisture. *Izv. Akad. Nauk. Ser. Biol.* 296–302. (In Russian)

Zviagintsev, D.G. & Zenova, G.M. 2001. Ecology of actinomycetes. Izd. GEOS. 256 pp. (in Russian).

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The sustainable reuse of compost from a new type of olive mill pomace in replacing peat for potted olive tree

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Abstract. The attention for the replacement of peat in growing substrates is increasing due to its environmental and economic advantages. The aim of the present work was to evaluate the impact of peat substitution by new type olive mill pomace and its compost on the vegetative activity of potted olive trees. A new type of humid pomace (hP) derived from an innovative two phase extraction system and its derived compost (C-hP) are used as total or partial (50% vol/vol) replacement of peat in olive growing substrate. The main chemical characteristics (moisture, water extractable organic carbon, total nitrogen, C/N ratio and electrical conductivity) of the hP and C-hP were measured. In order to evaluate the effects of the peat substitution on the plants vegetative activity, measurements of mortality, plant height, leaf net photosynthesis and plant growth (through final destructive measurements) were carried out. The use of hP resulted in a significant increase of the salinity of the substrate. The water extractable organic carbon concentration was higher in all substrates where peat was ewplaced and in particular when C-hP was used. The total replacement of peat with hP caused 100% mortality of the plants while C-hP can substitute peat up to 50% without causing a significative reduction of the final plant growth.

Key words: compost, olive pomace, olive tree, peat replacement.

INTRODUCTION

The olive oil extraction industry produces in a short period of time large amounts of oil mill waste (OMW), i.e. solid by-products (pomace) and olive mill water, whose gradual accumulation or incorrect disposal may cause serious environmental issues (Morillo et al., 2009; Regni et al., 2016). In the last decade, the composition and volumes of OMW has changed, as a result of innovative technologies used for olive oil extraction aiming at reducing water use during the various stages of the oil extraction. In fact, the traditional three-phase centrifugation system that generates olive oil, pomace and vegetation water is being gradually phased out in favor of a two-phase system that generates pomace having a very high water content as the only waste (TPOMW) (Alfano et al., 2008; Gigliotti et al., 2012). TPOMW, is a semi-solid lignocellulosic material with an abundance of potentially phytotoxic substances such as phenols, organic acids and salts (Cayuela et al., 2008). Several approaches for OMW disposal have been proposed for its valorization, such as direct land spreading, residual oil extraction, energy and biogas production, recovery of useful chemicals (Peri & Proietti, 2014; Proietti et al., 2015; Innangi et al., 2017; Regni et al., 2017). Since many of these approaches may be too costly for olive-oil producers worldwide, at present time, recycling solid OMW as soil amendment, either fresh or composted, represents the most convenient approach (Niaounakis & Halvadakis, 2006; Lopez-Pineiro et al., 2007; Rodrìguez-Lucena et al., 2010; Nasini et al., 2013; Ameziane et al., 2019). Co-composting of solid OMW, particularly TPOMW, with lignocellulosic bulking materials, such as pruning, strawor wool waste, is of particular interest for its operational simplicity and ability to transform this waste into a high-quality soil amendment characterized by loss of phytotoxicity and abundance of stabilized organic matter and nutrients for plants (Cayuela et al., 2010; Del Buono et al., 2011; Nasini et al., 2016; Ameziane et al., 2020). Among other uses, the TPOMWderived compost can be used as a cheap organic ingredient for growing substrate of potted plants that are now generally based on the rather expensive peat mixed with inorganic materials (Papafotiou et al., 2005). This valorisation strategy is particularly interesting considering that the rapid depletion of peat lands and the consequent environmental concern haveled the producing countries to limit the exploitation of this natural resource (Alexander et al., 2008). Indeed modern peat extraction methods can remove up to 22.5 cm of peat per annum but peat forms at only ca. 1 mm per annum resource (Alexander et al., 2008). Regarding the annual peat consumption of substrates, in Italy, it is around 5 million m^3, consisting largely of peat imported from abroad (Pinamonti & Cementero, 1997).

Furthermore, the European Commission in 2001 has excluded the issuance of the European 'Eco-label' for plants produced with growing substrates containing peat or derivatives. However, the use of TPOMW, especially if not-composted, as plant nursery substrate component may present problems due to their high organic load and mineral salt content, low pH and presence of phytotoxic compounds (Canet et al., 2008; Del Buono et al., 2011; Gigliotti et al., 2012). To the best of our knowledge, the effects of this new type TPOMW, composted or not-composted, on olive plant growth in nursery is not known.

A better understanding of this issue is therefore necessary. In this respect, the majority of studies have been conducted with olive mill wastewaters and aimed to evaluate their effects as soil amendment in field (Kotsou et al., 2004; Mekki et al., 2006; Di Serio et al., 2008). Meanwhile, the TPOMW used in this study was a humid pomace (hP) (water content between 64 and 78%) without any traces of olive pit obtained from an innovative two-phase extraction system. The high water content of hP made its use for oil extraction or energy production difficult. The two-phase extraction system 'DMF decanter' is rapidly spreading and therefore the production of hP is increasing, with consequent strong problems of disposal for oil exactrion or energy production due to its high levels of humidity. The aim of the present work was to evaluate the use as component of olive growing substrate in nursery of the new type of hP and its derived compost in total or partial (50% vol/vol) replacement of peat. We investigated the possibility to reduce the use of peat aiming at finding, as well, an environmentally friendly disposal of olive mill by-product.

MATERIALS AND METHODS

hP and derived compost (C-hP) characteristics

The hP used in this study was obtained from an innovative two-phase extraction system 'DMF decanter' ('Gruppo Pieralisi- MAIP', Jesi – Ancona, Italy) that does not need water addition and produces two types of by-products: a dehydrated pomace similar to that from a three-phase decanter (water content between 45 and 58%) and a hP (water content between 64 and 78%) without any traces of olive pit. For an appropriate composting of the hP, which had a water content of about 75%, bulking agents have been added in order to improve the bulk density. The C-hP was obtained by co-composting the humid pomace with shredded olive tree prunings (22%) and cereal straw (8%) (w/w) in a cubic pilot bioreactor (1 m^3) equipped with forced aeration (flow rate of 20 L min^{-1} for 10 min per hour). During the active phase (biooxidative phase), the temperature and the moisture were monitored continuously: this phase was considered finished when the mass temperature was similar to the room temperature and the re-heating did not occur (after about 50 d). The following curing phase was conducted in a trapezoidal pile for 80 more days with regular turning and wetting. The chemical characteristics of the hP and C-hP are reported in Table 1 (Gigliotti et al., 2012).

Table 1. Chemical properties of hP and C-hP (w/w of dry material) (Gigliotti et al., 2012)

	hP	C-hP
Moisture (%)	78.1 (3.8)	38.4 (0.8)
TOC (g kg^{-1})	524.3 (9.3)	473 (1.3)
TKN (g kg^{-1})	16.2 (0.6)	29 (0.5)
C/N	32.4	16.3
EC (dS m^{-1})	2.58 (0.08)	3.29 (0.4)

Environmental parameters and plant material

The research was conducted in Perugia–Central Italy (about 400 m a.s.l., 12°23’E longitude, 43°5’N latitude) under a shading structure. The climatic data were reported in Table S1.

In February 2016, one-year-old rooted cuttings of Olea europaea L., cultivar Leccino, obtained in a mist propagation system and then transplanted in pots (100 mL) containing a substrate composed of 60% (w/w) peat and 40% pumice, were individually potted in bigger black plastic pots (2.5 L) containing different substrates. All the substrates were added with the controlled-release fertilizer 'Osmocote' ('Scott Italia' 16:8:12 N:P:K) at the dose of 2 kg m^{-3} of substrate. The potted plants were maintained under the shading structure, on a bench, spaced 30×30 cm between and along the rows (north-south). Plants were regularly irrigated, depending on shading structure temperature and solar radiation, to avoid any symptoms of water stress.

Substrates characteristics

In the experiment, the following potting substrates compositions were used:

- 60% peat + 40% pumice (vol/vol), referred to as 'control';
- 60% hP + 40% pumice (vol/vol), referred to as 'hP60';
- 30% hP + 30% peat + 40% pumice (vol/vol), referred to as 'hP30';
- 60% C-hP + 40% pumice (vol/vol), referred to as 'C-hP60';
- 30% C-hP + 30% peat + 40% pumice + (vol/vol), referred to as 'C-hP30'.

The peat had organic C and N contents of 40 and 0.2% (DW), respectively, and a pH of 4.7. The pumice had a porosity of 65% and a pH of 7.0. Water-extractable organic carbon (WEOC) was obtained as described by Gigliotti et al., 2002. Electrical conductivity and pH were determined in a 1:10 compost:water extract ratio. Every 3 months, five pots per treatment were irrigated with 1 L of water, then the leached water was collected to determine the electrical conductivity (EC), using the conductimeter 'Hanna Instruments- HI 9033', and pH, using the pH-meter 'Radiometer Copenhagen-PHM 82)'.

Plant growth

At transplanting and 120 and 270 days after transplanting (DAT) the height of the plants was determined.

Every week, the number of dead plants in each substrate was determined. At 150 DAT, a pruning operation was carried out in order to remove basal shoots and shoots in competition with the principal axis. The fresh weight (FW) and dry weight (DW) of the pruning material were recorded.

At 270 DAT, nine plants per treatment were extracted from the substrate and the roots were washed. Then, roots, stem, lateral shoots and leaves were separated to determine their DW by oven-drying at 95 °C until constant weight was reached. Moreover, in these plants, the number of leaves, number and length of lateral shoots and stem diameter were measured. The development of root system was also assessed by measuring the number, length and diameter of principal roots.

Leaf net photosynthesis (P_n)

At transplanting, 30, 120 and 210 DAT, Pn was determined on cloudless days on seven well-lit current season leaves randomly selected in the middle part of shoots of seven plants per treatment. Measurements were made in the glasshouse, in the morning (from 9:00 to 10:30 h) at natural incident photosynthetic photon flux density (PPFD) that ranged from 414 µmol m^{-2} s^{-1}(February) to 656 µmol m^{-2} s^{-1} (May). Pn was measured using a portable IRGA (ADC-LCA-3, 'Analytical Development', Hoddesdon, UK) and a Parkinson-type assimilation chamber. Leaves were enclosed in the chamber and exposed to the sun's rays. The flow rate of air passing through the chamber was kept at 5 cm^3 s^{-1}. During gas-exchange measurements, the external CO2 concentration was about 360 cm^3 m^{-3} and the air temperature inside the leaf chamber was 2–3 °C higher than that in the atmosphere, varying from 11.0 °C (February) to 31.5 °C (August). Measurements were taken under steady state conditions (about 30 s). Pn was then expressed on a leaf-area basis.

Statistical analysis

The experiment was designed as a randomized block with nine blocks containing each three pots per treatment, resulting in twenty-seven pots for each experimental potting substrate.

Statistical analysis was performed using analysis of variance (ANOVA). Significant differences between values were determined at $P \leq 0.05$ by the Duncan's test.

RESULTS

Substrates characteristics

Table 2 shows the main chemical properties of substrates used in each tretment of the experiment. The pH was significantly different in substrates where the hP was used. The highest pH values were observed in the substrates prepared with compost, particularly for C-hP60. EC results showed similar values to the control in C-hP substrates. On the contrary, the use of hP resulted in a significant increase of the salinity. The WEOC concentration was higher in all substrates where the hP and C-hP were used. The same behaviour was found for the water soluble phenols, which were all significantly higher with respect to the control (hP60 > hP30 > C-hP60 > C-hP30).

Table 2. pH, EC, WEOC and soluble phenols in the substrates at the beginning of the experiment

	pH	EC	WEOC	Water soluble phenols
		mS cm^{-1}	mg g^{-1}	mg g^{-1}
Control	5.69 b	1.11 c	2.95 c	0.23 d
hP60	4.13 c	3.69 a	37.87 a	5.67 a
hP30	4.88 c	2.34 b	31.30 a	3.05 b
C-hP60	7.19 a	1.78 c	21.12 b	2.15 c
C-hP30	6.31 ab	1.42 c	14.69 b	1.41 c

Mean values followed by different letters are significantly different ($P < 0.05$).

Table 3 reports EC and pH of each treatment leaching water at 0, 90, 120 and 270 DAT. In the substrates containing hP or C-hP, the EC of the leaching water was initially higher than in control. Over time, it decreased in all substrates and the differences with respect to the control were no longer significant starting from 90 DAT. At the beginning of the experiment, pH of leaching water was similar in all the substrates but 120 days later, the substrate with hP30, C-hP30 and C-hP60 showed a pH higher than the control. At 270 DAT, the differences disappeared and the pH values of the leaching water were similar in all substrates.

Table 3. EC and pH in the leaching water of the substrates at 0, 90, 120 and 270 DAT

	EC (mS cm^{-1})			
	0	90	120	270
Control	0.82 c	0.65 a	0.73 a	0.74 a
hP60	3.81 a	-	-	-
hP30	3.90 a	0.95 a	0.88 a	0.82 a
C-hP60	1.54 b	0.54 a	0.77 a	0.71 a
C-hP30	1.22 b	0.62 a	0.55 a	0.53 a
		pH		
	0	90	120	270
Control	6.1 a	6.2 a	6.6 b	7.1 a
hP60	4.2 a	-	-	-
hP30	4.6 a	4.9 a	7.8 a	7.3 a
C-hP60	5.4 a	5.1 a	7.2 a	8.0 a
C-hP30	5.7 a	5.4 a	7.9 a	7.4 a

Mean values followed by different letters are significantly different ($P < 0.05$).

Plant growth

The mortality rate of plants grown with pomace and compost was low and generally slightly higher than in the control. hP60 substrate was the only exception causing the death of all plants after few days from transplanting (Table 4).

At 120 DAT, the control plants showed an increase in height greater than that of the plants grown in the substrates C-hP30 and C-hP60 and especially hP30. At 270 DAT, the plants grown in C-hP30 and C-hP60 showed a similar height to the Control while the height of plants grown in hP30 was significantly lower (Table 5).

Table 4. Mortality rate of the olive trees

	Mortality, %
Control	1 b
hP60	100 a
hP30	5.6 b
C-hP60	4.8 b
C-hP30	4.9 b

Mean values followed by different letters are significantly different ($P < 0.05$).

Table 5. Plant height at 0, 120 and 270 DAT

	Plant height (cm)		
	0	120	270
Control	10.9 a	58.5 a	118.7 a
hP30	12.5 a	23.8 c	64.4 b
C-hP60	12.1 a	49.6 b	98.5 a
C-hP30	11.4 a	41.2 b	113.9 a

Mean values followed by different letters are significantly different ($P < 0.05$).

The FW and DW of pruning material was higher in Control than in the substrates with hP and C-hP regardless the dose used for C-hP (Table 6).

Table 6. FW and DW of pruning material at 150 DAT

	FW	DW
	g	g
Control	95.5 a	33.6 a
hP30	65.5 b	23.4b
C-hP60	55.9 b	18.5 b
C-hP30	61.2 b	20.6 b

Mean values followed by different letters are significantly different ($P < 0.05$).

Table 7. Dry weight (DW) of different parts of olive plants at 270 DAT

	Roots DW, g	Stem+Shoots DW, g	Leaves DW, g
Control	17.35 a	38.86 a	23.95 a
hP30	13.80 a	21.70 b	19.75 a
C-hP60	8.60 b	15.25 c	21.35 a
C-hP30	13.17 a	21.35 b	22.94 a

Mean values followed by different letters are significantly different ($P < 0.05$).

Table 8. Development of aboveground parts

	Leaves n	Lateral shoots n	Mean length of lateral shoots cm	Stem diameter cm
Control	160.60 a	8.33 a	19.10 a	0.58 a
hP30	131.55 a	5.66 b	15.43 ab	0.58 a
C-hP60	91.66 b	4.00 b	9.59 b	0.50 a
C-hP30	116.00 ab	8.66 a	13.63 ab	0.55 a

Mean values followed by different letters are significantly different ($P < 0.05$).

At 270 DAT, the plants of hP30 showed a lower DW of aboveground parts due to a lower height, number and length of lateral shoots compared to the control plants, while C-hP60 plants had a reduced development of the roots system compared to the control plants due to a shorter root lengh. The plants grown in C-hP60 showed the lowest total DW while the Control plants showed the highest total DW. No significant differences were found regarding leaves DW and stem diameter (Tables 7, 8 and 9).

Leaf net photosynthesis (P_n)

At 30 DAT, Pn was higher in the Control plants while the plants of hP30 showed higher level of stress. Starting from 120 DAT, the differences in Pn rates between the different substrates was not significant (Table 10).

Table 9. Development of roots system

	Principal roots n	Mean length of principal roots cm
Control	4 a	29.19 a
hP30	3 a	20.61 b
C-hP60	4 a	10.72 c
C-hP30	3 a	19.95 b

Mean values followed by different letters are significantly different ($P < 0.05$).

Table 10. Leaf net photosynthesis (Pn) at 0, 30, 120 and 210 DAT

	Pn, µmol $(CO_2)m^{-2}s^{-1}$			
	0	30	120	210
Control	1.06 a	1.83 a	12.00 a	5.12 a
hP30	1.27 a	- 0.80 b	8.64 a	5.71 a
C-hP60	1.28 a	1.12 c	9.78 a	4.27 a
C-hP30	1.34 a	1.17 c	10.64 a	3.96 a

Mean values followed by different letters are significantly different ($P < 0.05$).

DISCUSSION

The results obtained indicate that hP especially if composted can be used for the realization of potting substrates without exceeding the 30% (v/v). In fact , after an initial growth reduction of the plants a satisfactory recovery occurred. Meanwhile, 60% (v/v) of hP in substrate composition is a too high dose since the plants died few days after transplant. The substrates obtained by using hP and composted hP showed different characteristics in terms of chemical properties. The low pH of hP substrates was caused by the slightly acidic pH of this type of feedstock (Roig et al., 2006; Gigliotti et al., 2012; Tortosa et al., 2012), which likely affect the mortality of plants. The increase of pH probably was due to the degradation of organic acids, oxidation of phenolic compounds, and the contemporaneous release of ammonium during the composting (Gigliotti et al., 2012). Although the hP substrates showed the highest EC values, the composting of hP did not result in an increase of soluble salts concentration in the respective substrates. The hP substrates showed the highest WEOC concentration, probably due to the degradation of labile, soluble C during the composting process (Said-Pullicino et al., 2007). The same trend was observed for the water soluble phenols, which are commonly found in high concentration in the olive mill wastewaters, causing phytotoxicity (Roig et al., 2006). It is also true that the total phenolic compounds in hP can decrease after its composting (Gigliotti et al., 2012), demonstrating the effectiveness of the aerobic process in their degradation. Moreover, it was found that soil microorganisms lead to a rapid degradation of WEOC and total phenol compounds after the agronomical reuse of the humid olive mill waste (Federici et al., 2017). Based on these considerations, it can be stated that the high mortality observed for hP60 substrate was probably more attributable to the combined effect of soluble phenol and salt concentration of this particular feedstock. These results are in agreement with Papafotiou et al., 2001 who used a pomace compost (moisture 25% w/w) to replace peat and observed that in *Syngonium podophyllum* L. the height was reduced only when 75% (in volume) of the peat was replaced with compost while in Ficus benjamina L. the height was not influenced at all by the amount of compost. The initial stress also evidenced by the decline of Pn can be related to the high CE and soluble phenol content (Papafotiou et al., 2001; Papafotiou et al., 2004; Barbera et al., 2013; Magdich et al., 2016). Successively, due to irrigations and consequent leaching, the EC decreased greatly and at 90 DAT it was similar to that of the control. In this way, considering the fact that olive has a medium resistance to salinity (Mousavi et al., 2019; Regni et al., 2019), the plants could overcome quite rapidly the initial stress.

In addition, the initial low pH in substrate with hP could have negatively affected the plant growth. Afterwards, the pH led to increase in all pots not showing any significant differences with the control. Whereas in the control, the pH behavior can be explained with the supply of carbonates through frequent irrigations, this phenomenon in the substrates with hP and C-hP, already observed in other similar experimentation (Proietti et al., 1995), probably is also a consequence of ammonia production following microbial degradation of organic matter present in the hP and C-hP.Considering that Pn is the integrated and symptomatic result of several processes, giving therefore important information about plant responses to environmental and agronomical factors (Bongi & Loreto, 1989), it is possible to affirm that the C-hP and, above all, the hP are initially the cause of stress. However, at 30 DAT for C-hP the phytotoxicty decreased and plants were able to recover their activity. The stronger negative effect on Pn and plant growth of hP, compared to the C-hP, is in agreement with results reported by Gigliotti et al., 2012 and Del Buono et al., 2011 who found that the composting greatly reduces the phytotoxicty of pomace through the degradation of phenols, lipids and toxic compounds, mainly contained in the water soluble organic matter fraction.

CONCLUSIONS

The results of the present study provide positive indications on the potential re-use of the composted humid pomace in replacing peat in growth substrate for potted olive trees. The compost, can be used as a co-substrates with peat, allowing strong reduction of its consumption in horticulture. This appears to be an important point since, in the last years, the use of peat has become a serious environmental and economic issue. Furthermore, the use of composted pomace in professional horticulture can contribute to the disposal of the pomace, a waste that will gradually increase over the next years, in an economic and environmentally friendly way. The utilization of not-composted humid pomace, on the contrary, cannot be recommended, unless used in small doses, since it strongly reduces plant activity causing even mortality.

Further studies would be useful to evaluate the possibility to use this type of compost for other plant species and to verify if its combinations at different percentageswith other components, such as agricultural soil, might allow further reduction of peat content in the substrates.

Supplementary materials

Table S1 Climatic conditions of the experimental site

Month	Average temperature (°C)	Rainfall (mm)
January	7.1	103.6
February	9.3	127.4
March	15.9	36.8
April	22.5	91.2
May	28.1	121.4
June	28.6	194.8
July	29.6	42.4
August	30.4	75.0
September	25.4	137.0
October	18.4	133.6
November	15.3	112.6
December	8.6	3.0

REFERENCES

Alexander, P.D., Bragg, N.C., Meade, R., Padelopoulos, G. & Watts, O. 2008. Peat in horticulture and conservation: the UK response to a changing world. *Mires Peat* **3**, Article 08

Alfano, G., Belli, C., Lustrato, G. & Ranalli G. 2008. Pile composting of two-phase centrifuged olive husk residues: technical solutions and quality of cured compost. *Bioresource Technology* **99**, 4694–4701

Ameziane, H., Nounah, A., Khamar, M. & Zouahri, A. 2019. Use of olive pomace as an amendment to improve physicochemical parameters of soil fertility. *Agronomy research* **17**(6), 2158–2171.

Ameziane, H., Nounah, A., Khamar, M. & Zouahri, A. 2020. Composting olive pomace: evolution of organic matter and compost quality. *Agronomy research* **18** in press https://doi.org/10.15159/ar.20.004

Barbera, A.C., Maucieri, C, Cavallaro, V., Ioppolo, A. & Spagna, G. 2013. Effects of spreading olive mill wastewater on soil properties and crops, a review. *Agricultural Water Management* **119**, 43-53. https://doi.org/10.1016/j.agwat.2012.12.009

Bongi, G. & Loreto, F. 1989. Gas-Exchange Properties of Salt-Stressed Olive (*Olea europea* L.) Leaves. *Plant Physiolog* **90**, 1408–1416

Canet, R., Pomares, F., Cabot, B., Chaves, C., Ferrer, E., Ribo, M. & Albiach, M.R. 2008. Composting olive mill pomace and other residues from rural southeastern Spain. *Waste Management* **28**(12), 2585–2592.

Cayuela, M.L., Millner, P.D., Meyer, S.L.F. & Roig, A. 2008. Potential of olive mill waste and compost as biobased pesticides against weeds, fungi, and nematodes. *Science of Total Environment* **399**, 11–18.

Cayuela, M.L., Sánchez-Monedero, M.A. & Roig, A. 2010. Two-phase olive mill waste composting: enhancement of the composting rate and compost quality by grape stalks addition. *Biodegradation* **21**, 465–473. https://doi.org/10.1007/s10532-009-9316-5

Del Buono, D., Said-Pullicino, D., Proietti, P., Nasini, L. & Gigliotti, G. 2011. Utilization of olive husks as plant growing substrates: phytotoxicity and plant biochemical responses. *Compost Science and Utilization* **19**, 52–60.

Di Serio, M.G., Lanza, B., Mucciarella, M.R., Russi, F., Iannucci, E., Marfisi, P. & Madeo, A. 2008. Effects of olive mill wastewater spreading on the physico-chemical and microbiological characteristics of soil. *International biodeterioration & Biodegradation* **62**(4), 403–407.

Federici, E., Massaccesi, L., Pezzolla, D., Fidati, L., Montalbani, E., Proietti, P., Nasini, L., Regni, L. & Gigliotti, G. 2017. Short-term modifications of soil microbial community structure and soluble organic matter chemical composition following amendment with different solid olive mill waste and their derived composts. *Applied Soil Ecology* **119**, 234–241.

Gigliotti, G., Proietti, P., Said-Pullicino, D., Nasini, L., Pezzolla, D., Rosati, L. & Porceddu, P.R. 2012. Co-composting of olive husks with high moisture contents: Organic matter dynamics and compost quality. *International biodeterioration & Biodegradation* **67**, 8–14. https://doi.org/10.1016/j.ibiod.2011.11.009

Gigliotti, G., Kaiser, K., Guggenberger, G. & Haumaier, L. 2002. Differences in the chemical composition of dissolved organic matter from waste materials of different sources. *Biology and Fertility of Soils* **36**, 321–329.

Innangi, M., Niro, E., D'Ascoli, R., Danise, T., Proietti, P., Nasini, L., Regni, L., Castaldi, S. & Fioretto, A. 2017. Effects of olive pomace amendment on soil enzyme activities. *Applied Soil Ecology* **119**, 242–249. https://doi.org/10.1016/j.apsoil.2017.06.015

Kotsou, M., Mari, I., Lasaridi, K., Chatzipavlidis, I., Balis, C. & Kyriacou, A. 2004. The effect of olive oil mill wastewater (OMW) on soil microbial communities and suppressiveness against *Rhizoctonia solani*. *Applied Soil Ecology* **26**, 113–121.

Lopez-Pineiro, A., Murillo, S., Barreto, C., Munoz, A., Rato, J.M., Albarran, A. & Garcia, A. 2007. Changes in organic matter and residual effect of amendment with two-phase olive-mill waste on degraded agricultural soils. *Science of Total Environment* **378**(1–2), 84–89.

Magdich, S., Abid, W., Boukhris, M., Ben Rouina, B. & Ammar, E. 2016. Effects of long-term olive mill wastewater spreading on the physiological and biochemical responses of adult Chemlali olive trees (*Olea europaea* L.). *Ecological Engineering* **9**7, 122–129. https://doi.org/10.1016/j.ecoleng.2016.09.004

Mekki, A., Dhouib, A. & Sayadi, S. 2006. Changes in microbial and soil properties following amendment with treated and untreated olive mill wastewater. *Microbiological Research* **161**(2), 93–101.

Morillo, J.A., Antizar-Ladislao, B., Monteoliva-Sánchez, M., Ramos-Cormenzana, A. & Russell, N.J. 2009. Bioremediation and biovalorisation of olive-mill wastes. *Applied Microbiology and Biotechnology* **82**, 25–39. doi:10.1007/s00253-008-1801-y

Mousavi, S., Regni, L., Bocchini, M., Mariotti, R., Cultrera, N.G.M., Mancuso, S., Googlani, J., Chakerolhosseini, M.R., Guerrero, C., Albertini, E., Baldoni, L. & Proietti, P. 2019. Physiological, epigenetic and genetic regulation in some olive cultivars under salt stress. *Scientific Reports* **9**, 1093. doi: 10.1038/s41598-018-37496-5

Nasini, L., De Luca, G., Ricci, A., Ortolani, F., Caselli, A., Massaccesi, L., Regni, L., Gigliotti, G. & Proietti, P. 2016. Gas emissions during olive mill waste composting under static pile conditions. *International Biodeterioration* & *Biodegradation* **107**, 70–76 https://doi.org/10.1016/j.ibiod.2015.11.001

Nasini, L., Gigliotti, G., Balduccini, M.A., Federici, E., Cenci, G. & Proietti, P. 2013. Effect of solid olive-mill waste amendment on soil fertility and olive (*Olea europaea* L.) tree activity. *Agriculture Ecosystem and Environment* **164**, 292–297.

Niaounakis, M. & Halvadakis, C.P. 2006. Olive Processing Waste Management Literature Review and Patent Survey, second ed. *Elsevier Ltd*., Kidlington, Oxford, pp. 65–73.

Papafotiou, M., Chronopoulos, J., Kargas, G., Voreakou, M., Leodaritis, N., Lagogiani, O. & Gazi, S. 2001. Cotton gin trash compost and rice hulls as growing medium components for ornamentals. *The Journal of Horticultural Science and Biotechnol*ogy **76**(4), 431–435.

Papafotiou, M., Phsyhalou, M., Kargas, G., Chatzipavlidis, I. & Chronopoulos, J. 2004. Olive-mill wastes compost as growing medium component for the production of poinsettia. *Scientia Horticulturae* **102**(2), 167–175. https://doi.org/10.1016/j.scienta.2003.11.016

Papafotiou, M., Kargas, G. & Lytra, I. 2005. Olive-mill waste compost as a growth medium component for foliage potted plants. *Horticultural Science* **40**, 1746–1750.

Peri, C. & Proietti, P. 2014. Olive mill waste and by-products. In: The Extra-Virgin Olive Oil Handbook; Peri, C. Ed.; John Wiley & Sons: Oxford, UK, pp. 283–302.

Pinamonti, F. & Centemero, M. 1997. Compost nella preparazione di terricci e substrati colturali. L'Informatore Agrario **44**, pp. 51–55.

Proietti, P., Federici, E., Fidati, L., Scargetta, S., Massaccesi, L., Nasini, L., Regni, L., Ricci, A., Cenci, G. & Gigliotti, G. 2015. Effects of amendment with oil mill waste and its derived-compost on soil chemical and microbiological characteristics and olive (*Olea europaea* L.) productivity. *Agriculture Ecosystem and Environment* **207**, 51–60.

Proietti, P., Palliotti, A., Tombesi, A. & Cenci, G. 1995. Chemical and microbiological modifications of two different cultivated soils induced by olive oil waste water administration. *Agricultura mediterranea* **125**, 160–171.

Regni, L., Del Pino, A.M., Mousavi, S., Palmerini, C.A., Baldoni, L., Mariotti, R., Mairech, H., Gardi, T., D'Amato, R. & Proietti, P. 2019. Behavior of Four Olive Cultivars During Salt Stress. *Frontiers in Plant Science* **10**, 867. doi:10.3389/fpls.2019.00867

Regni, L., Gigliotti, G., Nasini, L. & Proietti, P. 2016. Reuse of olive mill waste as soil amendment. In: Olive Mill Waste: Recent Advances for Sustainable Management; C. M. Galanakis Ed.; Elsevier-Academic Press: Oxford, UK, pp. 97–117.

Regni, L., Nasini L., Ilarioni L., Brunori A., Massaccesi L., Agnelli, A. & Proietti, P. 2017. Long Term Amendment with Fresh and Composted Solid Olive Mill Waste on Olive Grove Affects Carbon Sequestration by Prunings, Fruits, and Soil. *Frontiers in Plant Science* **7**, 2042.

Rodrìguez-Lucena, P., Hernandez, D., Hernandez-Apaolaza, L. & Lucena, J.J. 2010. Revalorization of a two-phase olive mill waste extract into a micronutrient fertilizer. *Journal of Agricultural and Food Chemistry* **58**, 1085–1092.

Roig, A., Cayuela, M.L. & Sanchez-Monedero, M.A. 2006. An overview on olive mill wastes and their valorization methods. *Waste Management* **26**(9), 960–969.

Said-Pullicino, D., Erriquens, F.G. &Gigliotti, G. 2007. Changes in the chemical characteristics of water extractable organic matter during composting and their influence on compost stability and maturity. *Bioresource Technology* **98**, 1822–1831.

Tortosa, G., Alburquerque, J.A., Ait-Baddi, G. & Cegarra, J. 2012. The production of commercial organic amendments and fertilisers by composting of two-phase olive mill waste (“alperujo”). *Journal of Cleaner Production* **26**, 48–55.

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Evaluation of feed conversion efficiency for different dairy cows breeds by milk yield, milk content and faecal amount

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Abstract. The objective of this study was to evaluation of feed conversion efficiency for Latvian Brown (LB) and Holstein Black and White (HM) dairy cows breeds to define optimal crude protein (CP) content in the feed. In the research study were completed three (A, B, C) dairy cows group (8 cows in each group) with LB and HM breed's cows in the early lactation period, from 10 till 30 lactation days. Each groups cows were feeder with total mixed ration (TMR) with different CP content (approx. 18.0%, 17.5%, 17.0% accordingly). In the research period were controlled the amount of feed fed and regularly collected feed samples. After 21 days feeding was controlled milk yield, collected milk samples for content testing, and faecal amount and samples. Milk samples were analysed for fat, total protein (%) and urea content (mg dL^{-1}). Milk samples for content parameters were analysed in an accredited milk quality laboratory. The statistical analyses were performed with the SPSS program package. The results acquired show that in all studied parameters were not significant differences between study groups. To evaluate the feed conversion efficiency during the study, we used the energy corrected milk (ECM) and the feed dry matter content during research and calculated the coefficient for each cow individually and on average in the study group. Milk yield, protein and faecal amount were significantly different among breeds. Milk urea content was average 28.5 mg dL^{-1} for all LB breed cows in all groups, for HM breeds it was 23.6 mg dL^{-1}. These results show that LB breed cows did not converse feed proteins wholesome. Total milk and faecal amount were decreased in a group with CP 17% in feed by 10% and 7% accordingly. By using this data, the farmer may make evaluations and forecast of farming efficiency; cows breed preference and environmental threats.

Key words: milk yield, milk urea, faecal, protein in feed.

INTRODUCTION

The volatility of the world dairy market and climate change are encouraging farmers to seek the most efficient use of available resources to reduce the environmental impact of their production process and thus reduce production costs. Therefore, it is important to evaluate the composition of the feed ration and to monitor its use.

Increasing the conversion efficiency of cattle feed is that less nutrients are excreted in the manure, so feed conversion efficiency affects both economic and environmental efficiency. One of parameter that recommended using for conversion efficiency control is milk urea content (Zhai et al., 2006; Gruber & Poetsch, 2012; Ruska et al., 2017). For an accurate assessment of feed conversion efficiency, is recommended to use Energy

Corrected Milk (ECM) to calculate productivity, which will allow comparisons to be made between cows, groups, or farms with different technologies and breeds. One of the recommended calculations of feed conversion efficiency is the ratio of ECM to dry matter intake, depending on lactation and day of lactation (Hutjens, 2005). According to the US National Science Council (NRC), farmers exceed on average 6.6% nitrogen in their diet, resulting in 16% increase nitrogen content in their urine and 2.7% increase nitrogen in their manure (Jonker et al., 2002). By feeding a balanced diet to dairy cows after calving, when the amount of feed ingested can also affect the animal's health, we can ensure control over productivity, health and costs.

One of the factors that stay influence on feed conversion efficiency coefficient is cow genotype. Results of previous studies confirm that Jersey and Holstein–Friesan crossbreed cows produce more milk from lower feed inputs (Coffey et al., 2017). While other study results with Holstein and Swedish–Red and Jersy or Holstesin crossbred dairy cows does not find interaction for any milk production parameters by milk solid yield (Ferris et al., 2018).

Research in the Netherlands at the end of the last century has shown that keeping dairy cows and thus milk production account for the greatest amount of nitrogen pollution in the surrounding environment. Calculations predicted an average nitrogen release from total nitrogen intake in 29% faecal, 50% in urine, 19% in milk and 2% in dairy cow to maintain body condition (Tamminga, 1992). Later, the results of the studies performed corrected this distribution for the following average nitrogen excretion in faecal 37%, urine 35%, milk 27% and 0% for body maintenance. The distribution of this may be affected by the influence of various factors that require in-depth study (Straalen, 1995).

The objective of this study was to evaluate difference of feed conversion rate between Latvian Brown (LB) and Holstein Black and White (HM) dairy cows breeds.

MATERIALS AND METHODS

The study was conducted from begin of May till the end of July 2019 at the research and study farm Vecauce of the Latvia University of Life sciences and Technologies (LLU MPS Vecauce). Twenty four dairy cows were completed in three groups within Latvian Brown and Holsten Black and white breeds in each group were presented. Cows were in early lactation phase from 10 to 30 lactation day, with second and third lactation. The cows were housed in a 3×3 Latin square design experiment, three diets over three periods each lasting 21 days. In this paper were analysed data from first phase of experiment (accordingly lactation phase from 10 to 40 days).

The cows fed with in farm used prepared total mixed rations (TMR) which differ by crude protein content in diet (A, B, C groups 18.0%; 17.5%; 17.0% accordingly). Feed compositions were completed within farm used maize silage, grain, soya seed, rapeseed cakes and mineral additives. All cows were fed *ad libitum*. Refused feed were collected and weighted every day separately for each cow. Water intake was recorded for each individual cow every day. To control the composition of the feed ration, TMR samples for testing are taken from the feed table every second or third day (n = 24 samples). In the Table 1 presented chemical composition of TMR is the average of the compound feed and not of each ingredient individually. Feed composition were analysed in accredited laboratory of LLU for dry matter (%), fat (%), protein (%), fibre content (%) etc.

Table 1. Chemical composition of feed compounds during the study

Traits	A group (n = 8)	B group (n = 8)	C group (n = 8)
Dry matter, %, included:	45.62	40.57	38.29
Crude protein in dry matter, %	18.02	17.89	16.99
Ash in dry matter, %	6.88	7.14	8.40
Crude fibre in dry matter, %	13.46	15.27	19.17
NDF, %	28.93	32.15	36.62
ADF,%	16.78	19.46	23.40
Fats in dry matter, %	3.30	3.20	3.17
Digestibility of organic substances, %	78.12	76.49	71.38

The crude protein content of the feed ranged from 16.99% to 18.02%, depending on the group to be fed. It meets the needs of dairy cows depending on milk yield. The crude fibre ranged from 13.46% to 19.17% and met the needs of high-yielding cows (NRC, 2001). The NDF content in all TMR groups meets the needs of dairy cows in begin of lactation.

Milk yield (kg) recording and sampling were on first, seven, eleven, fifteenth and twenty first days (n = 63 samples), separate for each milking time. Milk composition was analysed in accredited laboratory for milk quality control for content of fat (%), crude protein (%), urea (mg dL^{-1}) and somatic cell count for quality characterising.

Total faecal amount after 21 days were collected over 72 hours from each cow separately (n = 24 samples). Daily feces was collected, weighed, mixed thoroughly, and subsampled for each cows. Faecal sample composition were analysed in accredited laboratory of LLU for dry matter (%), nitrogen (N, %), phosphor (P, %), potassium (K, %) content.

For data processing, the feed and faecal dry matter and crude protein content, as measured as a percentage of dry matter in the laboratory were recalculated to amount (kg) according to ICAR guidelines (ICAR, 2017). Also content of dry matter and nitrogen in faecal was recalculated in compliance with formula (1).

$$Amount, kg = (yield, kg \times \% \, of \ content)/100 \qquad (1)$$

With an aim to compare and evaluate study results between groups and estimate feed conversion rate, milk yield and content were transformed in ECM (ICAR, 2017) by following formula:

$$ECM = (fat \ yield, kg \times 38.3 + protein \ yeild, kg \times 24.2 + \\ + milk \ yield, kg \times 0.7832)/3.14 \qquad (2)$$

Statistical processing of the data was carried out with *MS for SPSS* (SPSS Inc. Chicago, Illinois, USA) and *MS Office* programme *Excel*. Images were created with *MS Office* programme *Excel*.

RESULTS AND DISCUSSION

The choice of cow breed is important in terms of farming model, feeding and productivity. Dairy cows breed may have significant influence on milk productivity and quality traits, therefore feed and water intake by cows. The research groups consisted of two breeds of cows and evaluated the influence of the breed on productivity and feed consumption parameters. The results of the study show significant differences in productivity, feed utilization and faecal output in all study groups between breeds. Average milk productivity traits per cow in the control day in study are present in the Table 2.

Table 2. Average cow milk productivity traits by breed in experiment first phase

Traits	Study groups					
	A		B		C	
	Breeds					
	LB (n = 6)	HM (n = 15)	LB (n = 6)	HM (n = 15)	LB (n = 6)	HM (n = 15)
Milk yield, kg	28.9 ± 2.52[a]	47.5 ± 1.92[b]	28.2 ± 2.73[a]	46.8 ± 1.94[b]	26.1 ± 2.27[a]	47.8 ± 2.74[b]
Fat content, %	4.00 ± 0.015[a]	3.14 ± 0.181[b]	3.94 ± 0.104[a]	3.18 ± 0.338[b]	4.14 ± 0.149[a]	2.49 ± 0.309 [b]
Crude protein content, %	3.50 ± 0.162[a]	2.91 ± 0.086[b]	3.28 ± 0.95[a]	2.64 ± 0.287[b]	3.18 ± 0.080[a]	1.85 ± 0.304[b]
Urea content, mg dL^{-1}	28.3 ± 3.55 [a]	26.3 ± 1.14 [a]	28.7 ± 2.04 [a]	21.5 ± 3.14 [a]	28.6 ± 3.42[a]	22.9 ± 4.88[a]
ECM, kg	29.1 ± 2.54[a]	40.6 ± 1.81[b]	27.6 ± 2.47[a]	39.3 ± 2.81[b]	26.2 ± 2.62[a]	33.2 ± 2.98[b]

[a; b] – productivity indicators with unequal letter differed significantly among the breeds in separate group ($p < 0.05$).

The milk yield on the control day differs significantly between breeds in all study groups but does not differ between groups within the breed.

The LB breed yield ranged from 26.1 kg in C group to 28.9 kg in A group. The HM yield ranged from 46.8 kg in group B to 47.8 kg in group C. The fat and crude protein content of milk differed significantly between breeds, but there was not significant difference between the groups within one breed. The fat content of LB breed milk were ranged from 3.94% in group B to 4.14% in group C. The HM breed fat ranged from 2.94% in group C to 3.18% in group B. The crude protein content of LB breed milk ranged from 3.18% in group C to 3.50% in group A. The HM crude protein content was significantly lower and ranged from 1.85% in group C to 2.91% in group A. Other scientists have also conducted studies to compare the milk composition of Red and White and black cows. It was found that Swiss brown cows had significantly higher crude protein content in milk compared to Holstein cows (DeMarchi et al., 2008). Estonian red cow's milk has been found to have a higher content of crude protein than Estonian Holstein cows (Joudu et al., 2008).

The mean urea content in milk during the study was within optimal limits for all breeds, 21.5 mg dL^{-1} to 28.7 mg dL^{-1}. In Europe, the optimal urea content in milk is considered to be 15 mg dL^{-1} to 30 mg dL^{-1} (Bijgaart, 2003). The milk urea contents are not significantly different between breeds and groups. Previous studies with Holstein, Jersey and Switzerland Brown cows show a significant difference in crude protein and urea content in milk depending on the breed of dairy cow (Carroll et al., 2006).

Average feed and water intake differs significantly between breeds in all study groups, but not between groups (Tabel 3).

Table 3. Average feed traits and water intake per day by breed in experiment first phase

Traits	Study groups					
	A		B		C	
	Breeds					
	LB (n = 72)	HM (n = 120)	LB (n = 72)	HM (n = 120)	LB (n = 72)	HM (n = 120)
Feed intake, kg	35.5 ± 1.56ᵃ	38.8 ± 2.90ᵇ	34.7 ± 1.03ᵃ	42.6 ± 3.69ᵇ	37.6 ± 0.82ᵃ	47.7 ± 1.26ᵇ
Dry matter intake, kg	16.2 ± 0.71ᵃ	17.7 ± 1.32ᵇ	14.1 ± 0.42ᵃ	17.3 ± 1.49ᵇ	14.4 ± 0.31ᵃ	18.3 ± 0.49ᵇ
Crude protein intake, kg	2.92 ± 0.130ᵃ	3.19 ± 0.239ᵇ	2.52 ± 0.078ᵃ	3.09 ± 0.268ᵇ	2.44 ± 0.053ᵃ	3.10 ± 0.082ᵇ
Water intake, L	80.2 ± 6.20ᵃ	113.2 ± 3.79ᵇ	76.9 ± 3.15ᵃ	127.7 ± 15.54ᵇ	74.7 ± 2.30ᵃ	110.1 ± 9.89ᵇ

[a; b] – traits with unequal letter differed significantly among the breeds in separate group ($p < 0.05$).

Feed intake for LB breed cows were ranged from 34.7 kg in group B to 37.6 kg in group C. The HM breed feed intake were from 38.8 kg in group A to 47.7 kg in group C. Feed dry matter intake were significantly high for the HM breed cows (17.3 kg to 18.3 kg), but does not achieve nutritional requirement for milk yield of experiment cows. Dry matter intakes are affected by many factors: physiological, diet, environmental. Is it possible predict dry matter intake by dairy cows lactation, lactation day, milk yield etc. Daily dry matter intake could be less on begin of lactation than in the end by 5 kg per day (Weiss, 2015). Dry matter intake for LB breed cows were from 14.1 kg to 16.2 kg which provide nutritional requirement for their milk yield. Feed crude protein intake were ranged from 2.44 kg to 2.92 kg for LB breed cows and was significantly lower than for HM breed cows were ranged from 3.09 kg to 3.19 kg. Water intake is depending on dry matter intake. Is it possible to predict water intake by estimation. Our study results conform previous studies where average dry matter intake 18.3 kg per day require 75.2 kg water per day for lactating cows (Appuhamy et al., 2016). There we find significant differences between LB and HM breeds cows water intake.

Found out differences between breeds in feed intake and milk productivity consequence related to faecal output (Tabel 4). Faecal amount was significantly lower for LB breed cows (ranged from 29.7 kg to 27.6 kg), but there was not differences among study groups. The HM breeds cow faecal amount was higher and ranged from 39.5 kg in group A to 49.9 kg in group C.

Table 4. Average faecal traits output per day by breed in experiment first phase

Traits	Study groups					
	A		B		C	
	Breeds					
	LB (n = 6)	HM (n = 15)	LB (n = 6)	HM (n = 15)	LB (n = 6)	HM (n = 15)
Faecal, kg	29.7 ± 1.17ᵃ	39.5 ± 1.56ᵇ	30.1 ± 1.07ᵃ	45.0 ± 6.99ᵇ	27.6 ± 1.89ᵃ	49.9 ± 4.19ᵇ
Dry matter, kg	3.85 ± 0.058ᵃ	5.36 ± 0.203ᵇ	3.64 ± 0.176ᵃ	5.93 ± 0.856ᵇ	3.41 ± 0.355ᵃ	6.02 ± 0.497ᵇ
Nitrogen, kg	0.13 ± 0.004ᵃ	0.17 ± 0.004ᵇ	0.11 ± 0.006ᵃ	0.19 ± 0.030ᵇ	0.10 ± 0.010ᵃ	0.18 ± 0.020ᵇ

[a; b] – traits with unequal letter differed significantly among the breeds in separate group ($p < 0.05$).

Feed conversion efficiency is estimation of how much produced for each unit of feed consumed. For evaluation of feed conversion efficiency of dairy cows usually calculate the ratio of milk yield to feed dry matter intake. Feed efficiency is useful tool to control feed costs. Therefor breeding organisation start to use it for selection works, to control potential for lower maintenance requirements of the cow herd, reduce overall feed intake, improve feed conversion ratio, reduce methane emissions and reduce manure nitrogen, phosphorus and potassium production (Berry & Crowley, 2013). To estimate feed conversion efficiency lactating animals is more complicated than for growing animals. Lactating cows under lactation curve characterized by rapid catabolism of body reserves after calving than anabolism of body reserves until next calving (Roche et al., 2009).

To evaluate the conversion efficiency of the feed during the study, we used the ECM and the feed dry matter content and calculated the coefficient for breeds and on average in the study group (Fig. 1).

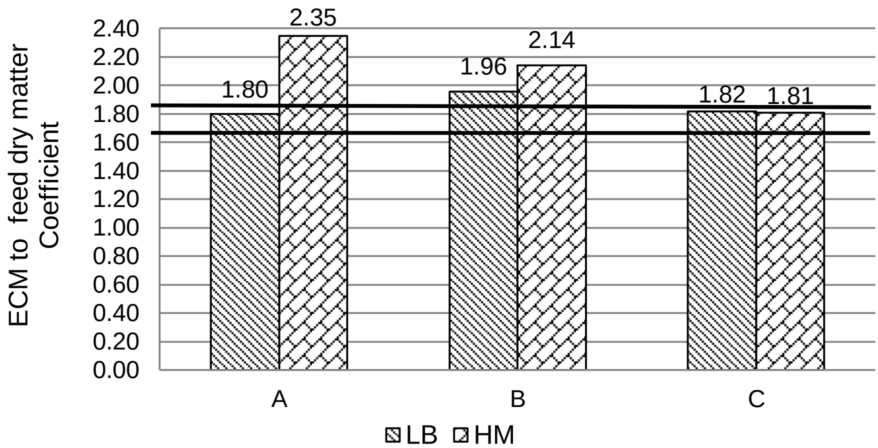

Figure 1. Feed conversion rate by dairy cows breeds in study groups.

The calculated coefficients show deficit of the dry matter in the feed ration, which was reflected in the reduction in live weight of individual cows as internal body reserves were used to provide milk yield. The average coefficients for LB breed cows were similar in all groups and did not differ significantly between groups and breeds. The average coefficients of groups A and B for HM breed cows were above the recommended level, and the coefficient of group C was within the recommended range corresponding to level 1.6–1.8 of second lactation, the initial lactation phase (Hutjens, 2005; Arndt et al., 2015). In the study feed conversion efficiency for group C cows for both breeds was most effective.

On farms, it is appropriate to apply the feed conversion efficiency coefficient at the herd or group level rather than for individual animals. The calculation of the coefficient requires weekly data on the dry matter content of the feed, the amount of uneaten feed and the milk productivity traits (ECM). The farm needs to record feed quality and consumption in order to be able to make the necessary changes to feed rationing in a timely manner.

Limited data are available on the comparative feed conversion efficiency of lactating LB and HM cows. In study where compare Friesian (F) and Jersey (J) breed cows were established that F cows was consumed more feed dry matter per day than J. Estimate efficiency parameter in this study (amount of milk produced per kg of dry matter consumed) was not find significant differences between breeds (Mackle et al., 1996).

Feed conversion efficiency possible to evaluate by crude protein utilization. In our study estimated crude protein amount in milk to crude protein intake (Table 5). Crude protein conversion coefficient of crude protein were ranged from 0.28 to 0.45 for HM breeds cows and form 0.34 to 0.36 for LB breed cows difference was significant between breeds. Study results are related with previous researcher estimation they predict 27% of total intake nitrogen extraction in milk and highly variable of this estimation 10% to 40% (Straalen, 1995; Calsamiglia et al., 2010).

Table 5. Feed crude protein conversion efficiency by dairy cows breeds in study groups

Breeds	A	B	C
LB	0.35 ± 0.024[a]	0.36 ± 0.028[a]	0.34 ± 0.020[a]
HM	0.45 ± 0.043[b]	0.39 ± 0.039[b]	0.28 ± 0.037[b]

[a; b] – traits with unequal letter differed significantly among the breeds in separate group ($p < 0.05$).

To evaluate feed conversion efficiency is it possible to analyse other productivity and utilisation parameters (Arndt et al., 2015).

CONCLUSIONS

Milk productivity traits and feed intake were different between LB and HM dairy cows. Milk yield, crude protein content, feed and water intake and faecal amount significantly differ between cows breeds. The conversion efficiency of the feed during the study was optimal for LB breeds cows in all study groups. For HM breeds cows this rate was optimal in C group, in A and B group coefficient was higher than recommended value.

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REFERENCES

Appuhamy, J.A.D.R.N., Judy, J.V., Kebreab, E. & Kononoff, P. J. 2016. Prediction of drinking water intake by dairy cows. *Journal of Dairy Science* **99**, 7191–7205. doi.org/10.3168/jds.2016-10950

Arndt, C., Powell, J.M., Aguerre, M.J., Crump, P.M. & Wattiaux, M.A. 2015. Feed conversion efficiency in dairy cows: Repeatability, variation in digestion and metabolism of energy and nitrogen, and ruminal methanogens. *Journal of Dairy Science* **98**, 3938–3950. doi.org/10.3168/jds.2014-8449

Berry, D. P. & Crowley, J.J. 2013. Genetics of feed effi ciency in dairy and beef cattle. *Journal of Animal Science* **91**, 1594–1613. doi:10.2527/jas2012-5862

Bijgaart, H. van den. 2003. Urea. New applications of mid–infra–red spectrometry. *Bulletin of the IDF*, No. 383, pp. 5–15.

Calsamiglia, S., Ferret, A., Reynolds, C.K., Kristensen, N.B. & van Vuuren, A.M. 2010. Strategies for optimizing nitrogen use by ruminants. *Animal* **4**, 1184–1196. doi:10.1017/S1751731110000911

Carrol, S.M., DePeters, E.J., Taylor, S.J., Rosenberg, M., Perez-Monti, H. & Capps, V.A. 2006. Milk composition of Holstein, Jersey and Brown Swiss cows in response to increasing levels of dietary fat. *Animal Feed Science and Tehnology*, **131**, 451–473.

Coffey, E.L., Delaby, L., Fitzgerald, S., Galvin, N., Pierce, K.M. & Horan, B. 2017. Effect of stocking rate and animal genotype on dry matter intake, milk production, body weight, and body condition score in spring-calving, grass-fed dairy cows. *Journal of Dairy Science* **100**, 7556–7568. doi.org/10.3168/jds.2017-12672

DeMarchi, M., Bittante, G., DalZotto, R., Dalvit, C., Cassandro, M. 2008. Effect of Holstein Friesian and Brown swiss breeds on quality of milk and cheese. *Journal of Dairy Science* **91**, 4092–4102.

Ferris, C.P., Purcell, P.J., Gordon, A.W., Larsen, T. & Vestergaard, M. 2018 Performance of Holstein and Swedish-Red × Jersey/Holstein crossbred dairy cows within low- and medium-concentrate grassland-based systems. *Journal of Dairy Science* **101**, 7258–7273. doi.org/10.3168/jds.2017-14107

Gruber, L. & Poetsch, E.M. 2012. Modelling the nitrogen exretion of dairy cows. Agricultural research and Education Ventre, Raumberg – Gumpenstein, 8952 Irdning, Austria http://www.seepastos.es/docs%20auxiliares/Actas%20Reuniones%20escaneadas/Proceedings/sessions/Session%204/4.697.pdf Accessed 29.2.2012.

Guidelines for Dairy Cattle Milk Recording. 2017. ICAR International Committee for Animal Recording. https://www.icar.org/Guidelines/02-Overview-Cattle-Milk-Recording.pdf Accessed 20.12.2019.

Hutjens, M.F. 2005. Dairy Efficiency and Dry Matter Intake. *Proceedings of the 7th Western Dairy Management Conferenc*, pp. 71–76.

Jonker, J.S., Kohn, R.A.& High, J. 2002 Dairy herd management practices that impact nitrogen utilization efficiency. *Journal of Dairy Science* **85**(5), 1218–1226.

Joudu, I., Henno, M., Kaart, T., Pussa, T. & Kart, O. 2008. The effect of milk protein contents on the rennet coagulation properties of milk from individual dairy cows. *International Dairy Journal* **18**, 964–967.

Mackle, T.R., Parr, C.R., Stakelum, G.K., Bryant, A.M. & MacMillan, K.L. 1996. Feed conversion efficiency, daily pasture intake, and milk production of primiparous Friesian and Jersey cows calved at two different live weights. *New Zealand Journal of Agricultural Research* **39**,3, 357–370. doi: 10.1080/00288233.1996.9513195

National Research Council NRC. 2001. *Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, 2001*. Washington, DC: The National Academies Press. doi.org/10.17226/9825

Roche, J.R., Friggens, N.C., Kay, J.K., Fisher, M.W., Stafford, K.J. & Berry, D.P. 2009. Body condition score and its association with dairy cow productivity, health and welfare. *Journal of Dairy Science* **92**, 5769–5801. doi:10.3168/jds.2009-2431

Ruska, D., Jonkus, D. & Cielava, L. 2017. Monitoring of ammonium pollution from dairy cows farm according of urea content in milk. *Agronomy Research* **15**(2), 553–564.

Straalen, W.M. 1995. *Modelling of nitrogen flow and excretion in dairy cows*. Thesis. Landbouw Universiteit Wageningen. ISBN 90-5485-475-8

Tamminga, S. 1992. Nutrition management of dairy cows as a contribution to pollution control. *Journal of Dairy Science* **75**, 345–357.

Weiss, B. 2015. Optimizing and Evaluating Dry Matter Intake of Dairy Cows. *WCDS Advances in Dairy Technology* **27**, 189–200.

Zhai, S.W., Liu, J.X., Wu, Y.M., Ye, J.A. & XU, Y.N. 2006. Responses of milk urea nitrogen content to dietary crude protein level and degradability in Lactating Holstein dairy cows. *Czech Journal Animal Science* **51**(12), 518–522.

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https://doi.org/10.15159/AR.20.100

Coffee crop coefficient prediction as a function of biophysical variables identified from RGB UAS images

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Abstract. Because of different Brazilian climatic conditions and the different plant conditions, such as the stage of development and even the variety, wide variation may exist in the crop coefficients (Kc) values, both spatially and temporally. Thus, the objective of this study was to develop a methodology to determine the short-term Kc using biophysical parameters of coffee plants detected images obtained by an Unmanned Aircraft System (UAS). The study was conducted in Travessia variety coffee plantation. A UAS equipped with a digital camera was used. The images were collected in the field and were processed in Agisoft PhotoScan software. The data extracted from the images were used to calculate the biophysical parameters: leaf area index (LAI), leaf area (LA) and Kc. GeoDA software was used for mapping and spatial analysis. The pseudo-significance test was applied with $p < 0.05$ to validate the statistic. Moran's index (I) for June was 0.228 and for May was 0.286. Estimates of Kc values in June varied between 0.963 and 1.005. In May, the Kc values were 1.05 for 32 blocks. With this study, a methodology was developed that enables the estimation of Kc using remotely generated biophysical crop data.

Key words: *Coffea arabica* L., drone, irrigation, leaf area, uav (unmanned aerial vehicle).

INTRODUCTION

Brazil produces a significant share of coffee beans, contributing 63.4% of the world production, with 10.5 million tons in the 2018/2019 crop (USDA, 2019). It is the largest Arabica coffee producer, accounting for 46.9% of the world Arabica coffee production, and is the second-largest Robusta coffee producer, contributing 16.5% of its world production (USDA, 2019).

Some improvements in coffee management and production systems, such as irrigation, have led to the development of the crop, enabling the crop to be introduced in areas that were not previously suitable for coffee production. In addition, irrigation has provided an increase in productivity (Bonomo et al., 2008). Even in traditional areas for coffee crop production, irrigation can help reduce the effects of prolonged droughts during critical periods of water requirement by coffee (Silva et al., 2005; Lima et al., 2010; Vicente et al., 2015).

For a better quantification of the amount of irrigation to be applied, the coffee water intake is estimated mainly by the use of climatological variables, with the crop coefficient (Kc) being determined by the relationship between crop evapotranspiration (ETc) that is evaluated experimentally; by reference evapotranspiration (ETo), obtained by lysimeters or by the use of estimation models (Doorenbos & Kassan, 1979); or still, by adapting the soil water balance (Camargo & Pereira, 1994).

In addition to being an indicator of great physical and biological significance, the Kc reflects the local climate and crop characteristics (Doorenbos & Pruitt, 1997), and it depends on the architecture, plant cover, and plant transpiration (Sato, 2007; Oliveira et al., 2007).

However, due to the different Brazilian climatic conditions, variation in the Kc values exist in the literature. Notably, the adoption of a single Kc value may lead, according to Silva et al. (2011), depending on the time of year, to over- or underirrigation, both of which are detrimental to the crop. In addition, according to Oliveira et al. (2007), several varieties of coffee, planting systems, and sizes exist; therefore, a single value of Kc should not be established, and agronomic experiments are needed. Thus, Kc values should be determined for the different phases of the coffee phenological cycle, plant age, local climatic conditions, and crop management adopted (Oliveira et al., 2007). However, agronomic experiments are time-consuming and laborious.

The development of Unmanned Aircraft Systems (UAS) in the last decade has enabled the acquisition of remote sensing images with a high spatial and temporal resolution, as well as providing information with vegetation details and an observation angle different from field observations. With this type of image, general mosaics are possible, as well as Digital Elevation Models, Digital Terrain Models, and 3D Models of the crop. Thus, the acquisition of plant biophysical data and information through remote images from UAS could replace field sampling and agronomic experiments.

Thus, it is believed that it is possible to determine the Kc of a coffee crop from high-spatial-resolution images obtained by UAS. Therefore, the present study aimed to develop a methodology for estimating the crop coefficient (Kc) in the short term by using relationships between Kc and biophysical parameters of coffee plants (leaf area, plant density, and weed management); this was achieved by using data already available in the literature and comparing it to Kc determined from RGB (Red, Green, Blue) UAS image data and from field data.

MATERIALS AND METHODS

The study was conducted in June 2017 and May 2018 in a 0.4 ha Travessia cultivar coffee plantation, which was a remnant of an earlier experiment, whose treatments are described by Caldas et al. (2018). This plantation is located in the city of Lavras, Minas Gerais, Brazil (21°13'33"S, 44°58'17"W, altitude 936 m) (Fig. 1). The plantation was established in February 2009 with 2.60×0.60 m spacing and is irrigated by drip irrigation.

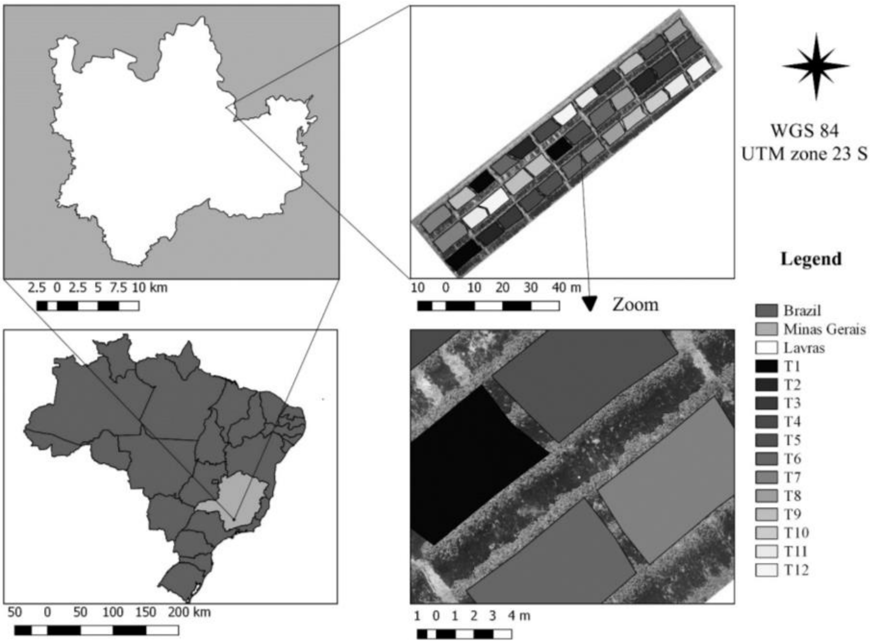

Figure 1. Study area location and treatment delimitation.

According to the Köppen classification, the regional climate is Cwa type, characterized by a dry season in the winter and a rainy season in the summer. The average annual rainfall is 1,460 mm, and the average annual temperature is 20.4 °C, with a minimum temperature of 17.1 °C in July and a maximum of 22.8 °C in February (Dantas et al., 2007). For the UAS image bank, six ground control points (GCPs) were evenly distributed throughout the field (Santos et al., 2019). The positions of the GCPs and the sampled plants were taken using a differential global positioning system (DGPS; Trimble Navigation Limited, Sunnyvale, California, USA), model SP60, with horizontal and vertical precision of 0.07 m. Later, the GCPs were identified in the images and used for georeferencing.

UAS-based data collection

The processing of images acquired by a UAS consists of a semiautomatic workflow, in which most of the software uses a similar workflow, following the process of calibrating the camera, aligning the images, and generating point clouds to generate the digital surface models (DSM) and the digital terrain model (DTM) (Hugenholtz et al., 2013; Nex & Remondino, 2014).

The software Agisoft PhotoScan Professional Edition version 1.2.4 (Agisoft LLC, St. Petersburg, Russia) was used to create the orthomosaic and to generate the DSM and DTM. This software identifies homologous points in the image and creates a continuous region by using stereoscopy to generate a point cloud.

The classification of the point cloud was performed to obtain the DSM and DTM, following the methodology described by Panagiotidis et al. (2016) and Surový et al. (2018); the parameters defined were maximum angle (deg) = 15, maximum distance (m) = 0.1, and cell size (m) = 40.

The DSM, DTM, and orthomosaic created in PhotoScan software were exported in a GeoTiff file from Quantum GIS software ver. 2.16.3 (QGIS Development Team, Open Source Geospatial Foundation); georeferenced in the Universal Transverse Mercator (UTM) coordinate system as WGS 84 zone 23 S datum, according to the coordinates of the control points collected in the study area; and used to draw a polygon of the area of interest in the shapefile (.shp) format.

To obtain the plant height, the methodology proposed by Panagiotidis et al. (2016) was used, in which a canopy height model (CHM) is obtained by subtracting the DSM from the DTM. For the correct extraction of plant height values, the Focal Statistics tool of ArcGIS version 10.5 (ESRI, Redlands, California, USA) was used, which identifies the highest pixel value in the CHM tree canopy, while avoiding lower or larger pixel values in the tree canopy. The plug-in Point Sampling Tool of QGIS software was used to obtain the plant height data already tabulated.

The crown diameters of the plants were extracted from the orthomosaic using the QGIS measurement tool.

All processing was performed for the 2 months of data obtained through the UAS.

Field measurements

The tree height measurement (hm) data and the crown diameter measurement (dm) data were collected in the same period of image acquisition, June 2017 and May 2018, with the aid of a measuring tape in 1.0 cm increments, with a maximum length of 2.50 m. We selected 144 plants, following the sampling methodology proposed by Ferraz et al. (2017).

All plants sampled were georeferenced using a DGPS receiver.

Statistics and data analysis

For the calculation of the biophysical parameters, Eq. (1) for the Leaf Area Index (LAI) was used, as reported by Favarin et al. (2002). This parameter was calculated using field data and data extracted from the UAS images.

$$LAI = 0.0134 + 0.7276 \times {D_c}^2 \times h \quad (1)$$

where Dc – crown diameter (m) and h – plant height (m).

To calculate the biophysical parameter Leaf Area (LA), Eq. (2) was used:

$$LA = LAI \times (DR \times DP) \quad (2)$$

where DR – Distance between rows (m) and DP – Distance between plants (m).

For the estimation of the crop coefficients (Kc), Eq. (3) was used, as proposed by Villa Nova et al. (2002):

$$Kc = 0.347 \times LA \times \left(\frac{Np}{10{,}000}\right) + kcd \times \left(1 - \frac{0.785{D_c}^2}{DP \times DR}\right) \quad (3)$$

where Np – Number of plants (0.641 plants ha^{-1}); LA – Leaf Area; D_c – Crown diameter (m); DR – Distance between rows (m); DP – Distance between plants (m), and kcd –Crop coefficient representative of the plant cover between rows (kcd = 1 in the presence of transpiring vegetation cover, and kcd = 0.5 absence of transpiring vegetation cover).

To create the Kc map, the average of the four plants of each block was used for the months of June and May. Electronic spreadsheets were used to calculate Kc and organize the data, and the GeoDA free software was used for the spatial analysis (Anselin, 2006).

To estimate the spatial variability of the data from the study area, the spatial weight matrix was calculated using GeoDA, using the 'queen' criterion; that is, second-order neighbors are considered. Thus, W_{ij} = 1.0 if the i-th block shares at least one side with the j-th block; otherwise, $W_{ij} = 0$. The pseudo-significance test was applied with $p < 0.05$ to validate the statistic.

Moran's global autocorrelation Index (I) proposed by Bailey & Gatrell (1995) describes the spatial arrangement of objects given by Eq. (4):

$$I = \frac{n}{W} \times \frac{\sum_i \sum_j W_{ij} z_i z_j}{\sum_i {z_i}^2} \; \forall \; i \neq j \qquad (4)$$

where n – number of observations; W_{ij} – element of the neighborhood matrix for pair i and j; W – sum of the weights of the matrix; z_i and z_j – deviations from the mean (z_i-z), (z_i-z), and z – mean.

According to Ponciano & Scalon (2010), the *i* ranges from 0 to 1.0, indicating positive direct autocorrelation, and from 0.0 to –1.0, indicating indirect and negative autocorrelation.

The regression and correlation of the Kc data obtained in the field and by the UAS were performed using the average of the four plants from each block. To evaluate whether the estimates were significant at $p < 0.05$, a t-test was applied. The Root Mean Square Error (RMSE) were also calculated. The descriptive statistical analyses were performed in the statistical software R (R Core Team, Vienna, Austria).

RESULTS AND DISCUSSION

The Kc data obtained by the UAS images, on average, were lower than the data obtained in the field; that is, the values obtained by the UAS images were underestimated for June 2017 and May 2018. However, the correlation was strong, with Pearson correlation coefficient with R equal to 0.85, coefficient of determination (R^2) equal to 0.73 was found for the Kc values in June 2017. In May 2018 Pearson correlation coefficient with R equal to 0.89, the coefficient of determination (R^2) equal to 0.79 was found for the Kc values (Fig. 2).

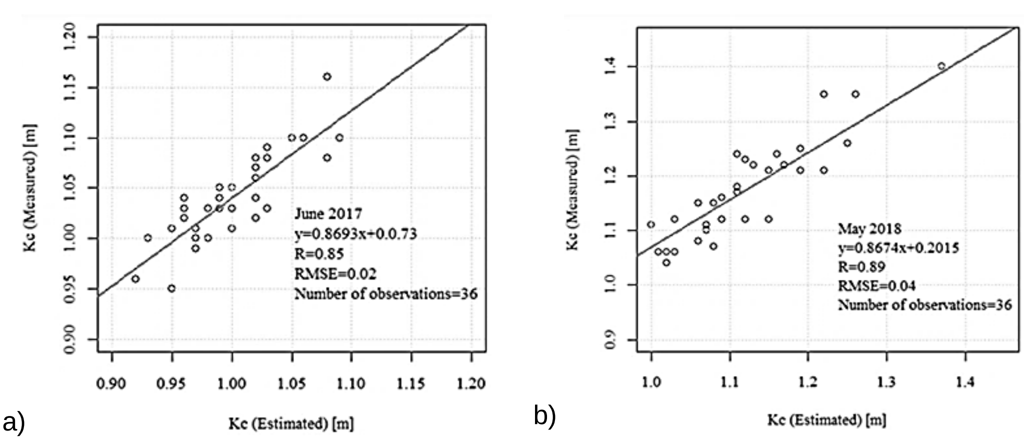

Figure 2. Regression between Kc data measured in the field and obtained by the UAS: a) of June 2017 and b) of May 2018.

Through linear regression, Eq. (5) is proposed to correct the coffee Kc data estimated from the UAS image, with the correlation of 72 numbers of observations, with an R value equal to 0.93 and a coefficient of determination (R^2) equal to 0.86. According to Eq. (5), it is possible to estimate the Kc of the coffee plant from Kc data obtained by the aircraft. This type of equation has practical relevance since it provides an indirect way of obtaining crop data.

$$Kc_m = 0.9618 \times Kc_e + 0.0879 \quad (5)$$

where Kc_m – Kc measured (m) and Kc_e – Kc estimated by the UAS (m).

The Kc measured in the field and that obtained from the UAS image were significantly different, with a p *value* < 0.02.

Kc map (Fig. 3) was proposed based on the biophysical characteristics of the coffee plants, following the methodology proposed by Villa Nova et al. (2002) and using UAS data.

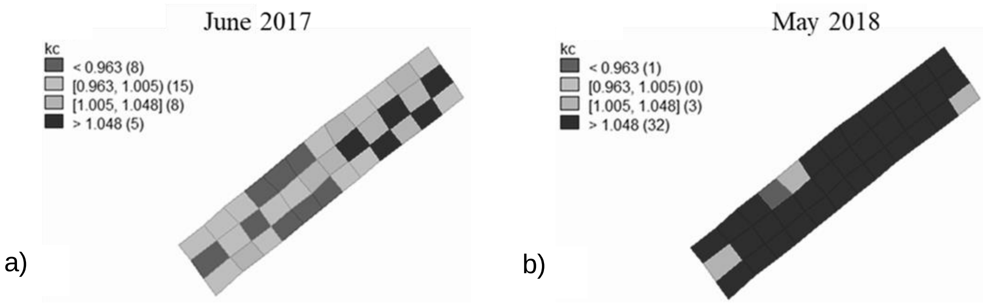

Figure 3. Kc map for the months (a) of June 2017 and (b) May 2018.

The Kc differed significantly between months; that is, the null hypothesis was rejected, and the Kc spatial autocorrelation in the blocks was considered; this autocorrelation increased with time. In June 2017, a I = 0.228 was observed, and in May 2018, I = 0.286.

As observed in Fig. 3, most of the blocks at the beginning of the experiment, in June 2017, when the coffee plants were in the vegetative period during the flower maturation stage, showed Kc values between 0.96 and 1.0. This variation is dependent on the plant height and crown diameter. For the month of May 2018, when the plants were in the reproductive period during the fruiting phase, the Kc values were 1.05 for 32 blocks, indicating a homogeneous Kc. According to Oliveira et al. (2007), in the period of crop establishment, the Kc curve has low values, and when the crop reaches a maximum canopy, the curve tends to stabilize with close values, a trend consistent with the data found in this study.

Some studies on Kc recommend values according to coffee crop development, such as Doorenbos & Pruitt (1997), who proposed a mean Kc value between 0.9 and 1.1 for adult coffee trees at all stages of development, without specifying the location and conditions under which these values were obtained. Those values are within a range greater than that found and recommended in this study (0.96 to 1.05), which is due to the variety and/or the method used for obtaining Kc.

Arruda et al. (2000) found Kc values equal to 0.73 and 0.75 in the first years of coffee production and Kc values of 0.87 and 0.93 for coffee plants 7 and 8 years of age, respectively. The present study found Kc values consistent with the values from that study, considering that mean Kc values between 0.96 and 1.05 were obtained for coffee plants 9 and 10 years of age, respectively.

For coffee plants with adequate management and heights of 2.0 to 3.0 m, in a sub-humid climate, Allen et al. (1998) proposed a Kc between 0.90 and 0.95 and for soil without plant cover and in the presence of weeds, they proposed a Kc between 1.05 and 1.10, respectively, with reference evapotranspiration estimated by the Penman-Monteith method - FAO. Those results were slightly higher than those found in the present study because the maximum Kc value found herein was 1.05, with weeds present in the interrows; this slight difference in the results can also be attributed to the method used for obtaining the Kc values.

Villa Nova et al. (2002), for Mundo Novo coffee crops, observed Kc values between 0.5 and 1.2 and between 0.9 and 1.2, without weeds and with weeds, respectively, with a density of 4,000 plants ha^{-1}. Despite using a different cultivar, that study obtained a Kc range close to that found in the present study, when considering the Kc results with weeds in the interrow.

As observed, the Kc values obtained herein were consistent with the literature, with some variations according to the stage, age, and variety. Furthermore, the method used to obtain the Kc values takes into account the plant biophysical characteristics, showing the sensitivity and consistency of the data, thus confirming its efficiency and adoption for rational irrigation management in coffee crops (Villa Nova et al., 2002).

Notably, the generated Kc map followed the equation proposed by Villa Nova et al. (2002), and it was developed for a specific variety. This equation may not represent Kc for all varieties; however, this methodology is feasible for generating Kc maps using biophysical data obtained remotely, which would be very useful in irrigation management.

According to the literature, studies for the quantification of Kc for coffee crops were performed using agronomic experiments. The advantage of using the methodology proposed herein is the ability to obtain biophysical parameters of coffee crops remotely, which is an indirect and nondestructive method of data collection. In addition, it is necessary to quantify the Kc at the different phases of the phenological cycle of coffee plants of different ages, which is difficult to do in the field. Thus, using remotes methods to obtain data from coffee plants allows the collection of crop data in almost real-time, determining the spatial variability of the crop data, and creating a historical series, thus favoring more efficient and assertive management.

CONCLUSIONS

This study developed a methodology for estimating Kc using biophysical data obtained remotely. It is an indirect methodology to obtain data in a non-destructive way.

The estimative showed a strong correlation, R of 0.93, so it is possible to estimate the Kc of the coffee plant from the Kc data obtained by the aircraft. In addition, the Kc values found in the study ranged from 0.96 to 1.0 in the vegetative period and from 1.05 in the reproductive period.

The data found were consistent with the literature; thus, this method is useful for estimating Kc and for assisting in irrigation management of coffee crops.

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REFERENCES

Allen, R.G., Pereira, L.S., Raes, D. & Smith, M. 1998. *Crop evapotranspiration-Guidelines for computing crop water requirements*. FAO, Rome, pp. 1–50.

Anselin, L., Syabri, I. & Kho, Y. 2006. GeoDa: An Introduction to Spatial Data Analysis. *Geographical Analysis* **38**, 5–22.

Arruda, F.B., Iaffe, A., Sakai, E. & Calheiros, R.O. 2000. Annual results of coffee crop coefficient in a trial in Pindorama – SP. In: *Brazilian Coffee Research Symposium*. Poços de Caldas, pp. 2:790 (in Portuguese).

Bailey, T.C. & Gatrell, A.C. 1995. *Interactive spatial data analysis. Essex: Longman Scientific*, 413 pp.

Bonomo, R., Oliveira, L.F.C., Silveira Neto, A.N. & Bonomo, P. 2008. Productivity of arabica coffee trees irrigated in Goiás cerrado. *Pesquisa Agropecuária Tropical* **38**, 233–240 (in Portuguese).

Caldas, A.L.D., Lima, E.M.C., Rezende, F.C., Faria, M.A.D., Diotto, A.V. & Júnior, M.C.R.L 2018. Productivity and quality of coffee cv crossing in response to irrigation and phosphate fertilization. *Revista Brasileira de Agricultura Irrigada* **12**, 2357–2365 (in Portuguese).

Camargo, A.P. & Pereira, A.R. (1994). *Agrometeorology of coffee crop*. Geneva: World Meteorological Organization, 92 pp.

Dantas, A.A.A., Carvalho, L.G. & Ferreira, E. 2007. Classification and climate trends in Lavras, MG. *Ciência e Agrotecnologia* **31**, 1862–1866 (in Portuguese).

Doorenbos, J. & Kassan, A.H. 1979. *Yield response to water*. FAO, Rome. 193 pp.

Doorenbos, J. & Pruitt, O. 1997. *Guidelines for predicting crop water requeriments*. FAO, Rome. 179 pp.

Favarin, J.L., Neto, D.D., García, A.G., Nova, N.A.V. & Favarin, M.G.G.V. 2002. Equations for estimating coffee leaf area index. *Pesquisa Agropecuária Brasileira* **37**, 69–773 (in Portuguese).

Ferraz, G.A.S., Silva, F.M., Oliveira, M.S.D, Silva, F.C.D. & Carvalho, L.C.C. 2017. Comparison between soil chemical attributes conventionally and mesh sampled. *Coffee Science* **12**, 17–29 (in Portuguese).

Hugenholtz, C.H., Whitehead, K., Brown, O.W., Barchyn, T.E., Moorman, B.J., Leclair, A. & Hamilton, T. 2013. Geomorphological mapping with a small unmanned aircraft system (sUAS): Feature detection and accuracy assessment of a photogrammetrically-derived digital terrain model. *Geomorphology* **194**, 16–24.

Lima, L.A., Custódio, A.A.P. & Gomes, N.M. 2008. Coffee yield and yield in the first five seasons irrigated by center pivot in Lavras, MG. *Ciência e Agrotecnologia* **32**, 1832–1842 (in Portuguese).

Nex, F. & Remondino, F. 2014. UAV for 3D mapping applications: a review. Applied *Geomatics* **6**, 1–15.

Oliveira, L.F.C.D., Oliveira, R.Z., Borges, L.B., Wehr, T.R. & Bonomo, R. 2007. Crop coefficient and water relations of coffee, cultivar Catucaí, under two irrigation management systems. *Pesquisa Agropecuária Tropical* **37**, 154–162 (in Portuguese).

Panagiotidis, D., Abdollahnejad, A., Surový, P. & Chiteculo, V. 2016. Determining tree height and crown diameter from high-resolution UAV imagery. *International Journal of Remote Sensing* **3**, 2392–2410.

Ponciano, P.F. & Scalon, J.D. 2010. Spatial analysis of milk production using a conditional autoregressive model. *Semina: Ciências Agrárias* **31**, 487–496 (in Portuguese).

Santos, L.M.D., Andrade, M.T., Santana, L.S., Rossi, G., Maciel, D.T., Barbosa, B.D.S. & Ferraz, G.A.S. 2019. Analysis of flight parameters and georeferencing of images with different control points obtained by RPA. *Agronomy Research* **17**, 2054–2063.

Sato, F.A., Silva, A.M., Coelho, G., Silva, A.C. & Carvalho, L.G. 2007. Crop coefficient (Kc) of coffee (Coffea arabica L.) in the autumn-winter period. *Engenharia Agrícola* **27**, 383–391 (in Portuguese).

Silva, A.C.D., Lima, L.A., Evangelista, A.W.P. & Martins, C.D.P. 2011. Evapotranspiration and crop coefficient of central pivot irrigated coffee. *Revista Brasileira de Engenharia Agrícola e Ambiental* **15**, 1215–1221 (in Portuguese).

Silva, A.M.D., Coelho, G. & Silva, R. 2005. Irrigation and fertilizer installment seasons on coffee yield in four seasons. *Revista Brasileira de Engenharia Agrícola e Ambiental* **9**, 314–319 (in Portuguese).

Surový, P., Ribeiro, N.A. & Panagiotidis, , D. 2018. Estimation of positions and heights from UAV-sensed imagery in tree plantations in agrosilvopastoral systems. *International Journal of Remote Sensing* **39**, 4786–4800.

United States Department of Agriculture (USDA). Avaible in:< http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1801> Access in: Dezembro de 2018.

Vicente, M.R., Mantovani, E.C., Fernandes, A.L.T., Delazari, F.T. & Figueredo, E.M. 2018. Effect of different irrigation depths on the development and production variables of central pivot irrigated coffee. *IRRIGA* **20**, 528–543 (in Portuguese).

Villa Nova, N.A., Favarin, J.L., Angelocci, L.R. & Dourado Neto, D. 2002. Estimation of coffee crop coefficient (Kc) as a function of climatological and phytotechnical variables. *Bragantina* **61**, 81–88 (in Portuguese).

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Embryo transfer results in endangered cow breeds in Latvia

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Abstract. Since 2017 multiple ovulation (MO) and embryo transfer (ET) were used to save endangered cow breeds in Latvia. The aim of this work was to analyse results to establish factors influenced recipients' pregnancy obtained up to now. Recipients age, induced (IRC) or native reproductive cycle (NRC), level of estradiol (E2) and progesterone (P4) in recipients' blood on ET day, stage and quality of embryo, fresh or thawed embryo usage and person provided ET were taken into account. Repeated artificial insemination (AI) had been provided in 19 (22.1%) recipients before ET. Pregnancy was accepted in 23 out of 76 recipients (30.3%) until now. Higher pregnancy results were obtained using fresh embryos (19 out of 53 (35.8%)) vs thawed embryos (4 out of 23 (17.4%)), ($P < 0.05$). The IRC was more productive than NRC ($P < 0.05$). The development stage, quality of embryos, E2 level were significant factors to reach pregnancy ($P < 0.05$) but recipients age, P4 level and person provided ET did not influence the pregnancy rate in the present study ($P > 0.05$). More successful pregnancies were reached using embryos obtained from donors which had a higher glucose and cholesterol level in blood ($P = 0.05$). In conclusion, our newly educated MOET team should gain their experience and results could be improved using IRC in recipients, fresh embryos, and more attention could be paid to the donor-cows management in order to reach more qualitative embryos. Somatic cell count in the donors' milk could be one of the indicator to avoid unsuccessful embryo obtaining.

Key words: cow embryo, embryo transfer, recipients.

INTRODUCTION

Thanks to ERAF project No.1.1.1.1/16/A/025, cow embryo collection and transfer is a developing field since 2017 in Latvia. Our main aim is to save endangered cow breeds in Latvia. A new staff was educated to provide multiple ovulation (MO), embryo flushing and embryo transfer (ET) in the cow.

In Latvia endangered cow breeds (Latvian Brown, Latvian Blue and Danish Red) are on extinction line. Mainly our endangered breed cows are kept on small farms without well-established management but it is important factor for cows' health and productivity despite the modesty of the native breeds (Cielava et al., 2017). Latvian Blue cows are suffering never from *Bovine leukaemia*, and mastitis is not common for them (Grīslis, 2006). Latvian Brown cows inherit durable leg, foot and hoof health from their ancestors (Šematoviča et al., 2017). Feed's composition and quality besides amount of milk, influences milk fat and protein content (Frorip et al., 2012; Šematoviča et al., 2017a). It is reported that Latvian native breed cows have high milk fat content, milk fat and protein ratio (Paura et al., 2012; Smiltiņa et al., 2015). Small number of animals exist and this was the main reason why almost all intended cows were used to obtain embryos despite the fact that many of them did not meet conditions necessary for a good donor cow status.

Successful ET depends on the embryo quality and subsequently on the donor-cow factors such as: genetic qualities, nutrition, health and housing conditions (Hasler et al., 1987; Son et al., 2012; Vieira et al., 2014; Diskin et al., 2016; Mikkola, 2017; Abdelatty et al., 2018). The housing system directly affects dairy cows' longevity, health of the udder and calving performance (Leso et al., 2019).

Recipient depending factors are very important and also widely investigated. Some of them are ovarian functionality and hormonal status (Hasler et al., 1980; Rao & Yelisetti, 2013). Within the framework of the present work because of Latvian society was not informed enough about MO, embryo flushing (EF) and ET procedures influence on animal's health, the animals' owners quite often allowed us to use heifers for ET which were after unsuccessful artificial insemination (AI) or quite old ones. Although heifers-recipients used were without clinical signs of illness.

The aim of this work was to analyse recipients' pregnancy results in relation to different factors. Recipient factors investigated were: age and reproduction history, natural or induced reproductive cycle using, estradiol and progesterone concentration in blood. Factors of embryos were: fresh or thawed embryo transferring, development stage and quality. There were taken into account person who provided embryo transfer procedure and season. The background of embryos was analysed: glucose and cholesterol level in donor-cow blood on embryo obtaining day and somatic cell count in milk on the nearest milk recording day.

MATERIAL AND METHODS

More than 150 heifers (different breeds and crossbreed) were accepted for a recipient role. These animals usually were kept on other farms than donor-cows. Different housing system (tied - 39 (51.3%) and free - 37 (48.7%)) and different feeding were provided (totally mixed ration and conventional feeding). Water was free available by automatic waterers. Animals were active and interacted with the personnel and each other appropriately depending on their individual character. Only 86 heifers (13–37 months of age, 330–400 kg bodyweight) became the recipients. It was too early to detect pregnancy in 10 recipients (11.6%). Cloprostenol (*Oestrophan*, Bioveta) was used to synchronize the oestrus cycle in recipients using two injections with 11 days of interval in 60 (69.8%) recipients, but in 26 (30.2%) recipients the 7th day of native reproductive cycle was used. It was a random day of the reproductive cycle when heifers received the first injection of Cloprostenol. An epidural anaesthesia in recipients was done using 2.0 mL Procamidor (*Procaine hydrochloride* 20.0 mg mL^{-1}, *Richter Pharma*) before ET.

Blood samples were taken to evaluate the ovaries' functional activity in heifers on the 7th day of the reproductive cycle. Concentration of E2 and P4 were analysed in the blood sera using Enzyme-Linked Fluorescent Assay method in the accredited laboratory (LVS EN ISO/IEC 17025:2005) at the Institute of Food Safety, Animal Health and Environment 'BIOR'. Some parameters of embryo donors were taken into account (blood glucose, cholesterol, P4 and somatic cell count in milk).

Heifers' ovaries were investigated by rectal palpation on the 7th day of the reproductive cycle (common ovarian status, presence, location and consistency of *corpus luteum* (CL)) before the potential process of ET. Poor quality of CL was the reason to decline a heifer to become a recipient because of inappropriate reaction to hormonal treatment through synchronization process or NRC. If the CL was well expressed, at least 10–12 mm large and compact, ET was carried out. Embryos were transferred to the same uterus horn to the current CL. If fresh embryos were used, it was done 6–8 h after EF from the donor cow. If frozen embryos were used, thawing was provided shortly before ET.

Embryos were evaluated and classified according to the International Embryo Technology Society standards and related guidelines (Bo & Mapletoft, 2013). The quality of totally 86 transferred embryos was: good - 38 (44.2%), fair - 44 (51.2%) and poor - 4 (4.7%), but their development stages were: stage III - morula (3 embryos, 3.5%), stage IV - compact morula (49 embryos, 57%), stage V - early blastocysts (19 embryos, 22.1%), stage VI - blastocysts (9 embryos, 10.5%), and stage VII - expanded blastocysts (6 embryos, 7%).

Donors mainly were located on other farms than recipients. Before transferring, embryos were flushed out from the donors using BoviFlush Recovery medium (Minitube) and stored at room temperature in BoviHold medium (Minitube). Embryos for cryopreservation were put in straws with BoviFreeze medium (Minitube) and embryo thawing was provided using Portable Incubator (Minitube). Embryos were carried to recipients in the ET devices heater V2.0 with a carrying strap, +25 °C (Minitube), and ET was done immediately.

Fresh embryos transfer was done in 57 (66.3%) recipients and thawed embryos were transferred to 29 (33.7%) recipients. Transferring was provided by three persons (A - 48 recipients, 55.8%), B - 2 recipients, 2.3%) and C - 36 recipients, 41.9%).

Data are expressed as the mean ± SD, percentage and independent samples *t-tests* were performed for statistical analysis considering the significance level of $P < 0.05$ using *IBM SPSS Statistics 21* software.

RESULTS AND DISCUSSION

Pregnancy was accepted in 23 out of 76 recipients (30.3%) by manual palpating 60-75 days after ET. Usually pregnancy should be approved earlier but our native breed cows are located in very different regions of Latvia. Significantly higher pregnancy results were obtained using fresh embryos (19 pregnancies out of 53 (35.8%) in comparison with of using thawed embryos (4 out of 23 (17.4%)), ($P < 0.05$). The cryopreservation is a physico-chemical process which has a negative influence on fertility and quality of embryos. A tremendous improvement was carried out to ensure a quality and fertility of embryos within the last decades (Saragusty & Arav, 2011; Huang et al., 2019). Nowadays cryopreservation allows to reach good pregnancy results in recipients using thawed embryos, and methodology is still improving (López-Damián et al., 2020).

Recipient factors could be very important for a successful ET outcome (Ferraz et al., 2016). Despite the unsuccessful AI was provided in 19 recipients (25%), before they were accepted for the recipients' role, it was not a statistically significant factor for the successful ET outcome ($P > 0.05$). Thirteen recipients (17.1%) expressed oestrus in the following reproductive cycle, 11 recipients (14.5%) were in heat in the second reproductive cycle, but 29 recipients (38.2%) were in the heat just after 3 and more reproductive cycles after ET. It remains unknown exactly how many of these recipients did not save transferred embryo at all, how many of them saved pregnancy, and how many micro-abortions occurred. It could be suspected that embryo survive failure is recognisable by too late rebreeding (Diskin et al., 2016). In our work it could be 38.2%, but it should be taken into account how accurately the owners of recipients had evaluated heifers' oestrus signs.

The IRC (54 recipients, 71.1%) was more productive than that of the NRC (22 recipients, 28.9%), ($P < 0.05$). A higher pregnancy rate using IRC was also noticed by other scientists (Hasler et al., 1987). Weak statistically significant correlation was established between pregnancy rate and recipients housing system ($r = 0.32$, $P < 0.001$). In tethered heifers group pregnancy saved 9 out of 39 (23.1%), but in free heifers group 14 out of 37 (37.8%). It could be explained with the more comfortable obstacles for expression of estrus signs to establish the first reproductive cycle day and better welfare conditions to save pregnancy after ET on the 7th day of the reproductive cycle.

In the present work, the development stage of embryo might have been a quite significant factor to reach recipients' pregnancy ($P = 0.07$) because most of pregnancies obtained were using compact morula (18 pregnancies out of 23 (78.3%). However, it should be mentioned that compact morulas totally were used in 45 recipients and 27 of them did not became pregnant ($P > 0.05$). An early blastocysts were used in 16 recipients, and a successful outcome was in three recipients (18.8%). Nine blastocysts were transferred and one pregnancy was detected (11.1%). Six expanded blastocysts were transferred and only one pregnancy was approved (16.7%). All pregnancies obtained by thawed embryos were fulfilled using embryos of the stage of the compact morula.

Table 1. Progesterone and estradiol level in recipients' blood on ET day

	Progesterone (nmol L^{-1})	Estradiol (pg mL^{-1})
Pregnant altogether (*n* = 23)	19.8 ± 7.87	9.7 ± 1.81
Un-pregnant altogether (*n* = 53)	22.7 ± 12.08	13.3 ± 10.83*
Pregnant using fresh embryo (*n* = 19)	18.2 ± 6.80	9.7 ± 1.93
Pregnant using thawed embryo (*n* = 4)	27.4 ± 9.15*	9.7 ± 1.35
Un-pregnant using fresh embryo (*n* = 34)	21.1 ± 9.79	12.2 ± 8.41
Un-pregnant using thawed embryo (*n* = 19)	25.4 ± 15.18	15.2 ± 14.15
Induced reproductive cycle (*n* = 54)	20.5 ± 10.81	11.5 ± 6.85
Natural reproductive cycle (*n* = 22)	25.2 ± 10.78	13.9 ± 13.56
Pregnant using natural reproductive cycle (*n* = 4)	29.7 ± 7.9	9.7 ± 1.35
Pregnant using induced reproductive cycle (*n* = 19)	17.7 ± 6.2	9.7 ± 1.93
Un-pregnant using natural reproductive cycle (*n* = 18)	24.1 ± 11.5	14.9 ± 14.96
Un-pregnant using induced reproductive cycle (*n* = 35)	22.0 ± 12.45	12.5 ± 8.28

* ($P < 0.05$).

Recipients which become pregnant were younger than unsuccessful pregnancy recipients (16.6 ± 2.33 and 18.0 ± 4.11 months respectively, ($P > 0.05$)).

Steroid hormone level in recipients' blood on ET day are presented in Table 1. In the present study, on the ET day the E2 level in blood was lower in recipients which became pregnant in comparison to the recipients which did not became pregnant (9.7 ± 1.81 and 13.3 ± 10.83 pg mL^{-1} respectively, $P < 0.05$). The level of steroid hormone P4 is very important to save the pregnancy and it depends on CL functional quality (Lonergan et al., 2007; Stevenson & Lamb, 2016). It was established that in the recipients which had become pregnant after ET, the level of P4 was higher on the 7th day of the reproductive cycle than recipients which failed to be pregnant (Rao & Yeliseti, 2013). In our study, P4 level had no statistically significant differences for a successful pregnancy and unsuccessful pregnancy in the recipients' blood (19.8 ± 7.87 and 22.7 ± 12.08 nmol L^{-1}, respectively, ($P > 0.05$)). The similar observation was revealed by other scientists who established no significant differences regarding P4 level in the heifers' blood which became pregnant and heifers which did not save a pregnancy later (Hasler et al., 1980). A more important factor established is CL quality, diameter and size (Nogueira et al., 2012). We noticed that a quite strong functionality of CL was necessary to save pregnancy using thawed embryos. Level of P4 to obtain only 4 pregnancies using thawed embryos were 27.4 ± 9.15 nmol L^{-1}.

Pregnancy rate was not different significantly between the person A and B providing ET ($P > 0.05$), but ET procedures provided by person C were unsuccessful ($P < 0.05$), (Table 2). Training and experience are very important conditions besides the factors related with donors, recipients, embryos, equipment, and methods used (Seidel, 1984).

Table 2. Pregnancies obtained by different persons A, B and C

	ET/ pregnancies totally	Pregnancies totally %	Fresh ET/ pregnancies	Fresh ET pregnancies %	Thawed ET/ pregnancies	Thawed ET pregnancies %
A	41 / 10	24.4	28 / 8	19.5	13 / 2	15.4
B	33 / 13	39.4	25 / 11	33.3	8 / 2	25.0
C	2 / 0	0.0*	0 / 0	0	2 / 0 *	0.0
TOTAL	76 / 23	30.3	53 / 19	35.8	23 / 4	17.4

* ($P < 0.05$).

We have found out that the background of embryos is important because more successful ET results were reached if embryos were obtained from donors which had a higher level of glucose in the blood (3.1 ± 0.39 and 2.8 ± 0.42 mmol L^{-1} respectively), higher level of cholesterol in the blood (5.6 ± 0.91 and 4.9 ± 1.11 mmol L^{-1}) and lower somatic cell count in milk (214.5 ± 121.94 and 486.4 ± 1,073.88 thousand mL^{-1}). Pregnancy rate between those donors' embryos was statistically significant ($P < 0.05$). Interrelation between the udder health and reproductive performance regarding pregnancy loss is actual still now (Dahl et al., 2020). The positive influence of the embryo donor blood glucose and cholesterol level on embryo harvest was reported earlier (Choe et al., 2012).

Season is mentioned as important factor for a successful MO, EF and ET (Hasler et al., 1987; Vieira et al., 2014). In the present work, 44 (58.7%) ET were provided in the winter-spring season, but 31 (41.3%) ET were done in summer-autumn period. Sixteen pregnancies were obtained in the winter-spring season but only seven pregnancies in summer-autumn period. This difference is not statistically significant because of number of unsuccessful ET at these periods.

Because of MOET was restarted after more than 35 years' interruption in Latvia the new staff had been trained. The experienced professionals have reached 70% pregnancy rate using fresh and 60% using thawed embryos (Gadisa et al., 2019). We did not find the individual factors that were decisive for the recipients' pregnancy. Using *IBM SPSS Statistics 21 software General Linear Model*, we have found the significance of interactions of individual components such as: recipient reproduction history, IRC and person provided ET. So we must to improve some areas of our work regarding technique, donors, recipients and embryos.

CONCLUSIONS

In conclusion, our newly educated and trained MOET team should improve experience to rise the rate of recipients' pregnancy. Very specific reasons were not revealed for quite poor results. Our success could be improved using induced reproductive cycle in recipients, fresh embryos, by improving technique of cryopreservation, and more attention could be paid to the donor-cows management in order to reach more qualitative embryos. Somatic cell count in the donors' milk could be one of the indicator to avoid unsuccessful embryo obtaining. Future monitoring and investigation must be provided to improve pregnancy results in recipients in our country.

REFERENCES

Abdelatty, A.M., Iwaniuk, M.E., Potts, S.B. & Gad, A. 2018. Influence of maternal nutrition and heat stress on bovine oocyte and embryo development. *International Journal of Veterinary Science and Medicine* **6**(supplement), S1–S5. doi: 10.1016/j.ijvsm.2018.01.005

Bo, G.A. & Mapletoft, R.J. 2013. Evaluation and classification of bovine embryos. *Animal Reproduction* **10**(3), 344–348.

Choe, C., Son, J., Jung, Y., Cho, S., Baek, K., Yoon, H., Lim, H., Kwon, E. & Kim, S. 2012. Relationship between transferable embryos and major metabolite concentrations in Holstein donor cows. Journal of Animal Reproduciton and Biotechnology 27(4), 229–235. doi:10.12750/JET.2012.27.4.229

Cielava, L., Jonkus, D. & Paura, L. 2017. Lifetime milk productivity and quality in farms with different housing and feeding systems. *Agronomy Research* **15**(2), 369-375.

Dahl, M.O., De Vries, A., Galvão, K.N., Maunsell, F.P., Risco, C.A. & Hernandez, J.A. 2020. Combined effect of mastitis and parity on pregnancy loss in lactating Holstein cows. *Theriogenology* **143**, 57–63. doi: 10.1016/j.theriogenology.2019.12.002

Diskin, M.G., Waters, S.M., Parr, M.H. & Kenny, D.A. 2016. Pregnancy losses in cattle: potential for improvement. *Reproduction, Fertility and Development* **28**(2), 83-93. doi: 10.1071/RD15366

Ferraz, P.A., Burnley, C., Karanja, J., Viera-Neto, A., Santos, J.E.P., Chebel, R.C. & Galvão, K.N. 2016. Factors affecting the success of a large embryo transfer program in Holstein cattle in a commercial herd in the southeast region of the United States. *Theriogenology* **86**(7), 1834–1841. doi: 10.1016/j.theriogenology.2016.05.032

Frorip, J., Kokin, E., Praks, J., Poikalainen, V., Ruus, A., Veermäe, I., Lepasalu, L., Schäfer, W., Mikkola, H., & Ahokas, J. 2012. Energy consumption in animal production – case farm study. *Agronomy Research Biosystem Engineering*, Special Issue **1**, 39-48.

Gadisa, M., Furgasa, W. & Duguma, M. 2019. Review on embryo transfer and its application in animal production. *Asian Journal of Medical Science Research & Review* **1**(1), 4–12.

Grīslis, Z. 2006. Latvian Blue Cow in Vidzeme, Jelgava. BŠSA '*Zilā govs*': 36 pp. (*In Latvian*).

Hasler, J.F., Bowen, R.A. & Nelson, L.D. 1980. Serum progesterone concentrations in cows receiving embryo transfers. *Reproduction* **58**(1), 71–77.
Hasler, J.F., McCauley, A.D., Lathrop, W.F. & Foote, R.H. 1987. Effect of donor-embryo-recipient interactions on pregnancy rate in a large-scale bovine embryo transfer program. *Theriogenology* **27**(1), 139–168. doi: 10.1530/jrf.0.0580071
Huang, Z., Gao, L., Hou, Y., Zhu, S. & Fu, X. 2019. Cryopreservation of farm animal gametes and embryos: recent updates and progress. *Frontiers of agricultural science and engineering* **6**(1), 42–53. doi: 10.15302/J-FASE-2018231
Leso, L., Pellegrini, P. & Barbari, M. 2019. Effect of two housing systems on performance and longevity of dairy cows in Northern Italy. *Agronomy Research* **17**(2), 574–581. doi: 10.15159/ar.19.107
Lonergan, P., Woods, A., Fair, T., Carter, F., Rizos, D., Ward, F., Quinn, K & Evans, A. 2007. Effect of embryo source and recipient progesterone environment on embryo development in cattle. *Reproduction, Fertility and Development* **19**, 861–868.
López-Damián, E.P., Jiménez-Medina, J.A., Alarcón, M.A., Lammoglia, M.A., Hernández, A., Galina, C.S. & Fiordelisio, T. 2020. Cryopreservation induces higher oxidative stress levels in *Bos indicus* embryos compared with *Bos Taurus*. *Theriogenology* **143**, 74–8. doi: 10.1016/j.theriogenology.2019.12.001
Mikkola, M. 2017. *Superovulation and embryo transfer in dairy cattle – effect of management factors with emphasis on sex-sorted semen*. Academic dissertation, 79. pp.
Nogueira, E., Cardoso, G.S., Marques Junior, H.R., Dias, A.M., Ítavo, L.C.V. & Borges, J.C. 2012. Effect of breed and corpus luteum on pregnancy rate of bovine embryo recipients. *Revista Brasileira de Zootecnia* **41**(9), 2129–2133. doi: 10.1590/S1516-35982012000900022
Paura, L., Jonkus, D. & Ruska, D. 2012. Evaluation of the milk fat to protein ratio and fertility traits in Latvian Brown and Holstein dairy cows. *Acta argiculturae Slovenica*, Supplement **3**, pp. 155–159.
Rao, M.M. & Yelisetti, U.M. 2013. Progesterone Profile in Embryo Transfer Recipient Cows. *The Indian Veterinary Journal* **90**(1), 59–60.
Saragusty, J. & Arav, A. 2011. Current progress in oocyte and embryo cryopreservation by slow freezing and vitrification. *Reproduction* **141**(1), 1–19. doi: 10.1530/REP-10-0236
Seidel, G.E. 1984. Applications of Embryo Transfer and Related Technologies to Cattle. *Journal of Dairy Science* **67**, 2786–2796. doi: 10.3168/jds.S0022-0302(84)81635-5
Šematoviča, I., Eihvalde, I. & Kairiša, D. 2017a. Reticulo-ruminal pH and temperature relationship between dairy cow productivity and milk composition. *Agronomy Research* **15**(2), 576–584.
Šematoviča, I., Grāvere, B. & Līdaks, M. 2017. Latvian Brown genepool characteristics and resources in 2017. *Proceedings of Conference on 'Current events in veterinary research and practice'*, pp. 26–30.
Smiltiņa, D., Bāliņš, A. & Grīslis, Z. 2015. A review of Latvian Blue (LZ) cows from the list of animal genetic resources in Latvia. *Acta Biologica Universitatis Daugavpiliensis* **15**(151).
Stevenson, J.S. & Lamb, G.C. 2016. Contrasting effects of progesterone on fertility of dairy and beef cows. *Journal of dairy science* **99**(7), 5951–5964. doi: 10.3168/jds.2015-10130
Vieira, L.M., Rodrigues, C.A., Mendanha, M.F., Sá Filho, M.F., Sales, J.N., Souza, A.H., Santos, J.E. &Baruselli, P.S. 2014. Donor category and seasonal climate associated with embryo production and survival in multiple ovulation and embryo transfer programs in Holstein cattle. *Theriogenology* **82**(2), 204–212. doi: 10.1016/j.theriogenology.2014.03.018

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Variation in the mass and moisture content of solid organic waste originating from a pig complex during its fermentation

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Abstract. The focus of the study was the fermentation of an organic waste mixture originating from a pig-rearing complex. The organic waste was processed in the laboratory-scale drum fermenter. Through the fermentation process, the fermented material was weighed by a system of four strain gauges installed under the fermenter. In our previous study, the following initial mixture characteristics were justified to have the fermentation process going – 65% to 70% moisture content and 400–600 kg m^{-3} density. The optimal operation mode of the fermenter was identified depending on the initial mixture composition – aeration frequency of 5 min h^{-1}; air consumption of 11.3 m^3 h^{-1} per 1.7 m^3 of the fermenter volume; drum rotation interval – three times every 12 hours. Under this operating mode, the mass loss was 3% already on the third fermentation day and 7% – on the fifth day. As a result, the mass of the finished organic fertiliser was 9% smaller than that of the loaded mixture. The moisture content of the processed material also decreased: under the average moisture content of the loaded mixture of 68.7%, the average moisture content of the organic fertiliser was 66.4%. Based on the resulting experimental data, the mathematical models describing the dependence of the mass and moisture content of the processed material on the fermentation time were created. The study outcomes allow concluding that the solid-state aerobic fermentation is one of the promising options for the utilisation of the solid fraction of pig slurry.

Key words: organic waste, slurry, organic fertiliser, fermentation, mass, moisture content.

INTRODUCTION

Intensification of pig farming and the herd expansion lead to an increase in the mass of pig slurry produced. The properly processed pig slurry must comply with the State Standard GOST R 53117-2008 'Organic fertilisers based on animal waste: Specifications'. In this case, it can be applied to the fields as an organic fertiliser to improve the soil fertility.

The most common pig slurry processing technology in the North-West of Russia is its solid-liquid separation followed by the passive composting of the solid fraction in clamps and the long-term storing (maturing) of the liquid fraction (Briukhanov & Uvarov, 2015).

Composting is an aerobic process that provides the comfort conditions for the activity of mesophilic and thermophilic microorganisms (Rodhe & Jonsson, 1999). Passive composting has to meet several mandatory requirements for the guaranteed processing of organic components. All parts of the clamp should gain the temperature of at least +50 °C within 10 days after the clamp formation (Uvarov et al., 2019). The processing should last for at least 2 months in the warm season and at least 3 months – in the cold season.

Considering a large number of uncontrollable external factors, such as precipitation, low ambient temperature, etc., the passive composting cannot ensure the high quality of the resulting organic fertiliser. The closed fermenters, free from the outside influences, can significantly improve the quality of the final product and reduce the processing time to several days (Aboltins et al., 2019).

Fermenters with a working body in the form of a horizontal rotating drum provide the mixing and additional aeration of the processed material, thereby avoiding its compaction and the oxygen-deficient pockets in some parts inside the fermenter. This guarantees the stable processing modes and the uniform compost maturation.

An additional advantage of drum fermenters is the higher dry matter content in the final product (Afanassiev et al., 2000) and, consequently, the higher nutrients concentration compared to the fertiliser produced by the passive composting (Poulsen et al., 2006; Sindhöj et al., 2013). The dry matter content of about 30%–50% in the organic fertiliser produced by the fermentation allows to granulate it without additional processing costs, thereby significantly increasing its rational transportation distance.

A pig-rearing complex is a large man-made facility with a significant environmental impact (Priekulis et al., 2019). Along with the basic pig production, the complex also generates different waste: solid household waste, sewage, slury, fodder waste, etc. Each waste is usually processed separately in compliance with its physical and chemical composition and regulated utilisation practices. There are technologies, however, which allow processing a mixture of several wastes, thereby increasing the processing efficiency and reducing the utilisation costs (Shalavina et al., 2017; Dąbrowska et al., 2019). One such technology is the aerobic solid-state fermentation.

The previous studies demonstrated that the recommended physical and chemical characteristics of the mixture, namely 65%–70% moisture content, 400–600 kg m^{-3} density, and C/N ratio of (15–30)/1, ensured the highly intensive fermentation process, complete processing and guaranteed disinfection of the mixture (Shalavina et al., 2019).

The objective of further research was to study the effect of fermentation temperature on the mass and some physical parameters of the fermented mixture.

MATERIALS AND METHODS

A pig-rearing complex located in the North-West Federal District was selected as a pilot farm. It produced two types of the solid fraction of pig slurry: the solid fraction coming from the screw separator and the solid fraction coming from the decanter centrifuge. In addition, the complex generated the waste from the mechanical cleaning of grain. The fermented mixture consisted of these three types of waste (Fig. 1).

The combination of the components was justified in our exploratory research: the solid fraction of pig slurry coming from the screw separator – 60%; the solid fraction of pig slurry coming from the decanter centrifuge – 32%; grain mechanical cleaning waste – 8% (Shalavina et al., 2019). Depending on the specific composition of the mixture to be fermented, the variation pattern of the mass and moisture content of the processed material is different.

Physical and chemical properties of the fermented mixture are shown in Table 1.

Experiments were conducted at IEEP – branch of FSAC VIM from February to June 2019 following the approved programme and methodology on a laboratory-scale drum fermenter (Fig. 2).

Table 1. Physical and chemical properties of the starting components

Indicator	Unit	Value
The solid fraction of pig slurry coming from the screw separator		
Moisture content	%	68.2
pH	-	8.5
Ash content	%	7
N_{total}	mg kg^{-1}	5,600
P_{total}	mg kg^{-1}	2,070
The solid fraction of pig slurry coming from the decanter centrifuge		
Moisture content	%	70.5
pH	-	9.9
Ash content	%	36.4
N_{total}	mg kg^{-1}	8,206
P_{total}	mg kg^{-1}	6,206
Grain mechanical cleaning waste		
Moisture content	%	13.9
pH	-	6.9
Ash content	%	12.5
N_{total}	mg kg^{-1}	1,036
P_{total}	mg kg^{-1}	3,300

Figure 1. The components of the fermented mixture: 1 – the solid fraction of pig slurry coming from the screw separator; 2 – the solid fraction of pig slurry coming from the decanter centrifuge; 3 – grain mechanical cleaning waste.

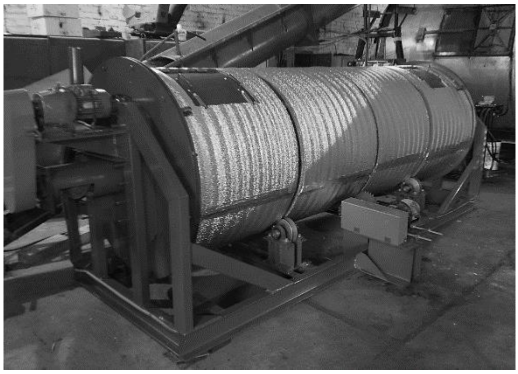

Figure 2. Laboratory-scale drum fermenter.

At the first stage, the initial components were mixed in the justified proportions. The average mass of the loaded mixture was 1,287 kg. The average moisture content of the mixture was 68.7%.

The mass of the processed material was measured by a weighing system of four strain gauges installed under the fermenter. The temperature was measured at six points: two measuring points per the loading, central and unloading parts of the fermenter. The speed and humidity of the supplied air were measured at the aeration air duct inlet using TKA-PKM/60 heat loss anemometer and humidity sensor. The measuring points of the required parameters are shown in Fig. 3.

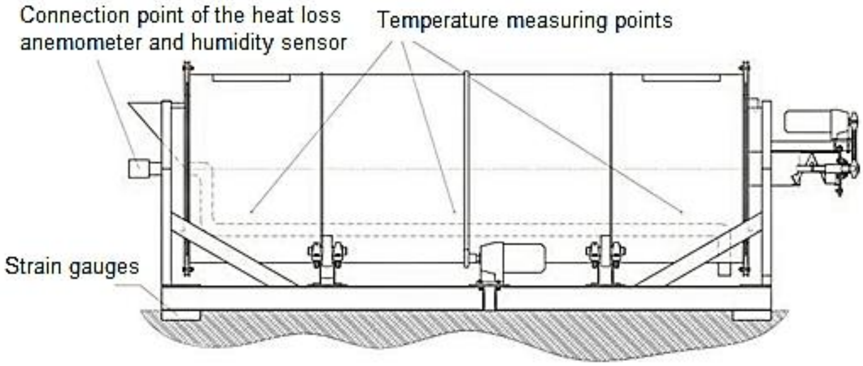

Figure 3. Scheme of the measuring points of the required parameters on the laboratory-scale fermenter.

The moisture content in the selected samples was determined in the laboratory of analytical methods of environmental engineering of IEEP – branch of FSAC VIM following the State Standard GOST 26713-85 'Organic fertilizers: Method for determination of moisture and dry residue'.

After the mixture loading and the mass measurement, the artificial aeration of the fermented mixture was launched. The optimal operation mode of the fermenter was identified in our previous studies depending on the initial composition of the mixture: aeration frequency of 5 min h^{-1}, air consumption of 11.3 m^3 h^{-1} per 1.7 m^3 of the fermenter volume; drum rotation interval – three times every 12 hours.

The fermentation, i.e. processing the mixture into an organic fertiliser, lasted for seven days. The ready organic fertiliser was unloaded on the 7^{th} day after the sampling and the mass measurement was finished.

The processed mixture mass was measured and the samples to determine the moisture content were taken every day at 9:00 A.M.

The experiment had three replications (Valge et al., 2015). The experimental data were processed in *Microsoft Excel* and *Statgraphics Centurion* programmes. The error in the mean values was estimated by Student's *t*-test. The true value of the mathematical expectation with probability *P* was in the range

$$P\left[\bar{x} \pm t_V \cdot \frac{\sigma}{\sqrt{n}}\right] = 1 - \alpha \quad (1)$$

where $\bar{x}$ – mean value; t_V – the tabular value for Student's *t* (for 0.9 probability level the value was 2.92); $1 - a$ – pre-chosen probability (for the fermentation process the value was 0.9); n – number of array elements (in this experiment $n = 3$); σ – mean-square deviation.

RESULTS AND DISCUSSION

The experiment started on 28 February 2019. The dynamic pattern of the changes in the fermentation temperature of the mixture averaged over the three replications under the specified operating mode is shown in Fig. 4.

A rapid temperature increase was observed: the temperature of the mixture reached +22.8 °C within 24 hours after the experiment started. After 48 hours it increased to +48.5 °C, and after 65 hours a threshold of +55 °C was reached and the temperature never dropped below this value.

The obtained experimental data on the processed mixture mass and the results of their statistical parameters are presented in Table 2

The calculation results showed that the average values fell in the range of standard deviations, therefore, the data were reliable and no suspect data were available.

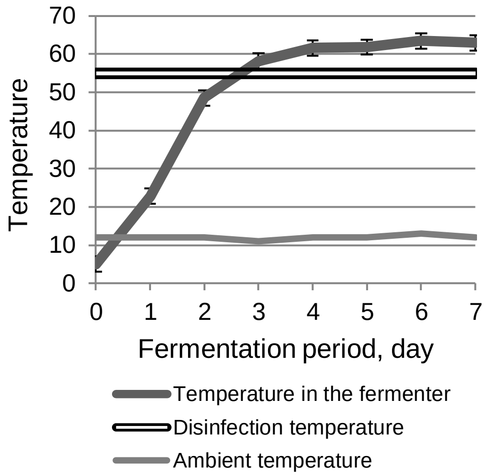

Figure 4. Dynamic pattern of the temperature variation.

Table 2. The data on the processed mixture mass in the drum-type fermenter

Fermentation stage	Average mass $\bar{x}$, kg	Dispersion, D	$\bar{x}+\sigma$	$\bar{x}-\sigma$	Upper interval limit, *P1*	Lower interval limit, *P2*
Mixture loading into the fermenter	1,287	0	1,287	1,287	1,287	1,287
Fermentation, day 1	1,277.7	30.8	1,283.3	1,272.1	1,287.2	1,268.2
Fermentation, day 2	1,269.3	21.5	1,273.9	1,264.7	1,277.1	1,261.5
Fermentation, day 3	1,248.7	6.8	1,251.3	1,246.1	1,253.1	1,244.3
Fermentation, day 4	1,214	20.6	1,218.5	1,209.5	1,221.6	1,206.4
Fermentation, day 5	1,195	16.6	1,199.1	1,190.9	1,201.9	1,188.1
Fermentation, day 6	1,184.7	50.8	1,191.8	1,177.6	1,196.7	1,172.7
Fermentation, day 7, unloading	1,176.3	42.8	1,182.8	1,169.8	1,187.3	1,165.3

The dependence of the average mixture mass on the fermentation time is shown in Fig. 5.

The graph of average values was found within the error range, verifying their reliability. The correlation coefficient was 0.98.

The mathematical dependence of the processed mixture mass on the fermentation time was described by the following regression expression:

$$M_m = 1{,}293.9 - t \cdot 17.83 \qquad (2)$$

where M_m – the processed mixture mass, kg; t – fermentation time, day.

As seen from Fig. 5, the average mass of the loaded mixture was 1,287 kg. Under the specified operating mode, the mass loss was 3% already on the third fermentation day and 7% – on the fifth fermentation day. As a result, the mass of the ready loose organic fertiliser was 9% smaller than the loaded mixture mass and amounted to 1,176.3 kg.

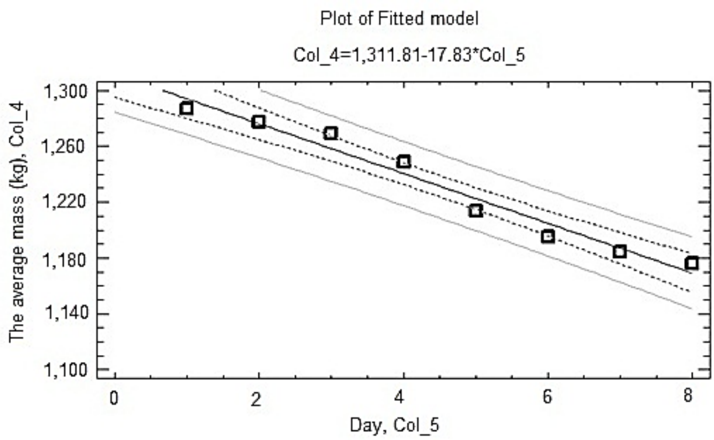

Figure 5. Dependence of the processed mixture mass on the fermentation time.

The obtained experimental data on the moisture content of the processed mixture and the results of their statistical analysis are presented in Table 3.

The results showed that the average values fell in the range of standard deviations, therefore, the data were reliable and no suspect data were available.

According to the study results, the mass of the processed mixture decreased due to changes in the moisture content.

Table 3. Moisture content data of the mixture processed

Fermentation stage	Average moisture content $\bar{x}$, %	Dispersion, *D*	$\bar{x}+\sigma$	$\bar{x}-\sigma$	Upper interval limit, *P1*	Lower interval limit, *P2*
Mixture loading into the fermenter	68.7	0.1	69.01	68.39	69.2	68.2
Fermentation, day 1	68.7	0.25	69.2	68.2	69.5	67.9
Fermentation, day 2	67	0.05	67.2	66.78	67.4	66.6
Fermentation, day 3	66.7	0.02	66.8	66.57	66.9	66.5
Fermentation, day 4	66.9	0.02	67.03	66.77	67.1	66.7
Fermentation, day 5	67.1	0.002	67.15	67.06	67.2	67
Fermentation, day 6	67	0.002	67.05	66.96	67.1	66.9
Fermentation, day 7, unloading	66.4	0.01	66.49	66.31	66.6	66.2

The mathematical dependence of the processed mixture moisture content on the fermentation time was described by the following expression:

$$MC = \sqrt{\left(4{,}394.4 + \frac{297.9}{t}\right)} \qquad (3)$$

where MC – moisture content of the processed mixture, %; t – fermentation time, day.

Moisture content (MC) was calculated from the experimental data in Table 4 by the least square method.

The specific capital and operating costs associated with the fermentation of the organic waste mixture originating from the pig-rearing complex were calculated. The calculations did not consider the construction and maintenance costs of the buildings and sites for the storage of starting components and ready products. The data obtained were compared with the indicators of the traditional processing technology – the passive composting on a concrete pad.

Specific capital costs of fermentation were 3,220 roubles t^{-1} (46 Euros t^{-1}), those of the passive composting – 1,500 roubles t^{-1} (21.4 Euros t^{-1}); specific operating costs of fermentation were 800 roubles t^{-1} (11.4 Euros t^{-1}), those of the passive composting – 355 roubles t^{-1} (5.1 Euros t^{-1}).

The organic waste fermentation in a drum-type installation has several advantages over the traditional passive composting:

– shorter time required – seven days of fermentation against up to three months of passive composting;

– fermentation guarantees the production of organic fertilisers with a specified quality irrespective of the uncontrollable external factors;

– smaller storages are required as the ready organic fertiliser can be immediately sold or stored on the field-edge sites before application.

CONCLUSIONS

The utilisation technology of the farm organic waste in a special installation, a drum-type fermenter, was tested. The microbiological transformation of waste in the aerobic process has important advantages over the composting – lower mass loss, significantly improved quality of the ready organic fertiliser and shorter processing period.

The optimal operation mode of the fermenter was identified in our previous studies depending on the initial composition of the mixture: aeration frequency of 5 min h^{-1}; air consumption of 11.3 $m^3 h^{-1}$ per 1.7 m^3 of the fermenter volume; drum rotation interval – three times every 12 hours.

When the temperature in the fermentation unit exceeded +55 °C, the required time for the bio-thermic treatment was three days. The further processing of the mixture in the fermentation unit allowed to reduce the mass and moisture content of the ready organic fertiliser with the higher nutrient content.

Under the specified operating mode, the mass loss was 3% already on the third fermentation day and 7% – on the fifth fermentation day. As a result, the mass of the ready loose organic fertiliser was 9% smaller than the mass of the loaded mixture.

The experimental data was used to create the mathematical models showing the dependence of the mass and moisture content of the processed raw material on the fermentation time. The models allow forecasting the processing of the organic waste from the pig farms into an organic fertiliser. The study outcomes allow concluding that the solid-state aerobic fermentation is one of the promising options for the utilization of the solid fraction of pig slurry.

REFERENCES

Aboltins, A., Melece, L. & Priekulis, J. 2019. Model for ammonia emissions' assessment and comparison of various dairy cattle farming systems and technologies. *Agronomy Research* **17**(2), 322–332. https://doi.org/10.15159/AR.19.081

Afanassiev, A., Afanassiev, V. & Kaliuga, V. 2000. Energy-saving technology of litter housing of pigs and two-step biofermentation of manure on pig farms. In: *Problemy intensyfikacji produkcji zwierzęcej z uwzględnieniem ochrony środowiska i przepisów UE. VI Międzynarodową Konferencja Naukowa*, Warszawa, Poland, pp. 421–427 (in English).

Briukhanov, A. & Uvarov, R. 2015. Advanced technologies of biofermentation manure / litter for North-West Russia. *Naucnoe obozrenie* **16**, 26–31 (in Russian).

Dąbrowska, M., Świętochowski A. & Lisowski A. 2019 Physicochemical properties and agglomeration parameters of biogas digestate with addition of calcium carbonate. *Agronomy Research* **17**(4), 1568–1576. https://doi.org/10.15159/AR.19.161

Poulsen, H., Lund, P, Sehested, J, Hutchings, N & Sommer, SG. 2006. Quantification of Nitrogen and Phosphorus in Manure in the Danish Normative System. In: *12th Ramiran International Conference 'Technology for Recycling of Manure and Organic Residues in a Whole-Farm Perspective'. DIAS Report no. 123.* Research Centre Foulum, Denmark: Danish Institute of Agricultural Sciences, vol. **II**, pp. 105–107.

Priekulis, J., Melece, L. & Laurs, A. 2019. Most appropriate measures for reducing ammonia emissions in Latvia's pig and poultry housing. *Agronomy Research* **17**(3), 797–805.

Rodhe, L. & Jonsson, C. 1999. *Provtagarutrustning för fastgödsel. [Sampler for solid manure]*. Swedish Institute of Agricultural and Environmental Engineering Report no. **252**, Uppsala, Sweden, (in Swedish).

Shalavina, E., Briukhanov, A., Uvarov, R. & Vasilev , E. 2017. Method for selection of pig manure processing technologies *Agronomy Research* **15**(3), 866–876.

Shalavina, E.V., Uvarov, R.A., Vasilev, E.V & Freidkin, I.A. 2019. Pilot study findings of bio-fermentation of the solid fraction of pig manure. *Technologies, machines and equipment for mechanised crop and livestock production* **99**, 326–334 (in Russian).

Sindhöj, E., Kaasik, A., Kuligowski, K., Sipilä, I., Tamm, K., Tonderski, A. & Rodhe, L. 2013. *Manure Properties on Case-Study Farms in the Baltic Sea Region.* Report 417, Agriculture & Industry, JTI – Swedish Institute of Agricultural and Environmental Engineering. Uppsala, Sweden. ISSN-1401-4963.

Uvarov, R., Shalavina, E., Vasilev, E. & Freidkin, I. 2019. Optimisation of substrate based on solid fraction of pig manure for solid-phase aerobic fermentation. *Technologies, machines and equipment for mechanised crop and livestock production* **100**, 187–196 (in Russian).

Valge, A., Dzhabborov, N. & Eviev, V. 2015 *Fundamentals of statistical processing of experimental data for research in mechanisation of agricultural production with examples in STATGRAPHICS and EXCEL.* Saint Petersburg; Elista: Kalmyk Univ. Publ. 140 pp. (in Russian).

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Study of influence of proteolytic action fodder additive in the composition of feed on productivity and development of broilers chickens' internal organs

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Abstract. In the system of comprehensive assessment of feed nutritional value, protein plays a special role. With the correct selection of proteolytic enzymes in the diet and with appropriate conditions (pH, temperature, duration and quantity of enzymes), most feed proteins can be degraded to preferred degree in the body: either partially, limited by proteolysis, or deep and complete – to individual amino acids, which is accompanied by an increase in animal productivity.
The objective was to study the efficiency of using the exogenous enzyme Sibenza DP 100 in the diet on productivity and development of broilers chickens' internal organs. The experimental part of the work was carried out under the industrial conditions of private limited company 'LISKoBroiler' on Ross 308 broiler chickens. When the proteolytic enzyme was added to the birds' diet without decreasing the nutritional value of crude protein and digestible aminoacids, higher values of the average daily gain were observed among all experimental groups compared to the control group, while reducing feed output per 1 kg of gain. While reducing the nutritional diet value in accordance with the matrix by 2.5% for crude protein and digestible amino acids and with the addition of the Sibenza DP 100 enzyme (500 g t^{-1}), growing broilers was characterized by a slight lag in live weight compared to the control group, but with the most effective consumption feed per 1 kg of gain. The abovementioned system for the proteolytic enzyme use had the most positive effect on the morphological structure of the pancreas of broiler chickens.

Key words: enzyme preparation, protease, broiler chickens, pancreas, nutrient digestibility, intestinal length.

INTRODUCTION

It is a fact that the nutritional value of feed cannot be expressed by one indicator – it must be comprehensive. In the system of a comprehensive assessment of the nutritional value of feed, protein plays a special role. The presence of protein in the diet in disproportionate amounts and with an imbalance in amino acids significantly affects the growth and development of birds and many metabolic processes.

Enzymes have been used in world animal husbandry since the 1970s. They were the first to be used in Scandinavian countries – Finland and Denmark (Fomina, 2008). In foreign countries, Clickner F.H. and Follwell E.H. in 1926 for the first time reported an improvement in the growth of chickens and an increase in egg production as a result of the addition of enzyme preparation to bird ration. In numerous experiments carried out by foreign specialists in feeding birds with enzymes, we see that all available enzymes at the initial stage of study were implemented into bird rations, not taking into account their activity and only in experimental manner and then laboratory studies revealed substrates for each enzyme (Hennig, 1976).

Previously, all enzymes with several activities had a proteolytic effect as concomitant, but not basic. From 2012 to 2019, 4 enzymes of proteolytic action were registered in Russia. Currently, the use of enzymes with protease activity in feeding poultry is of great interest among specialists.

With the right selection of proteolytic enzymes and with appropriate conditions (pH, temperature, duration and amount of enzyme), most feed proteins can degraded to preferred degree: either partially, limited by proteolysis, or deep and complete – to individual amino acids (Tsyperovich, 1971; Tikhonov & Yudina, 2014; Booker, 2015). Proteases have a relatively broad specificity. This means that they can decompose a variety of bonds in the protein molecules, while certain bonds in the peptide chains can be rapidly degraded, but, in addition, they can also decompose many other uncharacteristic bonds. Therefore, in each case it is necessary to carefully select the used proteinase complex or a combination of enzymes. Usually, the suitability of the enzyme system chosen for this use can be determined using special control experiments (Tsyperovich, 1971; Angel et al., 2011; Ajayi, 2015).

Metabolic processes, occurring in the body of birds, largely depend on the morphological and functional features of the digestive system (Fisinin, 2010; Fisinin & Egorov, 2011; Cowieson & Roos, 2014). In this regard, the subjective on the proteolytic enzyme influence on the productive indicators of broiler chickens and on the development of internal organs is relevant and has practical interest for poultry producers (Yu et al., 2007). The objective is to study the effectiveness of use of the exogenous proteolytic enzyme Sibenza DP100 in the diet of broiler chickens.

The aim is to study the influence of exogenous proteolytic enzyme Sibenza DP100 in the ration for productive indicators and the development of internal organs of broiler chickens.

MATERIALS AND METHODS OF RESEARCH

Under industrial conditions of private limited company 'LISKoBroiler' of Cherkizovo Group of Companies, Liskinsky District, Voronezh Region.

The material for carrying out scientific and industrial experiments was the broiler chickens of ROSS – 308 cross. Broilers breeding took place on a deep litter on the floor in specifically prepared mini insulators in windowless rooms with adjustable microclimate and with mechanization and automation of the drinking process. The litter is presented by husk of sunflower seeds 2–3 cm thick. All feeding and drinking equipment is presented and organized by BigDutchman. The microclimate in the housing is fully automated and controlled by viper computers by BigDutchman.

Technological parameters of keeping and feeding broiler chickens corresponded to cross breeding recommendations. Bird feeding was carried out manually, feed replenishing was done as it was eaten by birds. The live weight of chickens when starting the experiment in the daily age was on average 42 g. The landing density was 20, 22 heads m^{-2}.

Experimental birds were subjected to veterinary treatment according to the scheme of preventive measures adopted at the enterprise (annex 1).

For conducting all experiments chickens were selected in groups on the principle of analogues – the same in origin, age, general development. The bird intended for the experiment was individually weighed and distributed into groups by random sampling. The protease was introduced into the feed for the entire rearing cycle – 39 days. Feeding broiler chickens was carried out with full feed, which corresponded to the recommendations of Ross cross. All components of feed were subjected to zootechnical analysis immediately before preparation. Optimization of rations was carried out using программного 'CORMOPTIMA' complex. Rations based on corn, soybean protein meal, meat – feather meal (5.5% of each in the final ration) were used. Feeding broiler chickens was three-phase depending on the от period of cultivation:

Phase 1 – feeding aged 0–14 days – complete feed prepared on recipe PC-5-1.

Phase 2 – feeding aged 15–24 – complete feed prepared on recipe PC-5-2.

Phase 3 – of feeding aged 25–39 days – complete feed prepared on recipe PC- 6 1.

Table 1. Scheme of the scientific and economic experience

Groups	Amount of birds	Diet
Control	40	main diet (MD) – complete broiler fodder
1st experimental	40	nutritional value of MD is reduced in accordance with the matrix by 2.5% for crude protein and digestible amino acids without the inclusion of Sibenza DP 100
2nd experimental	40	MD + Sibenza DP 100 500 g t^{-1}
3rd experimental	40	nutritional value of MD is reduced in accordance with the matrix by 2.5% for crude protein and digestible amino acids with addition of Sibenza DP 100 in a dose of 500 g t^{-1}
4th experimental	40	nutritional value of MD is reduced in accordance with the matrix by 5% for crude protein and digestible amino acids with addition of Sibenza DP 100 in a dose of 500 g t^{-1}
5th experimental	40	nutritional value of MD is reduced in accordance with the matrix by 7.5% for crude protein and digestible amino acids with addition of Sibenza DP 100 in a dose of 500 g t^{-1}

According to the scheme of scientific and economic experience (Table 1), 5 groups of broiler chickens were formed: 1control group and 5 experimental groups, with 40 birds in each group. The control group received the main diet – complete fodder. In broilers of the 1st experimental group, the nutrition of the main diet was reduced by 2.5% for crude protein and digestible amino acids in accordance with the matrix recommended by the manufacturer, without the inclusion of the proteolytic Sibenza DP 100 enzyme. Chickens of the 2nd experimental group received the main diet with the addition of the Sibenza DP 100 enzyme in an amount of 500 g t^{-1}. The diet of broilers the 3rd experimental group was reduced in accordance with the matrix by 2.5% for crude protein and digestible amino acids with the addition of Sibenza DP 100 (500 g t^{-1}). Birds of the

4th experimental group was fed the main diet with a decrease in nutrition in accordance with the matrix by 5% for crude protein and digestible amino acids with the addition of the Sibenza enzyme DP 100 (500 g t^{-1}). In broilers of the 5th experimental group, the nutritional value of the main diet was reduced by 7.5% in crude protein and digestible amino acids with the addition of the Sibenza DP 100 enzyme (500 g t^{-1}).

The experimental chickens were reared on floor system in specially prepared mini isolators. The live weight of chickens averaged 42 g in the age of 1 day, when they were set for the experiment.

The digestibility of feed nutrients was studied in the course of balance experiments, according to the method of All-Russian Research and Technological Poultry Institute of Russian Academy of Sciences (Egorov et al., 2013).

At 24th day of age, 3 broiler males with weight corresponding to the group average were selected from each group. The birds were slaughtered and microscopic examined for the pancreas condition. The material was prepared by fixing in a 10% aqueous solution of neutral formalin and then preparing histological sections of 5–7 μm thickness on the MHC-2 microtome and staining them with Mayer hematoxylin and eosin stain.

The length of the intestine and the weight parameters of the pancreas were taken into account by individual measurements during slaughter on the 24th day and 35th day (3 animals from each group). The pancreas was weighed on a BM-313 electronic laboratory balance; division value of 0.001 g.

RESULTS

The research results showed that the introduction of protease into the diet without reducing the nutritional value of crude protein and digestible amino acids from 1 day of age to the end of fattening has a positive effect on the bird weight. So, in the 2nd experimental group, the chicken weight at the end of fattening (39th day) exceeded the control group value by 0.68% (Table 2). In other experimental groups at the end of the technological cycle, this indicator was lower than in the control group: in the 1st experimental group by 2.6%; in the 3rd experimental group by 0.68%; in the 4th experimental group by 3.7% ($P \leq 0.05$); in the 5th experimental group by 7.2% ($P \leq 0.001$). At the same time, in the 3rd experimental group that received a diet with a minimum reduction in nutritional value for crude protein and digestible amino acids in accordance with the matrix by 2.5% and with the addition of Sibenza DP 100 in a dose of 500 g t^{-1} of feed, the lag in weight gain was the most minimal if compared to the control group indicator.

Table 2. Productive indicators of broiler chickens

Indicator	Group					
	Control	1	2	3	4	5
Weight at 39 day of age, g	2,345 ± 26.9	2,284 ± 25.85	2,361 ± 27.15	2,329 ± 26.58	2,258* ± 26.27	2,177*** ± 31.70
Daily average weight gain, g	59.04	57.49	59.46	58.64	56.82	54.74
Viability, %	95.00%	95.00%	95.00%	95.00%	92.50%	95.00%
Feed output per 1 kg of weight gain, kg	1.704	1.739	1.683	1.67	1.741	1.76

*Note: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.*

Analysis of feed output per 1 kg of weight gain of the experimental bird showed (Table 2) that they were the smallest in the 3rd experimental group. In broilers of the 2nd experimental group, this indicator was also lower than in the control group – by 0.02 kg, which amounted to 1.68 kg. The best results in terms of feed output in the 2nd and 3rd experimental groups indicate a higher digestibility and the use of nutrients of complete fodder, which ensured the greatest increase in the weight gain in these groups. In the 1st, 4th and 5th experimental groups, feed output were higher in comparison with the control group by 0.035–0.056 kg.

The results of the balance experiment showed that the chickens of the 4th experimental group differed in the best digestibility of the dry matter; the difference with the control group for this indicator was 1.9%. Broilers of the 2nd, 3rd and 5th experimental groups were also characterized by higher digestibility of dry matter in comparison with the control group by 1.6%, 1.2% and 1%, respectively. In the 1st experimental group there was a decrease in this indicator in comparison with the control group by 0.4%. Digestibility of feed protein in broilers of the 2nd, 3rd and 4th experimental groups was higher than in the control group by 3.2%, 2.1% and 1.1%, respectively. In chickens of the 1st and 5th experimental groups were no differences in this indicator compared to the control group. The digestibility of fat in birds of the 2nd and 3rd experimental groups was higher than in the control group by 0.86% and 0.26%, respectively, and in chickens of the 1st, 4th and 5th experimental groups was lower by 1.72%, 1.24% and 1.0%, respectively. Fiber digestibility in the 2nd and 3rd experimental groups was higher than in the control group by 1.71% and 0.44%, respectively. Chickens of the 1st, 4th and 5th experimental groups showed a decrease in fiber digestibility in comparison with the control group in the range of 0.3–0.66%. The highest consumption of calcium was observed in the 2nd, 3rd, 4th experimental groups. The 1st and 5th experimental groups were characterized by a decrease in calcium absorption by 0.6% and 0.2%, in comparison with the control group. The phosphorus consumption by broilers of all experimental groups was slightly lower than that of the control group in the range of 0.1–0.88%.

Table 3. Digestibility and use of feed nutrients by broilers with the introduction of Sibenza DP 100 in the diet

Indicator	Group Control	1	2	3	4	5
Digestibility:						
of dry matter	74.6	74.2	76.2	75.8	76.5	75.6
proteins	89.6	89.7	92.8	91.7	90.7	89.4
fats	88.84	87.12	89.7	89.1	87.6	87.84
fibers	29.16	28.3	30.87	29.6	28.86	28.5
Consumption of:						
Calcium	51.5	50.9	52.54	52.13	51.8	51.36
Phosphorus	46.64	45.9	46.1	46.54	45.76	45.8

Based on the analysis of the nutrient digestibility, we can conclude that the best nutrient uptake was in the 2nd and 3rd experimental groups (in comparison with the control group), where the protease was added in addition to the main diet without reducing the nutritional value of the diet and with a minimum decrease in the nutritional value of the diet by 2.5% in crude protein and digestible amino acids. A tendency towards a decrease in the digestibility of food nutrients in chickens in the 4th and 5th experimental groups was observed, which indicates that a decrease in the nutritional value of the diet by 5.0% and 7.5% while feeding protease in an amount of 500 g/t is impractical. This statement is confirmed by a lower weight of chickens in these groups and an increase in feed output per 1 kg of gain.

The weight of the pancreas in broiler chickens is usually 3.55–3.81 g (Shneiberg et al., 1987). According to Somova (2012) the most active growth of this organ is recorded in chickens at age of 1 to 14 days (Somova, 2012). In general, the highest rates of pancreas growth in birds are recorded according to various authors up to 30 days of age, after which there is a gradual slowdown in the increase in the absolute weight of the organ (Bodrova, 2011; Matveev & Zhambulov, 2017).

When analyzing autopsy of chickens at 24 days (the period of changing from grower to finale diet) and before including meat and feather meal in the diet, in the conditions of this poultry farm, we see the following differences between the groups.

The pancreas weight in chickens of the 2nd and 3rd experimental groups was higher than the control by 2.79 and 9.22%, respectively, amounting up to 3.68 and 3.91 g. The pancreas weight in broilers of the 1st, 4th and 5th experimental groups was less than of the control group value by 0.84%, 7.2% and 12.29%, respectively (Fig. 1).

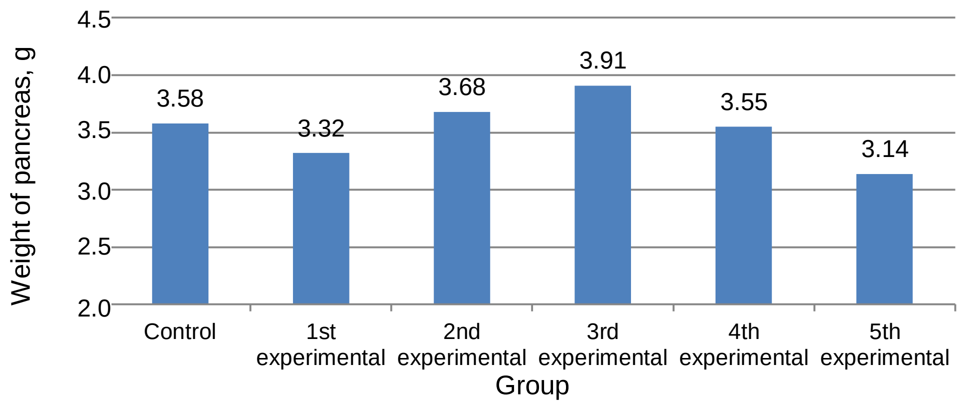

Figure 1. The weight of the broiler pancreas on 24th day, g (n = 3).

Hydrolysis of food nutrients occurs in all parts of the digestive tract, however, its highest intensity is observed in the small intestine. The feed uptake in broiler chickens is more intensive mainly due to the greater intestinal length, which ensures rapid growth and high meat yield of birds (Shestakov et al., 2012; Korsakov et al., 2019; Kablucheeva, 2000).

The intestinal length in broilers of the 2nd, 3rd, 4th experimental groups was 2.26%, 3.95% and 3.39% higher than that of the control group, and was estimated to 181–183 cm, compared with the length of the intestines of the control group, which was 177 cm (Fig. 2). The 5th experimental group showed no differences in the intestine length compared with the control group. In birds of the 1st experimental group, which received a nutritionally reduced diet in accordance with the matrix by 2.5% for crude protein and digestible amino acids and did not receive Sibenza DP 100, the intestinal length was 168 cm, which was 5.08% lower than in the control group. The established difference negatively affected the assimilation of nutrients, as evidenced by the results of the balance experiment.

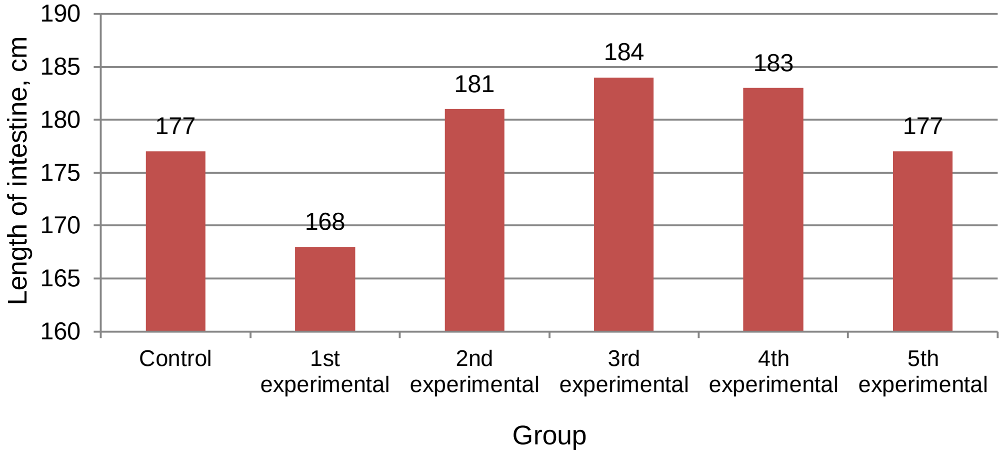

Figure 2. The length of the intestines of broilers on 24th day, cm (n = 3).

At 35 days of age, the weight of the pancreas in chickens of the 1st, 2nd, 3rd, 4th and 5th experimental groups was higher by 4.80%, 6.47%. 1.25%, 4.38% and 0.21%, respectively, than in the control group (Fig. 3).

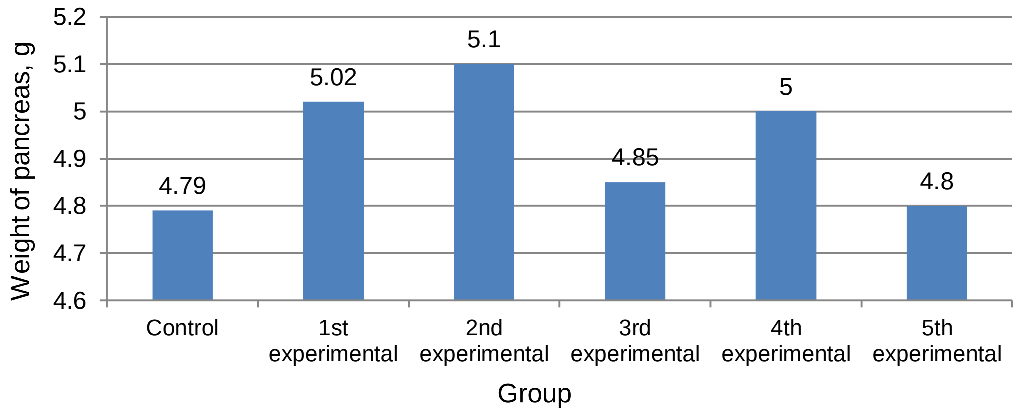

Figure 3. The weight of the broiler pancreas on 35th day, g (n = 3).

The intestinal length in broiler chickens of the 1st, 3rd, 4th and 5th experimental groups was lower by 13.68%, 1.35%, 8.97% and 13%, respectively, than in the control group, and amounted to 192.5, 220, 203 and 194 cm, respectively, while intestinal length of the control group was 223 cm. The length of the intestine in broilers of the 2nd experimental group, by the 35 day of age, exceeded the control group by 2.24%. The experimental group that consumed a diet with a minimal decrease in nutritional value for crude protein and digestible amino acids with the addition of protease had a slightly lower intestinal length by 1.35% compared with the control group (Fig. 4).

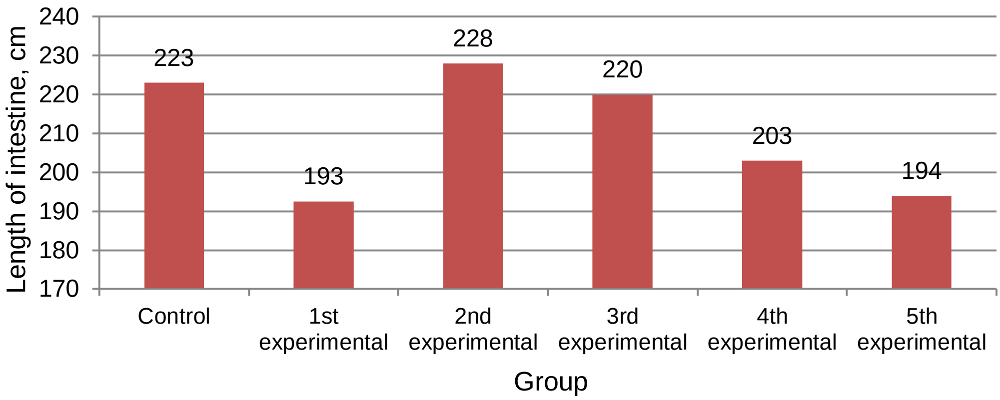

Figure 4. The length of the intestines of broilers on 35th day, cm (n = 3).

The pancreas is one of the vital organs of the digestive system, which produces enzymes that hydrolyze all the essential nutrients to monomers that can be absorbed into the blood and lymph. Digestive cells produce hydrolytic enzymes according to the general principles of protein synthesis. The endocrine part of the pancreas is represented by islets of Langerhans (Somova, 2007; Cyganova et al., 2008; Fisinin, 2017).

In a histological study of the pancreas in broiler chickens of the control group, we saw that the ducts are well functionally (Fig. 5), the islets of Langerhans were small and also had no physiological deviations (Fig. 6); the islets of Langerhans in one of the birds were larger with intensification functional activity.

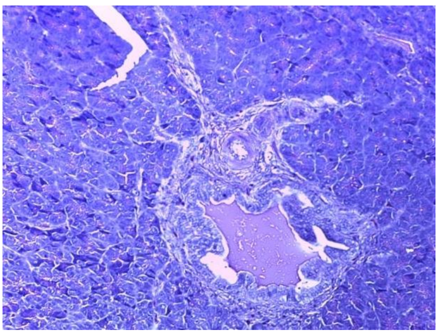

Figure 5. Histologic pattern of the pancreatic duct in chickens of the control group. Age 24 days. H&E stain. x400.

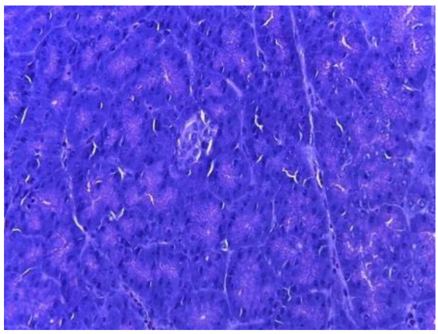

Figure 6. Histologic pattern of islets of Langerhans in broilers of the control group. Age 24 days. H&E stain. x400.

Birds of the 1st experimental group, which consumed a diet with a reduced nutritional value without protease administration, had inflammatory infiltrate in the pancreatic ducts (Fig. 7). Also, hyperemia of the vessels in the pancreas and an enhanced reaction of pancreatocytes was observed. The islets of Langerhans were activated.

When examining the pancreas of broiler chickens of the 2nd experimental group, we observed that the islets of Langerhans were small, normally functioning; the vessels were moderately blood-filled (Fig. 8). No inflammatory processeswere observed.

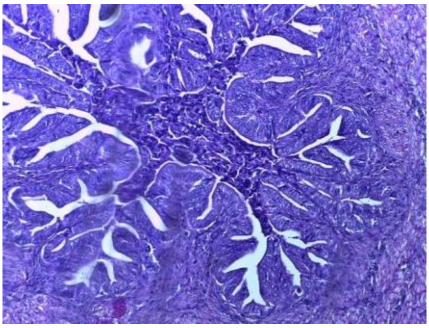

Figure 7. Histologic pattern of the pancreatic duct in chickens of the 1st experimental group. Age 24 days. H&E stain. x400.

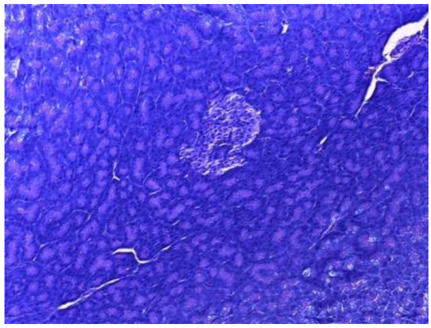

Figure 8. Histologic pattern of islets of Langerhans in chickens of the 2nd experimental group. Age 24 days. H&E stain. x400.

When examining the pancreas of the 3rd experimental group, we saw that the pancreas was somewhat activated, one of the ducts was without pathology (Fig. 9), in other – the epithelium was smoothed, outgrown; lumen of the duct there had a complex thick secret, islets of Langerhans were without pathology.

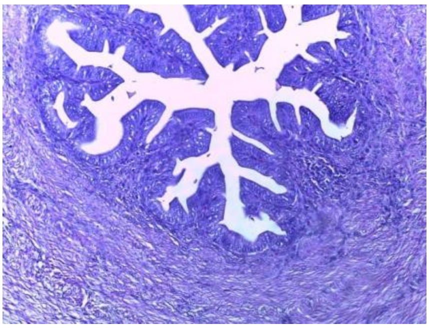

Figure 9. Histologic pattern of the pancreatic duct in chickens of the 3rd experimental group. Age 24 days. H&E stain. x400.

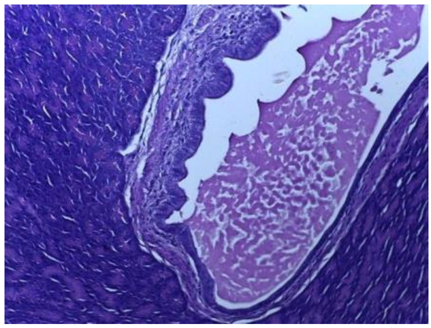

Figure 10. Histologic pattern of the pancreatic duct in chickens of the 4th experimental group. Age 24 days. H&E stain. x400.

The 4th experimental group that consumed a diet with reduced nutrition in accordance with the 5% matrix for crude protein and digestible amino acids and with the protease administration, had vascular hyperemia in the pancreas, the epithelium of ductswas smoothed, the wall was overgrown and there was a large amount of secretion in the lumen (Fig. 10), the islets of Langerhans had moderate secretion.

The 5th experimental group that consumed a diet with reduced nutrition in accordance with the matrix of 7.5%

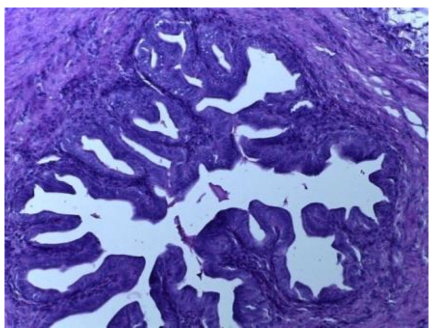

Figure 11. Histologic pattern of the pancreatic duct in chickens of the 5th experimental group. Age 24 days. H&E stain. x400.

in crude protein and digestible amino acids and with the protease administration, had the following changes in the pancreas: the duct epithelium was hyperplastic (Fig. 11), there was an increase in the number of islets of Langerhans per unit area.

CONCLUSION

On the basis of the conducted studies, it can be concluded that the introduction of the Sibenza DP 100 protease in addition to the main diet of broiler chickens, without reducing the nutritional value of crude protein and digestible amino acids, has a positive effect on the weight, feed output per 1 kg of weight gain and nutrient absorption. An analysis of the internal organs development indicates more intense metabolism in the body of broiler chickens in this experimental group. That is confirmed by the results of a morphology study of the pancreas, characterized by the best condition of the organ ducts and the absence of inflammatory processes.

The experimental group, which received a diet with a minimum decrease in nutritional value for crude protein and digestible amino acids in accordance with the matrix by 2.5% and the addition of Sibenza DP 100 – 500 g t^{-1}, was characterized by the smallest lag in weight gain against the background of the control group indicator, compared with groups where the nutritional value of the diet was reduced by 5% and 7.5% with the enzyme administration, while the best feed output per 1 kg of weight gain were noted.

The use of Sibenza DP 100 in broiler diets, with a decrease in nutrition in accordance with the matrices by 5% and 7.5% for crude protein and digestible amino acids in total of the studied parameters, was not effective, accompanied by a decrease in bird productivity.

REFERENCES

Ajayi, H.I. 2015. Effect of protease supplementation on performance and carcass weights of broiler chickens fed low protein diets. *Nigerian Journal of Agriculture, Food and Environment* **11**(1), 29–32.

Angel, R.A., Saylor, W., Vieira, S.L. & Ward, N. 2011. Effects of a monocomponent protease on performance and protein utilization in 7- to 22-day-old broiler chickens. *Poultry Science* **90**, 2281–2286.

Bodrova, L.F. 2011. *Clinical and hematological parameters and morphological characteristics of the internal organs of chickens of different crosses that received low-energy feed mixtures in an industrial environment*. Dissertation for the Doctor of Veterinary Sciences. 06.02.2001 Omsk, 497 pp.

Booker, I. 2015. It is possible to increase protein digestibility in broiler diets! *Fodder*. No. **10**. pp. 75–76.

Cowieson, A.J. & Roos, F.F. 2014. Bioefficacy of a mono-component protease in the diets of pigs and poultry: a meta-analysis of effect on ileal amino acid digestibility. *Journal of Applied Animal Nutrition*. URL: https://www.cambridge.org/core/journals/journal-of-applied-animal-nutrition/article/ (Date of appeal: 20.03.2019).

Cyganova, O., Shackih, E. & Zhenihova, N. 2008. Morphofunctional condition of the thyroid gland and biochemical blood test chickens-broiler under influence of the different forms of the iodine. *Agrarian Bulletin of the Urals* **10**(52), 78–81.

Egorov, I.A., Manukyan, V.A., Lenkova, T.N, Okolelova, T.M., Lukashenko, V.S., Shevyakov, A.N., Ignatova, G.V., Egorova, T.V., Andrianova, E.N., Rozanov, B.L., Lysenko, M.A., Egorova, T.A., Grozina, A.A., Laptev, G.Yu., Nikonov, I.N., Alexandrova, I.L., Ilyina, L.A., Novikova, N.I. 2013. Methodology for conducting scientific and industrial research on feeding poultry. Molecular genetic methods for the determination of intestinal microflora. *All Sergiev Posad*, pp. 53.

Fisinin, V.I. 2010. Innovations in Russian poultry industry. *Bulletin of the Russian Academy of Agricultural Sciences* **1**, pp. 9–12.

Fisinin, V. & Egorov, I. 2011. Modern approaches to poultry feeding: *Ptitsevodstvo*, **3**, pp. 7–9.

Fisinin, V.I., Egorov, I.A., Vertiprahov, V.G., Grozina, A.A., Lenkova, T.N., Manukyan, V.A. & Egorova, T.A. 2017. The activity of digestive enzymes in the duodenal chyme and blood plasma in the starting lines and hybrids of meat chickens using dietary supplements in the diet Agricultural Biology, Volume 52, **6**, pp. 1226–1233.

Fomina, O. 2008. Enzymes not for everyone. *Agrotechnics and technology* URL: https://www.agroinvestor.ru/technologies/article/14693-fermenty-ne-dlya-vsekh/ (accessed on03/20/2019)

Hennig, A. 1976. *Mineral substances, vitamins, biostimulants in feeding farm animals*. *Kolos*, Moscow, 467 pp.

Kablucheeva, T.I. 2000. *Features of digestion in the blind processes of the intestines in young meat chickens with different levels of protein and the use of probiotics in the diet*. Dissertation for the Candidate of Biological Sciences, Krasnodar, 153 pp.

Korsakov, K., Simakova, I., Vasilyev, A., Lifanova, S. & Gulyaeva, L. 2019. The effect of humic acids on the natural resistance of the body of broiler chickens and the quality of their meat. *Agronomy Research* **17**(S2), 1356–1366.

Matveev, O.A. & Zhambulov, M.M. 2017. Morphometric indices of the digestive organs of broiler chickens of the cross Ross 308, *News-bulletin of Orenburg State Agrarian University*. No. **1**(63), pp. 119–122.

Shestakov, I.Yu., Ovchinnikov, D.K., Shvedov, S.I. & Krasnikova, L.V. 2012. Morphological aspects of intestinal growth of hens crosses “Siberian” and “Rhodonite-2”. *Omsk Scientific Bulletin*. No. **1**(108), pp. 210–212.

Shneiberg, Ya.I., Nikodimova, TV, Suleymanov, F.I. & Chaplygina, N.A. 1987. Changes in the correlation of the structure of organs in ontogenesis of chickens and when exposed to ergotropics. *Age and ecological morphology of animals in conditions of intensive animal husbandry*: scientific papers compilation. Publishing House of Ulyanovsk State Agricultural Institute, Ulyanovsk, pp. 160–163.

Somova, O.V. 2007. Micromorphology of the pancreas of hens in postnatal ontogenesis. *Scientific notes of Vitebsk State Academy of Veterinary Medicine*, Volume **43**(Issue 2), pp. 252–255.

Somova, O.V. 2012. Morphometric indicators of the exocrine pancreas of chickens at different age periods. *Scientific notes of Vitebsk State Academy of Veterinary Medicine* **1**(48), 142–145.

Tikhonov, G.P. & Yudina, T.A. 1971. The basics of biochemistry. MGAVT *Altair*, 2014, Moscow, 184 pp.

Tsyperovich, A.S. 1971. Enzymes. *Tekhshka*, Kiev, 360 pp.

Yu, B., Wu, S.T., Liu, C.C., Gauthier Robert, Chiou Peter, W.S. 2007. Effects of enzyme inclusion in a maize–soybean diet on broiler performance. *Animal Feed Science and Technology*. Volume **134**(3–4), 283–294.10.

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Response surface for biodiesel production from soybean oil by ethylic route

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Abstract. Petroleum has been the most consumed energy source in the world, but it tends to run out due its non-renewable character. Among biofuels, biodiesel has emerged as the main candidate to substitute petroleum diesel. The present study aimed to identify the maximum yield point of biodiesel production by generating a response surface using molar ratio, temperature and agitation time as independent variables, and yield as a dependent variable. From the response surface, it is observed that the increase in temperature and reaction time leads to reduced yield. The configuration that resulted in maximum yield of 93.30% was 12:1 molar ratio, 30 °C temperature and 30-minute reaction time. From the chromatographic analysis it was possible to identify five different fatty acids in the composition of the biodiesels. Total saturated fatty acids (palmitic and stearic acids) ranged from 41.53% to 42.09% and total unsaturated fatty acids including monounsaturated and polyunsaturated fatty acids (oleic, linoleic and linolenic acids) ranged from 57.92% to 58.48%. According to the results of the physicochemical analyses, the specific mass at 68°F is in agreement with Brazilian, American and European specifications, ranging from 877.46 kg m^{-3} to 879.64 kg m^{-3}. The kinematic viscosity at 104 °F ranged from 4.49 mm² s^{-1} to 4.82 mm² s^{-1}. The acid value obtained did not vary within the limits established by the norms, and values between 0.54 and 2.74 mg KOH g^{-1} were observed.

Key words: biofuels, ethanol, optimization, transesterification.

INTRODUCTION

The most consumed energy source in the world is fossil fuel, derived from petroleum, but this raw material tends to run out due to its non-renewable nature (AMBAT et al., 2018). In addition, its use has been causing severe damage to the environment, from the extraction process to the final consumption. Thus, the study of alternative, clean and renewable energy sources is extremely important.

Dantas et al. (2016) state that the use of biomass has been gaining significant relevance due to the possibility of being used in the production of heat, either for industrial thermal use, or for electricity generation and/or for the possibility of being transformed into other forms of solid energies (charcoal), liquid energies (bioethanol, biodiesel, bio-oil) and gaseous energies (biogas).

For Razack & Duraiarasan (2016), among biofuels, biodiesel has become increasingly important as a substitute for petroleum diesel. This is because, according to Ambat et al. (2018), as it is derived from renewable raw materials, such as vegetable oils and animal fats, biodiesel promotes sustainable development through energy savings, reduces the need to import diesel oil, in addition to presenting characteristics such as low toxicity and low emission of polluting gases.

For Knothe et al. (2006), biodiesel can be obtained through several technologies, namely: cracking, esterification and transesterification, which involve the management of variables such as the alcohol:oil molar ratio, temperature, reaction time and catalyst quantity, which are decisive for the efficiency of the production process of this fuel. According to Bet-Moushoul et al. (2016) the transesterification process has been the most used industrially. Transesterification consists of the reaction of triglycerides in vegetable oil and an alcohol, in the presence of a catalyst, which can be acidic or basic, and generates glycerol as a by-product (MUSA, 2016).

Biodiesel production can be carried out from a wide variety of raw materials, which include most vegetable oils and animal fats, as well as residual oils and fats (Sirviö et al. (2018). Among these raw materials, soy stands out, which in January 2017 was responsible for approximately 65% of the biodiesel production in Brazil (ANP, 2017).

According to Musa (2016) and Jokiniemi & Ahokas (2013), the most used alcohols for the transesterification of biodiesel are methanol and ethanol. Other types of alcohol can also be used, as, for example, propanol, butanol, isopropanol, tert-butanol, octanol and branched alcohols, but the cost is much higher. Still according to the author, despite the several advantages of using methanol over ethanol (shorter reaction time, greater reactivity, lower reaction consumption, etc.), its use demands greater care in handling due to its greater volatility and to its highly toxic character. The use of ethanol, on the other hand, has the advantages of producing a 100% renewable fuel, a greater number of ketanes in ethyl esters and greater oxidative stability.

According to Meneghetti et al. (2013), the basic homogeneous catalysts NaOH and KOH have been the most used for the industrial biodiesel production. In homogeneous catalysis, both the catalyst and the reagents are in the same phase, forming a uniform mixture and, thus, the catalyst effectively participates in the reaction, but at the end of the process it is not consumed.

According to Victorino et al. (2016), the composition of the glycerin generated as a by-product may vary according to the raw material chosen for transesterification. Containing from 50% to 60% of glycerol and other substances inherent to the process, glycerin has several uses, from its utilization in the food industry to its use as a substrate in fermentation processes.

The molar ratio directly influences the yield of the transesterification reaction, and although the stoichiometric ratio is 3:1 (three moles of alcohol to one mole of triglyceride), it is necessary to use excess alcohol (6:1, 9:1, etc.) so that the balance of the reaction is disturbed and favors the formation of esters, as observed in the works of Borges et al. (2014), Peiter et al. (2018) and Giuffrè et al. (2017), the reaction temperature also has great influence on transesterification, since, in addition to promoting high percentages of biodiesel conversions, it also reduces the time in which these reactions are carried out. Temperatures of alcohols below the boiling point are the most used, as observed in the work of Joshi et. al. (2017), who worked with temperatures in the range of 40 °C, 50 °C and 60 °C.

Knothe et al. (2006) explain that the biodiesel quality determines the good functioning and useful life of diesel engines. The fuel must present appropriate physicochemical properties to ensure complete combustion and adequate engine performance. At the laboratory level, the most observed features for the characterization of produced biodiesel are specific mass at 20 °C, kinematic viscosity at 40 °C, acid value, saponification, ester content, cold filter plugging point and flash point.

Aiming to optimize biodiesel production by ethylic route from soybean oil, the present work aims to determine the biodiesel production process with the best yield using the response surface methodology (RSM) by varying the production factors (molar ratio, reaction time and temperature).

MATERIAL AND METHODS

Experiments realization site

Biodiesel production and analysis were performed at the Agricultural Machinery Laboratory (LABMAQ) of the Agricultural and Environmental Engineering Department and at the Teaching Laboratory of the Chemical and Petroleum Engineering Department (LENTEQ), both located at the School of Engineering at the Federal Fluminense University (UFF).

Raw materials used

The oil used in the present work was industrialized soybean oil. The ethanol and the catalyst used were Ethanol P.A. with 99.5% purity and sodium hydroxide (NaOH) in micropearls, respectively.

Table 1. Variation sources used in the work

Oil	Soybean		
Catalyst	Sodium Hydroxide (NaOH)		
Molar ratio (Ethanol:Oil)	6:1	9:1	12:1
Reaction Temperature (°C)	30	40	50
Reaction Time (min)	30	60	120
[mbo] Oil Mass (g)	100	100	100
Catalyst Mass (g)	1	1	1
[mba] Ethanol Mass (g)	31.588	47.381	63.175

Experiment planning

The following factors were evaluated: molar ratio alcohol:oil, temperature and reaction time for three levels, according to Table 1. The values of the production factors were obtained through a bibliographic survey of previous studies on biodiesel production by ethylic route. The experiments were carried out in triplicate.

The amount of oil used in the procedures was fixed at 100 g in order to be able to determine the amount of alcohol used for each molar ratio. The amount of catalyst used was 1% in relation to the oil mass. The average molar mass of soybean oil was presented by Almeida (2016) with a value of 874.9 g mol^{-1}. The chemical composition in fatty acids of soybean oil is shown in Table 2. The molar mass of ethanol is informed by the manufacturer with a value of 46.06 g mol^{-1}.

Transesterification

Biodiesel was produced through the transesterification process in the presence of a basic catalyst. Initially, the necessary quantities of raw materials (oil, ethanol and sodium hydroxide) were weighed according to the treatment. The oil was then placed in the oven to be preheated to the treatment temperature. Meanwhile, NaOH was diluted in ethanol using a magnetic stirrer at a temperature of 45 °C, producing sodium ethoxide. As soon as the mixture of ethanol with NaOH became homogeneous and the oil reached the preheating temperature, the latter was slowly placed in sodium ethoxide and the treatment temperature was controlled with a thermometer inserted in the Erlenmeyer.

Table 2. Chemical composition in fatty acids of soybean oil used in the work

Fatty Acid		M/M (%)
C14:0	Miristic	0.08
C15:0	Pentadecanoic	0.05
C16:0	Palmitic	10.82
C16:1	Palmitoleic	0.09
C17:0	Margaric	0.09
C17:1	Cis-10-heptadecenoic	0.06
C18:0	Stearic	3.69
C18:1	Oleic	24.18
C18:2 trans	t-linoleic	0.10
C18:2	Linoleic	52.72
C18:3 trans	t-linolenic	0.30
C18:3	Linolenic	6.56
C20:0	Arachidic	0.37
C20:1	Eicosenoic	0.26
C22:0	Behenic	0.46
C24:0	Lignoceric	0.17

SOURCE: Adapted from Scamilhe; Pimenta; Pereira (2016).

After the transesterification process, the mixture was placed in a separating hopper, remaining for 24 hours for the separation of glycerol from biodiesel. At the end of the separation phase, the glycerin was packed in safe containers for proper disposal and the biodiesel needed to go through purification steps as it presented impurities such as unreacted glycerol, ethanol and catalyst residues.

The first stage of purification consists of washing the ‘dirty biodiesel’ with distilled water at a temperature of 50 °C and hydrochloric acid, allowing it to rest for 20 minutes before removing the washing liquid. This procedure was performed three times, following the proportions of 30 mL of distilled water and 3 drops of hydrochloric acid (HCL) for each 100 mL of biodiesel.

According to Mendow & Querini (2013) using different acids in the biodiesel washing process, concluded that the biodiesel samples obtained by any of treatments have similar acid values. A similar phenomenon took place during the washing with hydrochloric, carbonic, citric or phosphoric acid solution. For this reason, the biodiesel samples obtained by any of treatments have similar acid values and are considerably higher than the maximum permitted by the international standard (EN 14214 - acid value = 0.5 mg KOH g^{-1}). higher than the maximum permitted by the international standard.

In the second stage, the biodiesel went through a drying process in which it was placed in an oven at a temperature of 105 °C ± 3 °C for 2 hours, so that the rest of the distilled water could evaporate. In the last stage, the biodiesel was slowly inserted into a funnel with filter paper, in order to remove the last residues present in the fluid, thus obtaining clean biodiesel.

Production yield

The yield of each treatment was obtained through Equation 1, used by Ambat et al. (2018), just evaluating the values of the oil mass and the clean biodiesel mass produced in each batch.

$$\text{Yield} = \frac{\text{biodiesel mass}}{\text{soybean mass}} \quad (1)$$

Physicochemical characterization

The physicochemical characterization of the produced biodiesel was conducted in accordance with Brazilian, American and European regulations, as indicated by Resolution No. 45 of August 25, 2014 of the Brazilian National Agency of Petroleum, Natural Gas and Biofuels (Agência Nacional do Petróleo, Gás Natural e Biocombustíveis - ANP, 2014). For the characterization of the biodiesel at a laboratory level, the specific mass at 20 °C, the kinematic viscosity at 40 °C and the acid value were used. The reference values adopted, according to the aforementioned Resolution, were: specific mass at 20 °C with a limit between 805 to 900 kg m^{-3}; kinematic viscosity at 40 °C with a limit between 3.0 to 6.0 mm^2 s^{-1} and acid value (max.) with a limit of 0.50 mg $KOH.g^{-1}$, as shown in Table 3 (ANP, 2014).

Table 3. Biodiesel specifications established by Brazilian, American and European regulations

Characteristic	Unit	Limit	Method		
			ABNT NBR	ASTM D	EN/ISO
Aspect	-	LII	-	-	-
Specific Mass at 20 °C	Kg m^{-3}	850 to 900	7,148 14,065	1,298 4,052	EN ISO 3675 EN ISO 12185
Kinematic viscosity at 40 °C	mm^2 s^{-1}	3.0 to 6.0	10,441	445	EN ISO 3104
Acid value, máx.	mg $KOH.g^{-1}$	< 0.50	14,448	664	EN 14104

SOURCE: Adapted from ANP, 2014.

The specific mass was determined in LENTEQ, using the Anton Paar digital densimeter, model DMA 500.

The procedure for obtaining the kinematic viscosity was also carried out in LENTEQ, using the digital controller Schott Gerate, model ASV350, the thermostatic bath Schott Gerate, model CT52 at 40 °C ± 0.05 °C and a CANNON-Fenske viscometer capillary tube nº100 and constant C equal to 0.01481 mm^2 s^{-2}. The kinematic viscosity, ν, was calculated from the measured flow time, t, and the instrument constant, C, using Eq. 2:

$$\upsilon = C\,.\,t \quad (2)$$

where υ – kinematic viscosity, in mm^2 s^{-1}; C – viscosimetric tube calibration constant, in mm^2 s^{-2}; t – flow time, in seconds.

The procedure to determine the acid value consists of using 0.1M solution of potassium hydroxide (KOH) to titrate the free fatty acid in the sample. In each sample, 2 g of oil were weighed in 10 mL of ether and ethanol solution (2:1) and, after homogenization of the solution, it is titrated with a phenolphthalein indicator. The acidity calculation is determined by Eq. 3:

$$I = \frac{V\ .\ 0.1\ .\ f\ .\ 56.1}{P} \quad (3)$$

where I – acid value; f – correction factor for the KOH solution; V – volume of KOH to titrate the sample; 56.1 – gram equivalent of KOH; P – number of grams of the sample.

Analysis by Gas Chromatography coupled to Mass Spectrometry

The samples were qualitatively analyzed in the Gas Chromatograph coupled to Mass Spectrometry (CG-EM) GCMS-QP2010 (Shimadzu, Tokyo, JP) using the following conditions: injection with flow division in a 1:20 ratio; DB5-MS column (30 m × 0.25 mm D.I. and 1 μm of 5% phenyl-polydimethylsiloxane); the carrier gas used was He (99.999% pure) under a constant flow of 3.0 mL min^{-1}; oven temperature setting was 50 °C – 180 °C with heating rate of 8 °C min^{-1}, 180 °C – 230 °C with heating rate of 5 °C min^{-1}, 230 °C – 310 °C with heating rate of 20 °C min^{-1} followed by isotherm for 15 minutes. The chromatographic profiles were made by comparison with the Nist 147 library (US National Institute of Standards and Technology 147), indicating the presence of some methyl esters in the samples. The distribution of the observed substances was determined by normalizing the area of each peak, that is, in percentage of relative chromatographic area.

Statistical analysis

For the statistical analysis, the program SISVAR version 5.3 (FERREIRA, 2014) was used, applying the Tukey test at the significance level of 5%. The statistical analysis was performed using the experimental data obtained in the present study, with a total of 81 observations, estimating the effects on the biodiesel production yield of the studied variables and their interactions.

Making the response surface

To make the response surface, the Matlab R2015b software (academic version) was used. Initially, all data was tabulated using electronic spreadsheets, in which all input and output variables were inserted. After data tabulation, the file was exported to Matlab through a script to generate the response surface based on interpolation.

The response surface was obtained based on the three input variables ((molar ratio (x), stirring time (y) and temperature (z)) and as the output variable the yield. Therefore, the yield was a function of the three input variables, according to Eq. 4:

$$\text{Yield} = f(xq, yq, zq) \quad (4)$$

After obtaining the response surface, the maximum yield point was determined based on the inputs.

RESULTS AND DISCUSSION

Physicochemical characterization of Biodiesel

Specific mass at 20 °C [kg m^{-3}]

Table 4 presents the variance analysis of the effect of the studied variables and their interactions in the specific mass determined with digital densimeter. The obtained results show that the molar ratio and reaction time, as well as the molar ratio*time and time*temperature interactions cause significant changes in specific mass at 20 °C. The significance of each effect is considered by the probability value (*p-value*). The *p-value* was set at 0.05 or 5% for a 95% confidence level in this study. In this analysis, the effects with a *p-value* of less than 0.05 are considered significant effects.

Table 4. Variance analysis of the effect of the studied variables and their interactions on the specific mass determined with digital densimeter

F.V.	*G.L.*	*SQ*	*QM*	*FC*	*P-value*
Molar ratio	2	5.542141	2.77107	62.768	0.0000*
Time	2	0.854719	0.42736	9.680	0.0073*
Temperature	2	0.178319	0.08916	2.020	0.1950^{ns}
Molar ratio * Time	4	0.949570	0.23739	5.377	0.0212*
Molar ratio * Temperature	4	0.659437	0.16486	3.734	0.0533^{ns}
Time * Temperature	4	0.882659	0.22067	4.998	0.0257*
Error	8	0.353185	0.044148		
Total Corrected	26	9.420030			
CV	0.02				
Overall Average	878.4437				

*F.V. = variation source; G.L. = degree of freedom; SQ = sum of squares; QM = mean square; FC = calculated F; Pr > Fc = hypothesis test; * Significant; ns = not significant.*

From the statistical analysis of the molar ratio*reaction time interaction (Table 5), it is possible to observe that the highest specific mass values 879.14, 878.95 and 879.09 kg m^{-3} occurred for a 12:1 molar ratio, with no significant differences for reaction times. The lowest values of specific mass 877.63 and 877.72 kg m^{-3} were observed for a 6:1 molar ratio and the times of 60 and 120 minutes, respectively, with no significant differences between them.

Table 5. Statistical analysis for specific mass determined with densimeter considering molar ratio and reaction time

Time	Molar Ratio		
(min)	6:1	9:1	12:1
30	878.60 Ab	878.32 Aa	879.14 Ba
60	877.63 Aa	878.21 Ba	878.95 Ca
120	877.72 Aa	878.32 Ba	879.09 Ca

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

In the analysis of biodiesel by Peiter et al. (2018), the authors determined the specific mass at 20 °C of biodiesel produced from soybean oil by ethylic route using the molar ratio parameters of 1:10 oil:alcohol and reaction time of 30 minutes, and it was observed that the value of 873.4 kg m^{-3} is in accordance with ANP Resolution No. 45/2014. In the work of Borges et al. (2014), the authors determined the specific mass of soybean biodiesel produced by ethylic route using molar ratios of 9:1 and 12:1 and reaction times of 30 and 60 minutes, making it possible to observe that the specific mass values 870.0 kg m^{-3} and 891.8 kg m^{-3} are also in accordance with ANP Resolution No. 45/2014. Thus, the results obtained in the present study do not differ from those found by Peiter et al. (2018) and Borges et al. (2014) and are in accordance with the parameters of ANP Resolution No. 45/2014.

Kinematic Viscosity at 40 °C [mm^2 s^{-1}]

Table 6 presents the variance analysis of the effect of the studied variables and their interactions on kinematic viscosity. The results obtained show that the molar ratio, as well as the molar ratio*time interaction cause significant changes in the kinematic viscosity at 40 °C. The significance of each effect is considered by the probability value (*p-value*). The *p-value* was set at 0.05 or 5% for a 95% confidence level in this study. In this analysis, effects with a *p-value* of less than 0.05 are considered significant effects.

Table 6. Variance analysis of the effect of the studied variables and their interactions on kinematic viscosity

F.V.	*G.L.*	*SQ*	*QM*	*FC*	*P-value*
Molar ratio	2	0.055563	0.027782	9.147	0.0086*
Time	2	0.009289	0.004644	1.529	0.2739^{ns}
Temperature	2	0.009641	0.004821	1.587	0.2627^{ns}
Molar ratio * Time	4	0.048839	0.012210	4.020	0.0447*
Molar ratio * Temperature	4	0.024089	0.006022	1.983	0.1902^{ns}
Time * Temperature	4	0.015276	0.003819	1.257	0.3615^{ns}
Error	8	0.024297	0.003037		
Total Corrected	26	0.186994			
CV	1.18				
Overall Average	4.659339				

*F.V. = variation source; G.L. = degree of freedom; SQ = sum of squares; QM = mean square; FC = calculated F; Pr > Fc = hypothesis test; * Significant; ns = not significant.*

The interaction molar ratio*reaction time (Table 7) makes it possible to observe that the kinematic viscosity was higher for the molar ratio of 6:1 and time of 30 minutes, in which the value of 4.80 $mm^2 s^{-1}$ was found, differing significantly from other times and molar ratios.

The analysis of the molar ratio*temperature interaction (Table 8) highlights that the molar ratio of 9:1 and the temperature of 30 °C present the lowest value of kinematic viscosity 4.52 $mm^2 s^{-1}$, with significant differences for the other molar ratios.

Table 7. Statistical analysis for kinematic viscosity considering molar ratio and reaction time

Time	Molar Ratio		
(min)	6:1	9:1	12:1
30	4.80 Bb	4.59 Aa	4.66 Aa
60	4.64 Aa	4.60 Aa	4.69 Aa
120	4.64 Aa	4.59 Aa	4.72 Aa

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

Table 8. Statistical analysis for kinematic viscosity considering molar ratio and temperature

Temperature	Molar Ratio		
(°C)	6:1	9:1	12:1
30	4.71 Ba	4.52 Aa	4.69 Ba
40	4.71 Aa	4.65 Ab	4.69 Aa
50	4.65 Aa	4.61 Aab	4.68 Aa

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

In the work of Borges et al. (2014), the authors produced soybean biodiesel by ethylic route and obtained kinematic viscosity values of 4.3 and 4.4 $mm^2 s^{-1}$. The kinematic viscosity value of 5.0625 $mm^2 s^{-1}$ obtained by Peiter et al. (2018) also complies with Brazilian and European standards, which establish values from 3.0 to 6.0 $mm^2 s^{-1}$. Thus, the results obtained in the present study do not differ from those found by Peiter et al. (2018) and Borges et al. (2014), and are in accordance with the standards.

Acid value [mg KOH g^{-1}]

Table 9 presents the variance analysis of the effect of the studied variables and their interactions on the acid value. The results obtained for the acid value show that only the molar ratio causes significant changes. The significance of each effect is considered by the probability value (*p-value*). The *p-value* was set at 0.05 or 5% for a 95% confidence level in this study. In this analysis, effects with a *p-value* of less than 0.05 are considered significant effects.

Table 9. Variance analysis of the effect of the studied variables and their interactions on acid value

F.V.	*G.L.*	*SQ*	*QM*	*FC*	*P-value*
Molar ratio	2	7.915563	3.957781	52.733	0.0000*
Time	2	0.158141	0.079070	1.054	0.3925^{ns}
Temperature	2	0.145341	0.072670	0.968	0.4202^{ns}
Molar ratio * Time	4	0.456770	0.114193	1.521	0.2839^{ns}
Molar ratio * Temperature	4	0.253704	0.063426	0.845	0.5343^{ns}
Time * Temperature	4	0.166393	0.041598	0.554	0.7022^{ns}
Error	8	0.600430	0.075054		
Total Corrected	26	9.696341			
CV	24.87				
Overall Average	1.101482				

*F.V. = variation source; G.L. = degree of freedom; SQ = sum of squares; QM = mean square; FC = calculated F; Pr > Fc = hypothesis test; * Significant; ns = not significant.*

As shown in Tables 10 and 11, for the 6:1 and 9:1 molar ratios, the values do not present significant differences among them. The highest values of acid value were found for the 12:1 molar ratio, differing significantly from the values of the 6:1 and 9:1 molar ratios. The reaction time and temperature did not cause significant changes in the acid value.

Table 10. Statistical analysis for acid value considering time and molar ratio

Time	Molar Ratio		
(min)	6:1	9:1	12:1
30	0.70 Aa	0.70 Aa	1.84 Ba
60	0.59 Aa	0.85 Aa	2.18 Ba
120	0.65 Aa	0.84 Aa	1.57 Ba

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

Table 11. Statistical analysis for acid value considering temperature and molar ratio

Temperature	Molar Ratio		
(°C)	6:1	9:1	12:1
30	0.59 Aa	0.83 Aa	1.59 Ba
40	0.65 Aa	0.80 Aa	2.07 Ba
50	0.70 Aa	0.76 Aa	1.93 Ba

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

During the analysis of the biodiesel acid value, values were found well above the limit established by Table 3. This fact probably results from the procedure used to wash the biodiesel using three drops of hydrochloric acid and 30 mL of distilled water for each 100 mL of dirty biodiesel.

This procedure can work satisfactorily for washing biodiesel produced with methanol, because, according to Gomes (2011), it has smaller molecules (compared to ethanol), so the bonds between alcohol and glycerin are broken more easily and quickly, while ethanol has more stable bonds with glycerin, and therefore requires a more appropriate washing procedure. It is also worth noticing that the acid value of the soybean oil used with raw material was checked and that it had an acid value of 1.08 mg KOH g^{-1}, which, possibly, may also have contributed to the high acid value of biodiesels. According to Santos et al. (2017), the acid value is directly related to the oil's conservation status and the latter is influenced by the nature and quality of the raw material, the purity of the oil, the processing and, mainly, by the storage conditions, since the decomposition of glycerides is accelerated by heating and light.

Thus, the washing procedure adapted from Abbaszadeh et al. (2014) was tested, which consists of standardizing a 1 molar hydrochloric acid solution and using a 1:1 water: biodiesel ratio to wash. The molar ratio used in these tests was 6:1, with a temperature of 40 °C and 30 minutes of reaction.

In the first test, only two drops of the solution were added to the washing water in the first wash. In the other washes, distilled water was used without adding the hydrochloric acid solution. Biodiesel was washed until the waste water from the wash had a clear appearance. In the second test, the first two washes were made only with water and in the third wash two drops of the solution were added to the water and the biodiesel continued to be washed until the waste water from the wash had a clear appearance. In the third test, in every wash, two drops of the solution were added to the water and the biodiesel continued to be washed until the waste water from the wash was clear. Table 12 shows the number of times that each test took until the waste water had a clear appearance.

Table 12. Result of biodiesels washing tests

Variable	Test 1	Test 2	Test 3
Addition of two drops of the HCl solution	In the 1st wash	In the 3rd wash	In all washes
Number of washes	9	14	19
Yield (%)	88.37	87.48	87.91
Specific Mass (Kg m^{-3})	878.68	879.16	879.74
Kinematic viscosity (mm^2 s^{-1})	5.05	5.10	5.11
Acid value (mg of KOH g^{-1})	0.54	0.54	0.60

The tests showed positive results in relation to the acid value, as it was possible to observe that washing the biodiesel with distilled water and two drops of the HCl solution in the first wash resulted in a biodiesel with a lower acid value than the one previously produced, with a lower number of washes.

Observing the physicochemical characteristics of biodiesel, it is noticed that the values are close to those previously obtained. It is believed that this procedure is also capable of producing biodiesel that complies with the limits established in ANP Resolution No. 45/2014 for the other molar ratios of 9:1 and 12:1.

Analysis by Gas Chromatography coupled to Mass Spectrometry

For the Chromatography analysis, only the molar ratio was taken into account, therefore, the samples (of each molar ratio) were prepared by mixing all treatments of each molar ratio.

In the chromatographic profile of biodiesel produced from soybean oil by ethylic route with a 6:1 molar ratio, the signs of interest related to fatty esters are included in the interval from 16 to 21 minutes of elution (or retention), as shown in Table 13 and Fig. 1. The fatty acids that present the largest areas are Oleic and Stearic acids, with areas of 52.26% and 30.69%, respectively.

Table 13. Fatty acids table for a 6:1 molar ratio

Fatty acid	Nomenclature	Retention time (min)	Area (%)
C16:0	Palmitic	16.962	11.40
C18:0	Stearic	19.433	30.69
C18:1	Oleic	20.040	52.26
C18:2	Linoleic	20.645	5.30
C18:3	Linolenic	20.755	0.36

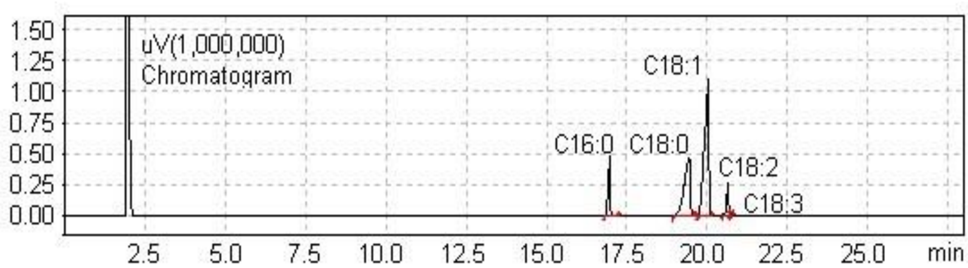

Figure 1. Chromatogram for a 6:1 molar ratio.

The Chromatogram of the biodiesel sample produced from soybean oil by ethylic route with a 9:1 molar ratio shows, in the interval between 16 and 21 minutes, the retention times of the fatty esters that most contributed to the biodiesel composition, as shown in Table 14 and Fig. 2. It can be seen that biodiesel with a 9:1 molar ratio has Oleic and Stearic acids as those with the largest areas, with 52.41% and 30.34%, respectively.

Table 14. Fatty acids table for a 9:1 molar ratio

Fatty acid	Nomenclature	Retention time (min)	Area (%)
C16:0	Palmitic	16.966	11.19
C18:0	Stearic	19.488	30.34
C18:1	Oleic	20.052	52.41
C18:2	Linoleic	20.651	5.59
C18:3	Linolenic	20.757	0.48

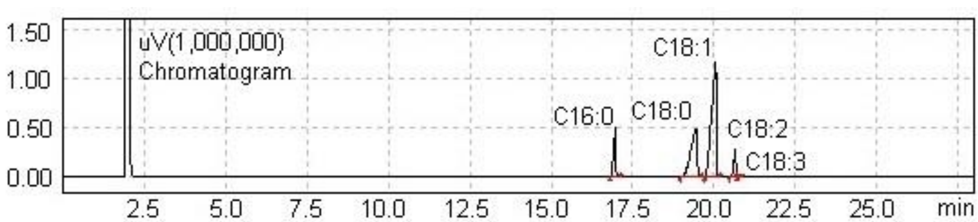

Figure 2. Chromatogram for a 9:1 molar ratio.

In the chromatographic profile of biodiesel produced from soybean oil by ethylic route with a 12:1 molar ratio, the signs of interest related to fatty esters are included in the interval from 16 to 21 minutes of retention, as shown in Table 15 and Fig. 3. It can be seen that biodiesel with a 12:1 molar ratio presents Oleic and Stearic acids as those with the largest areas, with 52.22% and 30.61%, respectively.

With the chromatographic analysis, from the determination of the elution times, it was possible to identify five different fatty acids in the composition of the biodiesels. Total saturated fatty acids (palmitic and stearic acids) ranged from 41.53% to 42.09% and total unsaturated, including monounsaturated and polyunsaturated (oleic, linoleic and linolenic acids) ranged from 57.92% to 58.48%.

Table 15. Fatty acids table for a 12:1 molar ratio

Fatty acid	Nomenclature	Retention time (min)	Area (%)
C16:0	Palmitic	16.972	11.25
C18:0	Stearic	19.468	30.61
C18:1	Oleic	20.069	52.22
C18:2	Linoleic	20.659	5.45
C18:3	Linolenic	20.761	0.47

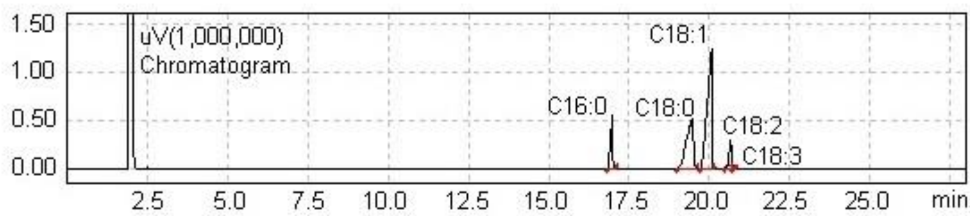

Figure 3. Chromatogram for a 12:1 molar ratio.

From the analysis of Table 16, it can be seen that biodiesel with a 6:1 molar ratio has, on average, a higher percentage of saturated fatty acids 42.09%, while the 9:1 molar ratio has the lowest percentage of saturated fatty acids 41.53%, and all molar ratios differ significantly from each other. On the other hand, the 9:1 molar ratio has the highest percentage of unsaturated fatty acids 58.48%, differing significantly from the other molar ratios.

Table 16. Statistical analysis for fatty acids areas

Fatty acid	Molar Ratio		
	6:1	9:1	12:1
Stearic	30.69 C	30.34 A	30.61 B
Linoleic	5.30 A	5.59 C	5.45 B
Linolenic	0.36 A	0.48 C	0.47 B
Oleic	52.26 B	52.41 C	52.22 A
Palmitic	11.40 C	11.19 A	11.25 B

Averages followed by the same letter on the line do not differ from each other at the level of 5% probability according to the Tukey test.

Yield

Table 17 presents the variance analysis of the effect of the studied variables and their interactions on the production yield. The results obtained show that the molar ratio, reaction time and temperature, as well as the molar ratio*temperature interaction, cause significant changes in yield. The significance of each effect is considered by the probability value (*p-value*). The *p-value* was set at 0.05 or 5% for a 95% confidence level in this study. In this analysis, effects with a *p-value* of less than 0.05 are considered significant effects.

Table 17. Variance analysis of the effect of the studied variables and their interactions on the production yield

F.V.	*G.L.*	*SQ*	*QM*	*FC*	*P-value*
Molar ratio	2	39.54295	19.771474	8.855	0.0004*
Time	2	62.07995	31.039974	13.902	0.0000*
Temperature	2	359.37242	179.686211	80.475	0.0000*
Molar ratio * Time	4	19.23380	4.808450	2.154	0.0847^{ns}
Molar ratio * Temperature	4	28.77455	7.193638	3.222	0.0182*
Time * Temperature	4	17.55756	4.389390	1.966	0.1108^{ns}
Error	62	138.43403	2.232807		
Total Corrected	80	664.99526			
CV	1.71				
Overall Average	87.28216				

*F.V. = variation source; G.L. = degree of freedom; SQ = sum of squares; QM = mean square; FC = calculated F; Pr > Fc = hypothesis test; * Significant; ns = not significant.*

From the analysis of the molar ratio * reaction time interaction (Table 18) it is possible to observe that the highest yields 89.72% and 88.37% were obtained for the time of 30 minutes and for the molar ratios of 9:1 and 12:1, not differing significantly from each other. It is also observed that the increase in time leads to a lower yield in production, except for the 6:1 molar ratio, whose yield does not differ significantly among times.

The molar ratio*temperature interaction (Table 19) highlights that the highest yields 91.16% and 89.51% were obtained for the temperature of 30 °C and for the molar ratios of 9:1 and 12:1, not significantly differing between them. It can be seen that the increase in temperature leads to a lower yield in production.

Table 18. Statistical analysis for yield considering time and molar ratio

Time	Molar Ratio		
(min)	6:1	9:1	12:1
30	87.12 Aa	89.72 Bb	88.37 ABb
60	86.55 Aa	87.71 Aa	87.29 Ab
120	86.31 ABa	87.35 Ba	85.13 Aa

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

Table 19. Statistical analysis for yield considering temperature and molar ratio

Temperature	Molar Ratio		
(°C)	6:1	9:1	12:1
30	88.61 Ab	91.16 Bc	89.51 ABb
40	87.58 ABb	88.70 Bb	86.16 Aa
50	83.80 Aa	84.92 Aa	85.12 Aa

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

In the interaction temperature*reaction time (Table 20), the maximum yields 90.87% and 90.09% are observed for the temperature of 30 °C and times of 30 and 60 minutes, and do not differ significantly from each other. It is also observed that the increase in temperature and reaction time leads to a decrease in yield, except for the temperature of 50 °C, in which the yield does not differ significantly between times.

Moradi et al. (2013) obtained the maximum yield of 93.2% for the 9:1 molar ratio and reaction temperature of 60 °C. In the work of Rahimi et al. (2014), the authors obtained the maximum yield of 94.78% using the 9:1 molar ratio and it was observed that the increase in the molar ratio from 6:1 to 9:1 causes an increase in yield; on the other hand, the increase in the molar ratio from 9:1 to 12:1 results in reduced yield, the same behavior observed in the present study.

Joshi et al. (2017) investigated the effects of the molar ratio on the biodiesel yield, keeping the catalyst quantity, temperature and reaction time constant. The authors observed that there is a constant increase in the biodiesel yield up to a 10:1 molar ratio; however, after 10:1, the yield remains practically constant. They attribute this to the fact that the glycerol produced during the reaction is dissolved in the excess alcohol, affecting the reaction balance.

Table 20. Statistical analysis for yield considering temperature and reaction time

Temperature	Time (min)		
(°C)	30	60	120
30	90.87 Bc	90.09 Bc	88.32 Ab
40	89.09 Bb	87.34 Ab	86.01 Aa
50	85.24 Aa	84.13 Aa	84.46 Aa

Averages followed by the same letter, uppercase on the line and lowercase on the column, do not differ from each other at the 5% level according to the Tukey test.

Morais et al. (2013) also observed that the increase in the molar ratio from 1:8 to 1:12 caused a reduction in yield, and associated this problem with excess alcohol and its boiling point, as it is likely that in larger molar ratios the evaporation of alcohol is more pronounced, causing the loss of part of the reagent and consequent reduction in conversion to esters.

Response Surface

The Response Surfaces present the behavior of the variables manipulated in this work (molar ratio, temperature and reaction time), as well as their interactions with the biodiesel production yield from soybean oil.

As shown in Fig. 4, the increase in temperature results in a decrease in yield. As represented by the dark blue color, the yields of biodiesels produced at a temperature of 50 °C were the lowest for all molar ratios and all stirring times, with values varying between 83% and 85%. Temperatures between 30 °C and 40 °C showed higher yields compared to the temperature of 50 °C. This fact can be explained, according to Morais et al. (2013), by the loss of alcohol during the reaction, as the increase in this variable favors the alcohol volatilization. On the other hand, Joshi et al. (2017), using a reflux condenser coupled to the system, observed an increase in the reaction yield when the temperature goes from 40 °C to 50 °C, because with the use of the reflux condenser the evaporated alcohol returns to the reaction.

The Fig. 5 shows another perspective of the same response surface. It is possible to notice that the molar ratios of 9:1 and 12:1 were the ones that presented the highest yields. The 9:1 molar ratio showed yields above 90% (90.85%, 91.19% and 91.44%) for 30, 60 and 120 minutes, respectively, with the temperature at 30 °C. With the temperature at 40 °C, the 9:1 molar ratio also showed yields above 90% (91.78%) for the time of 30 minutes. The 12:1 molar ratio showed the maximum yield of 93.30%, for the temperature of 30 °C and time of 30 minutes, represented by the dark red color.

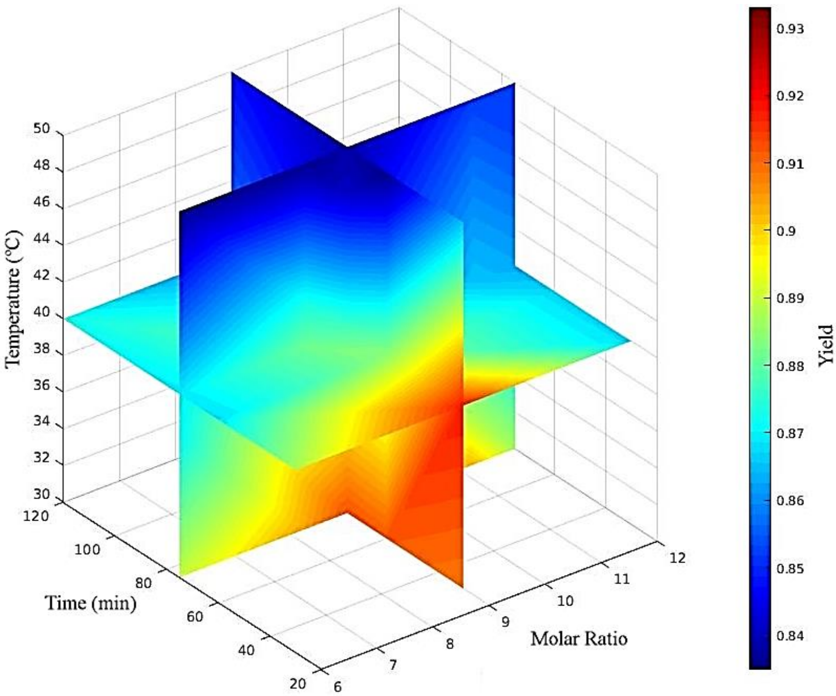

Figure 4. Response Surface of behavior of the variables manipulated (molar ratio, temperature and reaction time) at production yields effects.

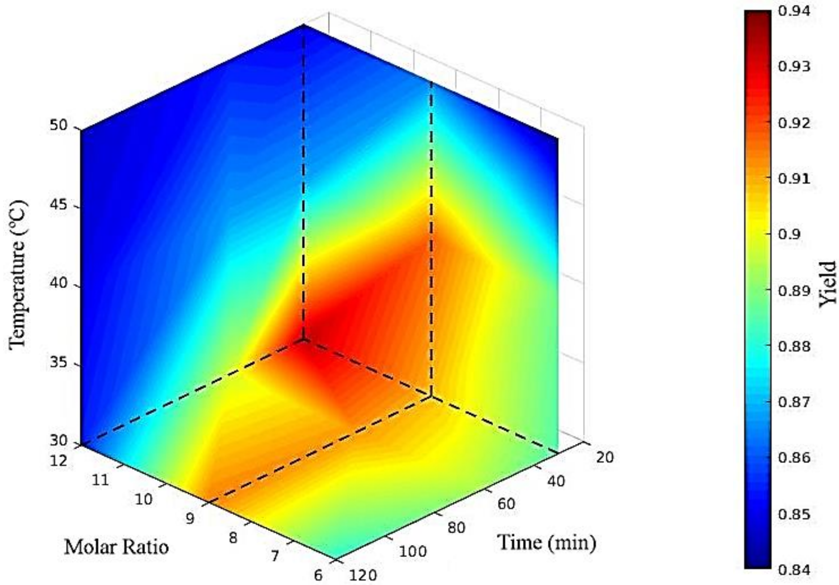

Figure 5. Response Surface - another perspective.

The increase in the molar ratio shows an increase in yield due to the greater amount of alcohol present in the reaction and consequently to the greater conversion into esters.

However, the increase in the molar ratio from 9:1 to 12:1 for higher temperatures showed a reduction in yield, a fact that according to Morais et al. (2013) is related to alcohol volatilization. Joshi et al. (2017) also explain that the transesterification reaction using high molar ratios favors the solubilization of glycerol in alcohol, which generates an imbalance in the reaction and consequently a reduction in yield. Although the 12:1 molar ratio was the one with the highest yield, this was only observed for the temperature of 30 °C and time of 30 minutes, and in the other times and temperatures this molar ratio showed unsatisfactory yields.

The Fig. 6 shows the normalized distribution of production yields, and it is possible to notice that for the 12:1 molar ratio, few values reached yields of 92% to 95%, and these refer to the region of maximum yield observed in Fig. 5. On the other hand, the 9:1 molar ratio showed a significantly greater amount of values between 90% and 93%. These values are justified by the better performance of the 9:1 molar ratio (in relation to the 12:1 ratio) at the different reaction times studied, as shown in Fig. 5.

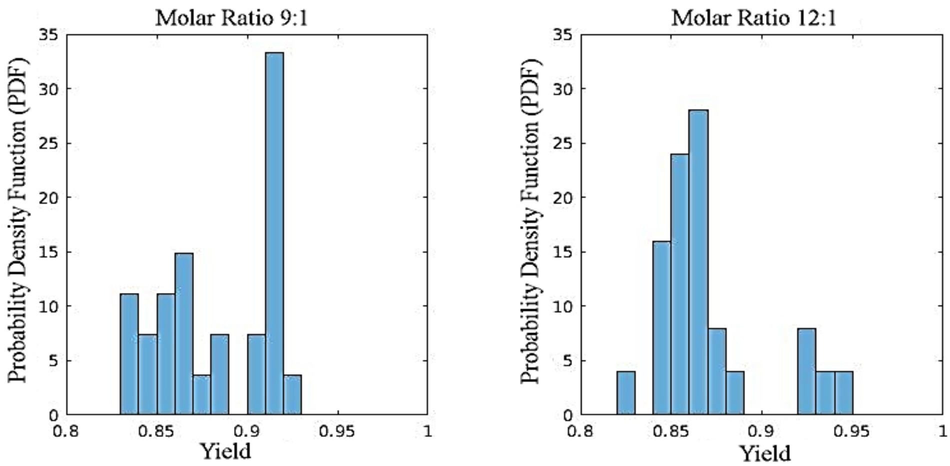

Figure 6. Normalized Probability Distribution of production yields for 9:1 and 12:1 molar ratio.

This study clearly shows that the response surface methodology was an adequate method to determine the best operating conditions, in order to maximize the production of ethyl esters. Higher yield results in less glycerin produced, therefore, in lower environmental impact. By determining the best yield point, the amount of alcohol used is optimized, which results in efficiency in both production cost and yield. Regarding the reaction time and temperature, it is possible to state the condition for obtaining greater yield in the biodiesel production and with low energy consumption.

CONCLUSIONS

In this work, the yield of biodiesel production from soybean oil was analyzed during the variation of the parameters molar ratio alcohol: oil, temperature and reaction time in laboratory conditions, making it possible to conclude that the configuration that results in maximum yield of 93.30% is a 12:1 molar ratio, temperature of 30 °C and reaction time of 30 minutes. The 6:1 molar ratio showed a higher yield of 89.30% at a temperature of 30 °C and a reaction time of 60 minutes, while the 9:1 molar ratio showed a maximum yield of 91.78% at a temperature of 40 °C and reaction time of 30 minutes. From the analysis of the response surface, it is observed that the increase in temperature and reaction time leads to a reduction in yield, a fact explained by the favor of alcohol volatilization, which impacts on the formation of ethyl esters.

According to the results of the physicochemical analyses, the specific mass at 20 °C is in accordance with Brazilian, American and European specifications, varying between 877.46 kg m^{-3} and 879.64 kg m^{-3}. The kinematic viscosity at 40 °C varied between 4.49 mm² s^{-1} and 4.82 mm² s^{-1}, and is also in accordance with the limits established by the standards. The acid value obtained did not vary within the limits established by the standards. Values between 0.54 and 2.74 mg of KOH g^{-1} were observed, but according to the standards presented, this value could not exceed the maximum limit of 0.5 mg of KOH g^{-1}.

Based on the results of the study, it can be said that the use of ethanol (as a substitute for methanol) combined with soybean oil, results in the production of a fuel from 100% renewable sources (ethanol derived from sugarcane and soybean oil), in addition to using a low toxicity reagent when compared to methanol.

REFERENCES

Abbaszadeh, A., ghobadian, B., Najafi, G. & Yusaf, T. 2014. An experimental investigation of the effective parameters on wet washing of biodiesel purification. *International Journal of Automotive and Mechanical Engineering (IJAME)* **9**, 1525–1537, January-June, ISSN: 2180–1606. doi: http://dx.doi.org/10.15282/ijame.9.2013.4.0126

Almeida, T.S. 2016. *Transesterification reaction of soybean oil and jatropha by methanolysis and ethanolysis using several catalysts*. Dissertação de Mestrado em Engenharia Mecânica, Faculdade de Engenharia de Ilha Solteira - Universidade Estadual Paulista (UNESP), **106** pp. (in Portuguese).

Ambat, I., Srivastava, V. & Sillanpää, M. 2018. Recent advancement in biodiesel production methodologies using various feedstock: A review. *Renewable and Sustainable Energy Reviews* **90**, 356–369. doi: https://doi.org/10.1016/j.rser.2018.03.069

ANP. Boletim mensal do biodiesel (Feb. 2017), 2017. Available in: <*http://www.anp.gov.br/publicacoes/boletins-anp/2386-boletim-mensal-do-biodiesel*>. Accessed in: 09/01/2019 (in Portuguese).

Bet-MoushouL, E., Farhadi, K., Mansourpanah, Y., Nikbakht, A.M., Molaei, R. & Forough, M. 2016. Application of CaO-based/Au nanoparticles as heterogeneous nanocatalysts in biodiesel production. *Fuel* **164**, 119–127. doi: https://doi.org/10.1016/j.fuel.2015.09.067

Borges, K.A., Squissato, A.L., Santos, D.Q., Borges Neto, W., Batista, A.C.F., Silva, T.A., Vieira, A.T., Oliveira, M.F. & Hernández-Terrones, M.G. 2014. Homogeneous catalysis of soybean oil transesterification via methylic and ethylic routes: Multivariate comparison. *Energy*, **67**, 569–574. doi: https://doi.org/10.1016/j.energy.2014.02.012

Brasil. Resolução ANP Nº 45 de 25/08/2014 - Especificação do Biodiesel. Available in: <*http://legislacao.anp.gov.br/?path=legislacao-anp/resol-anp/2014/agosto&item=ranp-45-2014*>. Accessed in 30/08/2019. (in Portuguese).

Dantas, J., Leal, E., Mapossa, A.B., Silva, A.S. & CostA, A.C.F.M.C. 2016. Synthesis, characterization and catalytic performance of mixed nanoferrites submitted to transesterification and esterification reaction using methyl and ethyl route for biodiesel production. *Revista Materia* **21**(4), 1080–1093. doi: http://dx.doi.org/10.1590/s1517-707620160004.0099

Ferreira, D.F. 2014. Sisvar: a Guide for its Bootstrap procedures in multiple comparisons. *Ciência e Agrotecnologia*. [online]. 2014, **38**(2), 109–112. doi: http://dx.doi.org/10.1590/S1413-70542014000200001

Giuffrè, A.M., Capocasale, M., Zappia, C. & Poiana, M. 2017. Biodiesel from tomato seed oil: transesterification and caracterisation of chemical-physical properties. *Agronomy Research* **15**(1), 133–143.

Gomes, C.S., Arroya, P.A. & Pereira, N.C. 2011. Biodiesel production from degummed soybean oil and glycerol removal using ceramic membrane. *Journal of Membrane Science* **378**, 453–461. doi: https://doi.org/10.1016/j.memsci.2011.05.033

Jokiniemi, T. & Ahokas, J. 2013. A review of production and use of first generation biodiesel in agriculture. *Agronomy Research* **11**(1), 239–248.

Joshi, S., Gogate, P.R., Moreira Junior, P.F. & Giudici, R. 2017. Intensification of biodiesel production from soybean oil and waste cooking oil in the presence of heterogeneous catalyst using high speed homogenizer. *Ultrasonics – Sonochemistry* **39**, 645–653. doi: https://doi.org/10.1016/j.ultsonch.2017.05.029

Knothe, G., Gerpen, J.V., KrahL, J. & Ramos, L.P. 2006. *Manual de Biodiesel*. 1.Ed. São Paulo: Editora Edgard Blücher, 352 pp. (in portuguese).

Mendow, G. & Querini, C.A. 2013. High performance purification process of methyl and ethyl esters produced by transesterification. *Chemical Engineering Journal* **228**, 93–101. http://dx.doi.org/10.1016/j.cej.2013.05.007

Meneghetti, S.M.P., Meneghetti, M.R. & Brito, Y.C. 2013. A Reação de Transesterificação, Algumas Aplicações e Obtenção de Biodiesel. *Revista Virtual de Química*. **5**(1), 63–73.

Moradi, G.R., Dehghani, S., Khosravian, F. & Arjmandzadeh, A. 2013. The optimized operational conditions for biodiesel production from soybean oil and application of artificial neural networks for estimation of the biodiesel yield. *Renewable Energy* **50**, 915–920. doi: https://doi.org/10.1016/j.renene.2012.08.070

Morais, F.R., Lopes, C.S., Lima Neto, E.G., Ramos, A.LD. & Silva, G. 2013. Influence of Temperature and Molar Ratio on Continuous Biodiesel Production. *Scientia Plena* **9**(10), 104–202.

Musa, I.A. 2016. The effects of alcohol to oil molar ratios and the type of alcohol on biodiesel production using transesterification process. *Egyptian Journal of Petroleum* **25**, 21–31. doi: https://doi.org/10.1016/j.ejpe.2015.06.007

Peiter, A.S., Lins, P.V.S., Meili, L., Soletti, J.I., Carvalho, S.H.V., Pimentel, W.R.O. & Meneghetti, S.M.P. 2018. Stirring and mixing in ethylic biodiesel production. *Journal of King Saud University - Science*. doi: https://doi.org/10.1016/j.jksus.2018.01.010

Rahimi, M., Aghel, B., Alitabar, M., Sepahvand, A. & Ghasempour, H.R. 2014. Optimization of biodiesel production from soybean oil in a microreactor. *Energy Conversion and Management* **79**, 599–605. doi: https://doi.org/10.1016/j.enconman.2013.12.065

Razack, S.A. & Duraiarasan, S. 2016. Response surface methodology assisted biodiesel production from waste cooking oil using encapsulated mixed enzyme. *Waste Management* **47**, 98–104. doi: https://doi.org/10.1016/j.wasman.2015.07.036

Santos, G.M., Brito, M.M., Sousa, P.V.L. & Barros, N.V.A. 2017. Determination of the acidity index in soybean oils sold in retail supermarkets. Revista Ciência e Saúde 2, 11–14 (in portuguese).

Sirviö, K., Heikkilä, S., Help, R., Niemi, S. & Hiltunen, E. 2018. Properties of local produced animal-fat based biodiesel and its blend with fossil fuel. *Agronomy Research* **16**(S1), 1237–1246. doi: https://doi.org/10.15159/AR.18.083

Victorino, T., Pereira, R. & Fiaux, S. 2016. Use of biodiesel glycerin obtained from frying oil for the cultivation of the *Aspergillus niger* fungus. *Revista Brasileira De Ciências Ambientais* (Online), **42**, 56–66. doi: https://doi.org/10.5327/Z2176-947820160107

Agronomy Research **18**(S2), 1516–1525, 2020
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A simple tool for resource availability optimization: A case study of dairy whey supply for single cell protein and oil production in Latvia

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Abstract. Single cell proteins (SCP) and oils (SCO) are promising alternatives for replacing conventional feed ingredients in animal and aquaculture fish feeds. The production costs of SCP and SCO need to be reduced by using inexpensive substrates (production by-products) suitable for cultivation of protein and oil producing microorganisms. This article reviews the availability of milk processing by-product – whey in Latvia, in 2019. Additionally, a simple production plant location optimization model is proposed, where no prior knowledge of location optimization or experience with dedicated software is required from the user. The case study demonstrated that the model is valid, and it can be used as a simple tool for resource acquisition from multiple sources to single production plant.

Key words: availability analysis, biomass supply chain, dairy waste, location optimization, whey.

INTRODUCTION

Single cell protein (SCP) and single cell oil (SCO) are recognized as one of the most promising alternatives for replacing animal feeds, in particular aquaculture fish feeds (Spalvins & Blumberga, 2018). However, current production costs of SCP and SCO hinder the wider use of these products in feed production (Spalvins & Blumberga, 2018). In order to make SCP and SCO production costs more economically viable, it is necessary to use cheap substrates for microbiological fermentations (Spalvins & Blumberga, 2018).

In order to evaluate the substrates for the production of SCP and SCO, it is necessary to perform data analysis and determine the total substrate volumes available in specific regions or globally as a whole. In addition, it is necessary to identify the current use for specific substrates and compare whether the current use has higher added value than the production of SCP and SCO. However, such a superficial analysis is not sufficient to determine whether the use of a particular substrate will indeed be economically justified. Additional in-depth availability analysis is needed to calculate procurement costs, local availability, required transportation and logistics systems.

Spalvins & Blumberga (2018) previously reviewed the most appropriate tools for substrate availability analysis at three different levels: biomass supply chain between producer and processing plant; biomass supply chain between multiple producers, biomass logistics system (biomass transporters) and single processing plant; supply chain of multiple applicable biomass types on national level for multiple processing plants. Biomass supply chain between producer and processing plant was based on the integrated biomass supply analysis and logistics model devised by Sokhansanj et al. (2006). Biomass supply chain between multiple producers, biomass logistics system (biomass transporters) and single processing plant was based on simulation and optimization model devised by Ebadian et al. (2013). Supply chain of multiple applicable biomass types on national level for multiple processing plants was based on biomass availability assessment model devised by Welfle et al. (2014). In this study simplified model of biomass supply chain between multiple producers, biomass logistics system (biomass transporters) and single processing plant was developed and utilized. A more detailed description of the availability models for each level is described in detail by Spalvins & Blumberga (2018), Sokhansanj et al. (2006), Ebadian et al. (2013) and Welfle et al. (2014).

Spalvins et al. have reviewed most of the cheap agricultural and industrial by-products applicable for SCP and SCO production (Spalvins et al., 2018a; Spalvins et al., 2018b; Spalvins & Blumberga, 2019; Spalvins et al., 2019), but information on the availability and potential price of each specific by-product, if used in fermentation, is very limited. Consequently, this publication analyses the availability of one of the potential substrates - dairy residues. Whey and other dairy processing by-products are characterized by high levels of chemical oxygen demand (~75 g kg^{-1}) and biological oxygen demand (54 g kg^{-1}), due to high concentrations of lactose (~4.7%) (Slavov, 2017), lipids and other organic compounds (Brião, & Granhen Tavares, 2007). In EU due to high COD and BOD levels whey cannot be released in local water bodies or even in local wastewater treatment systems without prior pre-treatment (Valorlact, 2012). Therefore, dairy industry is required to lower the COD and BOD levels before whey can be released in local wastewater systems, which in turn creates additional costs for dairy processing plants. Many companies are looking for solution to process whey generated from cheese and cottage cheese production. The solutions used in Latvia are the processing of whey into whey powder, whey protein powder, lactose powder, whey drinks etc., however, this solution involves high upfront investments and the use of spray drying is characterized by high energy consumption and relatively low efficiency (Cheng et al., 2018). Some companies in Latvia partner with local biogas plants to use generated whey as feedstock for anaerobic fermentations. Although, this is only possible if the biogas plant is nearby and biogas production in general is a solution with significantly lower added value than utilization of the same feedstocks in SCP or SCO production (Spalvins et al., 2018a).

To calculate the substrate availability and to find optimal location for the SCP or SCO plant, a simple and quick-to-use model was developed, which is based on the calculation and validation of the initial site location and validation to confirm the accuracy of the results.

MATERIALS AND METHODS

Data collection

Initially, all available information on the amount of whey generated in Latvia was collected. This was done mainly by summarizing the available information on the cheese production volumes of each milk processing company in Latvia. It was then assumed that approximately 9 and 0.6 litres were generated per kilogram of cheese and cottage cheese respectively.

Although in Latvia, according to NACE (Statistical classification of economic activities in the European Communities) rev. 2 classification, 61 companies correspond with 10.51 class (Operation of dairies and cheese making) (Lursoft, 2020), after interviewing the representatives from Central Statistical Bureau of Latvia (CSB), they informed us that statistics on cheese production are collected only from 22 companies. These companies are centralized medium to large capacity dairy plants, which produce the vast majority of aged and fresh cheese in the country. The rest are either not specialized in the production of cheese, but fall under NACE class 10.51 or are small domestic producers, farms or sole traders who have very small production capacities and whose products are sold mainly in local markets. Therefore, the exclusion of these remaining companies from the statistics in this study does not have pronounced effect on the accuracy of the simulation, because the whey collection in small volumes is not cost-effective and would have not be done in real life scenario as well. The sources of information were data of the Central Statistical Bureau of Latvia (CSB) (CSB, 2020), information on individual companies available in mass media and companies' websites, as well as interviews of dairy processing plant representatives. CSB provided the most detailed information on the monthly production volumes of aged and fresh cheese. Due to Latvian Statistics Law (OP number: 2015/118.3) which is prepared following the guidelines of the European Statistics Code of Practice, the data provided by CSB could not contain information that would allow to directly identify specific companies to their respective cheese production volumes. Therefore, the data received from CSB was arranged by the territorial entities and not the companies, thus allowing for indirect identification of specific companies and their production volumes, which is in line with Latvian Statistics Law if data is used for research. Apart from research this does not apply for other purposes, therefore in order to follow the limitation put in place by the law, in this publication no specific data on the production volumes will be given.

Optimization model

The initial optimization model was developed using MS Excel, MS Visio and Google Maps. On the map of Latvia, the locations of all milk processing plants (B_n) were marked, also the calculated whey volumes (W_ns un $W_n$su) were assigned for each relevant dairy plant. The map of Latvia was placed on XY axis and on each axis value ($A_{i,z}$) the distance (D_n) in a straight line was calculated using Pythagorean theorem (Fig. 1, B; Fig. 3; Eq. 1). Followed by calculation of the given company's (n) coefficient ($K_n^{A_{i,z}}$) for the given coordinates of the particular potential SCP/SCO plant (i, z) (Fig. 1, C; Fig. 3; Eq. 2). $K_n^{A_{i,z}}$ value is calculated for every company, for every possible coordinates of the potential SCP/SCO plant.

Formulas for the initial optimization model:

$$D_n = \sqrt{(A_i x - B_n x)^2 + (A_z y - B_n y)^2} \quad (1)$$

$$K_n^{A_{i,z}} = D_n \times (W_n s - W_n su) \quad (2)$$

$$P_{i_0 \dots i_{106}, z_0 \dots z_{64}} = \sum K_{n_1 \dots n_{22}}^{A_{i,z}} \quad (3)$$

where $A_{i,z}$ – SCP/SCO plant coordinates ($A_i x$, $A_z y$), i = {0…106}; z = {0…64}; B_n – particular dairy plant (n) coordinates ($B_n x$, $B_n y$), n = {1…22}; $W_n s$ – the quantity of generated whey in a given dairy plant (n), tonnes; $W_n su$ – consumption of whey in processes unrelated to SCP/SCO production in a given dairy plant (n), tonnes; D_n – distance from variable SCP/SCO plant (i,z) to particular dairy plant (n); $K_n^{A_{i,z}}$ – coefficient of the given company (n) for the given coordinates of the particular SCP/SCO plant (i,z); $P_{i,z}$ – SCP/SCO plant location (i,z) potential.

Summing the coefficients of all companies for the given SCO/SCP plant coordinates gives the potential of the each particular location (Fig. 1, D; Fig. 3; Eq. 3): the lowest calculated $P_{i,z}$ value represents the location where the largest amount of whey can be delivered to by traveling the shortest distance.

To validate the optimization model, initially the optimal coordinates (20 lowest $P_{i,z}$ values) were used to mark the area. This area was used to validate the optimization model. Settlements with a population over 100 were evenly marked in the area (n = 24). These settlements were used to recalculate real distances using national and local roads using Google Maps. This was done because the largest rivers of Latvia (Daugava, Gauja, Lielupe) can only be crossed at certain locations, so the real distance for some dairy plants may change significantly and thus the optimal location for the SCP/SCO plant may also change. The resulting distances from each company to each of the settlements in the optimal area were used in the calculations just as in the initial optimization calculation for calculating $K_n^{A_{i,z}}$ value, only this time it was done by replacing D_n value with real distances travelled via motorways.

Example for locating the lowest validated $P_{i,z}$ value (Fig. 1):

- Gather information on generated/available by-product in area of interest;
- Put the area of interest on XY axis using MS Visio, every coordinate on the XY axis is the potential SCP/SCO plant coordinates ($A_{i,z}$) (Fig. 1, A);
- Locate the main production plants (B_n) that generate the by-product ($W_n s$ – $W_n su$) (Fig. 1, A);
- Calculate the distance from production plant location to every XY coordinates (D_n) by using Pythagorean theorem (MS Excel) (Fig. 1, B);
- Calculate the initial $P_{i,z}$ values by multiplying distance values with amount of by-product generated and then summing all the $K_n^{A_{i,z}}$ values for the given SCP/SCO plant location (Fig. 1, C);
- Lowest $P_{i,z}$ value is the initial optimal SCP/SCO plant location (Fig. 1, D, grey square);
- Any number (in this case n = 6) of lowest initial $P_{i,z}$ values can be used to draw the initial optimal area from which populated locations will be Fig. 1, E, F).

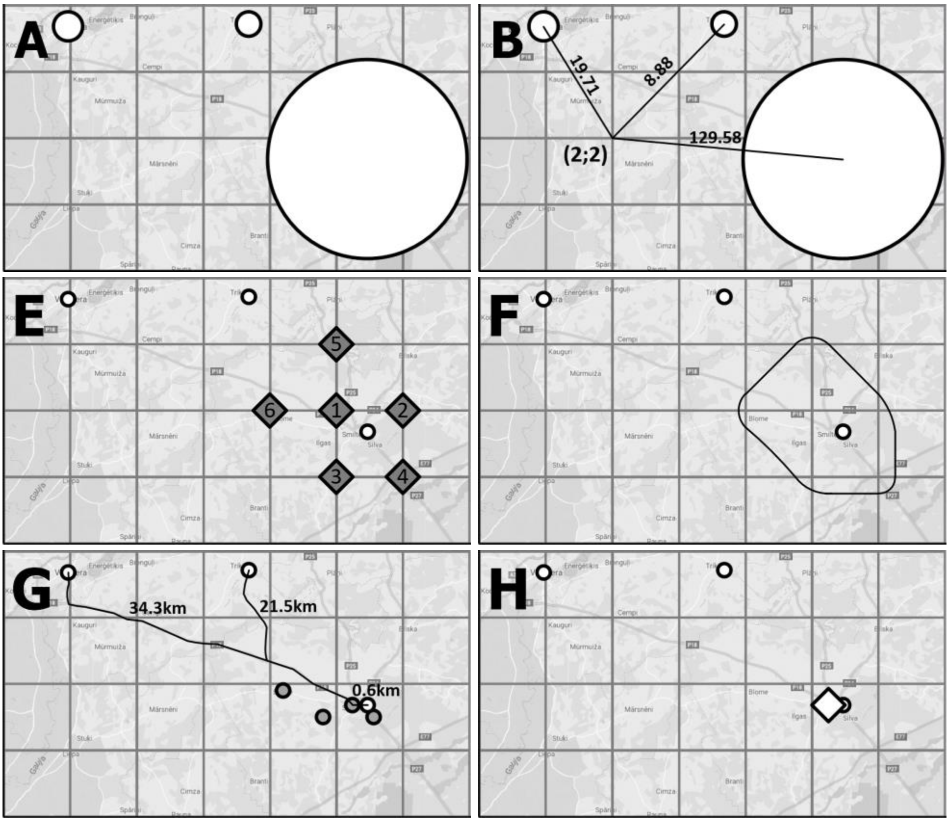

Figure 1. Visual example for locating and validating the optimal location for by-product processing plant with lowest validated $P_{i,z}$ value (white square). A – area of interest on XY axis, with by-product-generating plants (white circles proportional to generated substrate volumes); B – distance measurement using Pythagorean theorem from by-product generator to (2;2) coordinates in the area; C – calculated $P_{i,z}$ value for single coordinates; D – coordinates with the lowest $P_{i,z}$ value marked in the map (gray square); E – six lowest $P_{i,z}$ value marked in the map (gray squares); F – six lowest $P_{i,z}$ values used to mark initial optimal area from which populated locations will be selected; G – by using Google Maps distance via motorways is calculated for every populated location within initial optimal area (gray circles); H – populated location with the lowest $P_{i,z}$ value (white square) is the validated optimal location for the by-product processing plant.

- Distance from selected populated locations can be measured using Google Maps to every production plant (Fig. 1, G);
- If the maps lower left corner is aligned with XY (0:0) coordinates, the aligned locations for the production plant can be easily found in the shape coordinates in MS Visio;
- Settlements within the initial optimal area (Fig. 1, F) can be validated by recalculating the distance via motorways, thus obtaining the validated optimal SCP/SCO plant location (Fig. 1,G, white square);

- Calculation is finished by locating the populated location with the lowest $P_{i,z}$ value (see Fig. 1, H, white square). To this populated location the largest amount of by-product can be delivered while driving the shortest total distance.

RESULTS AND DISCUSSION

Data collection

The aggregated data on the amount of whey generated by Latvian dairy plants are shown in Fig. 2. From this data it can be concluded that the cheese production volumes in Latvian dairy plants are distributed evenly over Latgale and Vidzeme districts. In Kurzeme and partially in Zemgale districts situation is different due to large volumes of fresh milk that is being exported to neighbouring country Lithuania. This also outlines a problem within dairy industry of Latvia, where approximately 2,000 tonnes of fresh milk are produced every day, while only 1,300 tonnes are processed in local dairy plants (LSM, 2014). The other 700 tonnes are processed in neighbouring countries and then portion of it is sold back in Latvia. This way Latvian dairy industry and Latvian economy as a whole is losing vast amounts of potential revenue due to export of low added value raw material (fresh milk) and import of value added products (dairy products) while essentially the origin of both products is the same.

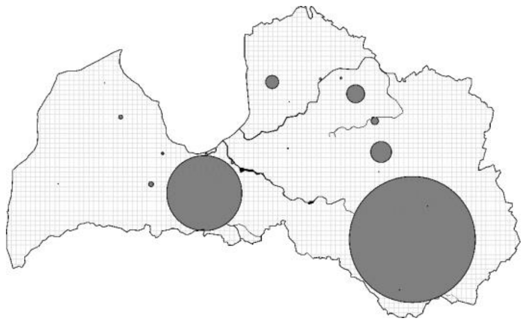

Figure 2. The aggregated data on the amount of whey generated by Latvian dairy plants. Circle sizes are proportional to the amount of whey generated in the specific dairy plant (n = 22).

Problem with the calculation of the total whey volume was that the amount of whey generated per kilogram of finished product differs significantly between aged and fresh cheese. Thanks to data provided by CSB it was possible to accurately calculate the volumes of generated whey from both types cheese. It is also important to distinguish aged and fresh cheese whey from one another not only because of the different amounts generated per kilogram of product, but also because fresh cheese whey has lower pH (6–7 pH and 4.5–4.8 pH for aged and fresh cheese whey respectively) which can significantly affect the SCP and SCO yields. In Latvia a total of 30961 tonnes of aged cheese and 19,977 tonnes of fresh cheese where produced in 2019. Which generated approximately 278651 tonnes of aged cheese whey and 11,986 tonnes of fresh cheese whey. Aged cheese whey constituted a majority of whey – 95.88%, while fresh cheese whey only constituted 4.12%. Therefore, a whey-utilizing SCP/SCO production technology should give the main focus on improving efficiency of utilizing solely whey from aged cheese.

During data collection it was also concluded that most dairy plants in Latvia are highly specialized for production of certain dairy products. Some almost exclusively produce aged cheese (Preilu siers, AS), some specialize in sweet cottage cheese bars

(Rigas piena kombinats, AS), while others mainly produce various types of yogurts (Tukuma piens, AS) etc.

Optimization model

The initial optimization model was used to find the optimal area (Fig. 3) and to mark a point in map with the lowest $P_{i,z}$ value (Fig. 3, grey square). By validating the optimization model and replacing the distance of straight lines with real distances via motorways, it was found that the lowest P value deviated by only 7.96 km (Fig. 3., black square) from the initial point with lowest $P_{i,z}$ value and the validated optimal location was still within the initially marked area. This indicates that the initial optimization model has, with very little deviation, indicated the optimal SCP/SCO plant location, which also confirms that this model can be used to find optimal production plant location.

Figure 3. Coordinates of the optimal location of the production plant, depending on the amount of whey generated in dairy processing companies in Latvia. Initial optimal area (white area) was located by marking 20 coordinates with the lowest $P_{i,z}$ values. Grey square – lowest initial $P_{i,z}$ value. Lowest validated $P_{i,z}$ value (black square) was located by recalculating distances from dairy plants to potential production plant sites inside initial optimal area via motorways. Model XY axis resolution: i = {0…106}; z = {0…64}.

In this case study the 20 lowest initial $P_{i,z}$ values were used to draw initial optimal area (Fig. 3), but since number of selected initial $P_{i,z}$ values is arbitrary selected it can be changed depending on particular case study and users preferences. If larger number of initial lowest $P_{i,z}$ values are used the number of selected populated areas will also be larger which in turn will make the validation process more time consuming, therefore balance with selected XY axis resolution (in this case i = {0…106}; z = {0…64}) and size of the initial optimal area need to be considered to keep the calculations manageable. In the case study a single step on XY axis was about 4.2 km, which might have been too high resolution since similar results could be obtained, for example, by using half of XY axis resolution (Fig. 4). Although the initial optimal area is less refined due to decreased resolution (i = {0…53}; z = {0…32}; single step on XY axis ~8.4 km; deviation from initial $P_{i,z}$ value to lowest $P_{i,z}$ value after validation was 14.72 km), the lowest validated $P_{i,z}$ value is still within the initial optimal area, which in this case was created from only

10 lowest initial $P_{i,z}$ values (Fig. 4). This shows that model can be successfully used even with significantly decreased XY resolution. The decreasing of XY resolution by half did not affect the final result of the optimal SCP/SCO plant location while also significantly decreasing the amount of calculations that are required to perform the analysis via MS Excel. With i = {0…106}; z = {0…64} XY resolution, the model required to calculate 6955 $P_{i,z}$ values, while with halved resolution i = {0…53}; z = {0…32}, the model required only 1782 $P_{i,z}$ values to be calculated thus decreasing the amount of required calculations almost 4 fold, while still providing with the same results final results. This shows that model can be adjusted for user's preferences while also making the calculations more manageable.

Figure 4. Optimization model repeated with halved XY axis resolution: i = {0…53}; z = {0…32}. Initial optimal area (white area) was located by marking 10 coordinates with the lowest $P_{i,z}$ values. Grey square – lowest initial $P_{i,z}$ value.

CONCLUSIONS

By interviewing two companies for this study, it became apparent that companies lack capacity to provide the researchers with requested data, since data acquisition takes a long time and usually employees are unwilling to spend additional hours for data gathering. Also, the provided information about the dairy plants current use of cheese and cottage cheese whey should be taken with some scepticism, as company representatives are unlikely to disclose if some of the whey is discharged into local wastewater system or in natural water bodies without prior treatment. Both companies stated that they process or treat 100% of the generated whey, although estimates (Valorlact, 2012) state that at least 25% of the generated whey in EU is not disposed of properly or is not reprocessed in new products. Of course, this might also not be the case in Latvia and possibly all of the generated whey is treated properly or reprocessed, but with currently conflicting information it is difficult to know for sure. Complete data for generated aged and fresh cheese volumes was provided by CSB, thus it was possible to perform analysis with accurate data for the generated whey amount in each of the 22 dairy companies.

The current model assumes that all generated whey is used in SCP/SCO production, which is not accurate, because in reality some of the dairy plants use whey for production of other value added products. Such products as whey powder, whey protein powder, lactose powder, whey drinks etc. are also products with higher added value than SCP or

SCO. Therefore, further data acquisition is required in the future to calculate the available whey volumes in each dairy plant. This could be done by further interviews for the production volumes of alternative products which use whey as main ingredient or by acquiring data via other sources – CSB, media etc. Since most of the generated whey in Latvia is aged cheese whey (95.88%) the SCP and SCO production should focus on using only this type of whey. By using the reported SCP and SCO yields when using whey as substrate (Paraskevopoulou et al., 2003; Vamvakaki et al., 2010; Yadav et al., 2014), in Latvia, it would be possible to produce up to 800 tonnes of pure SCP (Paraskevopoulou et al., 2003) or up to 2,250 tonnes of pure SCO (Vamvakaki et al., 2010). SCO also has wider possible applications (feeds, biofuel, building block chemicals etc.) (Ratledge, 2013; Spalvins & Blumberga, 2019) and higher market price (Spalvins & Blumberga, 2018), therefore it can be concluded that SCO is the preferable end-product if whey is used as a substrate.

After validation, the developed model confirms that it is possible to calculate the optimal SCP/SCO plant location. Use of model itself is simple, quick and does not require any prior knowledge in using dedicated optimization or dynamic modelling software. This model can be used to optimize the sourcing of any raw material, in any industry where the situation requires the raw material to be gathered from multiple sources and transported to single processing plant. Therefore, the proposed model can be used for modelling the sourcing of by-products not only applicable for SCP or SCO production, but also for other purposes, such as sourcing of starch rich by-products for ethanol fermentations, sourcing of vegetable oil rich by-products and wastes for fuel production (biodiesel), sourcing of lignocellulose rich by-products and waste materials for lignocellulose hydrolysis followed by ethanol production etc.

In the future, the model need to be refined by following improvements:

By taking into account the number and capacity of transporting trucks and optimization of the route from raw material supplier to another, if it is not possible to load the truck fully in single source;

By introducing a dynamic model of the SCP or SCO production process to find the optimal production capacity (bioreactor volumes), while also taking into account the amount of feedstock supplied daily and the very short shelf life of the feedstock (for whey it is 24 hours);

Improve the dynamic model of the transport and production process by introducing a cost estimation that could also demonstrate whey viability for use in SCP and SCO production, by taking into account the market price of the final product;

Ensuring that developed model is easily adjustable for different types of feedstocks applicable for SCP and SCO production.

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REFERENCES

Brião, V.B. & Granhen Tavares, C.R. 2007. Effluent generation by the dairy industry: Preventive attitudes and opportunities. *Brazilian Journal of Chemical Engineering* **24**(4), 487-497.

Central Statistical Bureau of Latvia. 2020. LL540m. Dairy product production volumes by month. Available at https://data1.csb.gov.lv/pxweb/lv/lauks/lauks__05Lopk__isterm/LL540m.px. (in Latvian).

Cheng, F., Zhou, X. & Liu, Y. 2018. Methods of improvement of the thermal efficiency during spray drying. *E3S Web of Conferences* **53**, 01031.

Ebadian, M., Sowlati, T., Sokhansanj, S., Townley-Smith, L. & Stumborg, M. 2013. Modeling and analysing storage systems in agricultural biomass supply chain for cellulosic ethanol production. *Applied Energy* **102**, 840–849.

LSM. 2014. Dairy industry in Latvia is growing, purchasing power of the population – low. Available at https://www.lsm.lv/raksts/zinas/ekonomika/piena-nozare-latvija-augosa-iedzivotaju-pirktspeja-zema.a85965/ (in Latvian).

Lursoft. 20 Search for NACE rev. 2 categories. Available at: https://nace.lursoft.lv/10.51/companies/?vr=3&old=0&v=en&o=companies&q=

Paraskevopoulou, A., Athanasiadis, I., Kanellaki, M., Bekatourou, A., Blekas, G. & Kiosseoglou, V. 2003. Functional properties of sigle cell protein produced by kefir microflora. *Food Res. Int.* **36**, 431–438.

Ratledge, C. 2013. Microbial oils: an introductory overview of current status and future prospects. *OCL* **20**(6) D602.

Slavov, A.K. 2017. General characteristics and treatment possibilities of dairy wastewater – A review. *Food Technol. Biotechnol.* **55**(1), 14–28.

Sokhansanj, S., Kumar, A. & Turhollow, A.F. 2006. Development and implementation of integrated biomass supply analysis and logistics model (IBSAL). Biomass & Bioenergy 30, 838–847.

Spalvins, K. & Blumberga, D. 2018. Production of fish feed and fish oil from waste biomass using microorganisms: overview of methods analyzing resource availability. *Environmental and Climate Technologies* **22**, 149–154.

Spalvins, K. & Blumberga, D. 2019. Single cell oil production from waste biomass: review of applicable agricultural by-products. *Agronomy Research* **17**(3), 833–849.

Spalvins, K., Vamza, I. & Blumberga, D. 2019. Single cell oil production from waste biomass: review of applicable agricultural by-products. *Environmental and Climate Technologies* **23**(2), 325–337.

Spalvins, K., Ivanovs, K. & Blumberga, D. 2018a. Single cell protein production from waste biomass: review of various agricultural by-products. *Agronomy Research* **16**(S2), 1493–1508.

Spalvins, K., Zihare, L. & Blumberga, D. 2018b. Single cell protein production from waste biomass: comparison of various industrial by-products. *Energy Procedia* **147**, 409–418.

Valorlact. 2012. VALORLACT - Full use of the whey producedby the dairy industryLIFE11 ENV/ES/000639.*http://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=4256&docType=pdf* Accessed 06.02.2020

Vamvakaki, A.N., Kandarakis, I., Kaminarides, S., Komaitis, M., Papanikolaou, S. 2010. Cheese whey as a renewable substrate for microbial lipid and biomass production by Zygomycetes. *Eng. Life Sci.* **10**(4), 348–360.

Welfle, A., Gilbert, P. & Thornley, P. 2014. Increasing biomass resource availability through supply chain analysis. *Biomass and Bioenergy* **70**, 249–266.

Yadav, J.S.S., Bezawada, J., Ajila, C.M., Yan, S., Tyagi, R.D. & Surampalli, R.Y. 2014. Mixed culture of *Kluyveromyces marxianus* and *Candida krusei* for single-cell protein production and organic load removal from whey. *Bioresource Technology* **164**, 119–127.

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Comparison of growth of maiden trees of cultivars and genotypes of Cornelian cherry (*Cornus mas* L.) in a nursery

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Abstract. Cornelian cherry (*Cornus mas* L.) is still not a very popular fruit plant in Poland. Fruit growers have been recently increasingly interested in the cultivation of plants with fruits that can be widely used in processing. Fruits of Cornelian cherry can be eaten raw, and processed in various ways: for tinctures, juices, jams, silage, candied, etc. Both the fruits and preserves are characterised by high pro-health properties due to the content of vitamins, anthocyanins, and loganic acid. *Cornus mas* adapts well to the climate and soil conditions in Poland. The only limitation of its broader cultivation is lack of good planting material. The experiment investigated the efficiency of budding on two-year-old seedlings (*Cornus mas* L.) of several cultivars and genotypes of Cornelian cherry. Moreover, the height of plants, stem diameter, average number of shoots, number of leaves on selected shoots, and quality of roots were determined. The cornelian cherry maidens obtained by budding with dormant bud in August on two-year-old seedlings of Cornelian cherry (*Cornus mas* L.) constitutes high quality material suitable for establishing commercial plantations. Maiden trees of particular cultivars and genotypes of cornelian cherry significantly differ in height, diameter, number of branches and leaves, as well as the size of the root system. The diameter of the trunk is a good indicator of the quality of Cornelian cherry maiden, because it is closely positively correlated with the height of plants and the number of shoots.

Key words: growth correlations, stem diameter, T-budding, quality of cornelian cherry maiden trees.

INTRODUCTION

Fruit production is a very important branch of human economy, because fruit is a significant ingredient of human diet. It is well known that the optimal diet should be varied. Only then can it provide the body with necessary nutrients used for growth, energy production, and repair processes. Cornelian cherry fruit can provide variety to human diet, and even serve as a protection against diseases. Especially when they are processed in a modern way using e.g. dehydration, which does not cause loss of vitamin C and flavonoids (Strizhevskaya et al., 2019).

Intensification of agriculture leads to the creation of monocultures. This simplifies the landscape, threatens the biodiversity of flora and fauna, and may cause soil and water pollution. Therefore, it has recently been emphasised that the maintainance of high biodiversity permits obtaining higher and more stable yields with high fruit quality, and reduction or elimination of the use of pesticides by farmers (Fedosova & D'Antuono, 2012). Cornelian cherry (*Cornus mas* L.) is an example of a fruit species that is not widely distributed in cultivation. Cornelian cherry comes from Little Asia and Eastern Europe. In the natural state, it also occurs in the south of Europe, near the Mediterranean – Turkey, Italy, Spain, Portugal, and Greece. It grows naturally in bushes, in oak and hornbeam forests, and river valleys. It prefers sunny positions, and can often be found in the mountains, therefore it is very resistant to difficult conditions such as drought and strong winds. It grows well on any type of soil as long as it is permeable, and shows high tolerance for the pH of the substrate (Bijelić et al., 2012). Larger crops are harvested from wild plants in countries such as Iran, Turkey, and Serbia. Nonetheless, raw material obtained from seedlings from free pollination is very diverse in terms of quality. They are small, of various shapes and colours. Moreover, they are often less juicy and tart. Most importantly, the fruits show no uniform pattern of ripening, which makes harvesting difficult. The harvest is one of the most labours and expensive practices in the orchard. However, due to its similarity to olives, mechanical harvesting can be optimized here (Bernardi et al., 2016).

Many countries, including Ukraine, Poland, Iran, the Czech Republic, Austria, Serbia, Turkey, and others, breeding works have started to select cultivars. In the case of cornelian cherry, breeding is carried out towards faster and more abundant yield, and production of fruit of larger sizes, with a high share of flesh and a small stone (Magdhradze et al., 2009). During selection, cultivars interesting in terms of shape and colour are distinguished. The date of fruit ripening, duration of the ripening period, strength of tree growth, resistance to drought, and self-fertility are also important.

Choosing a cultivar for a commercial plantation should depend on the planned fruit management. Fruits intended for direct consumption as a dessert should have the largest mass (it is worth reaching for the Ukrainian variety with the largest weight of over 7g), or be distinguished by the colour of the skin, i.e. yellow ('Bukovinskij', 'Flava', 'Jantarnyj', 'Neżnyj' and 'Pervenets'). Red fruit varieties suitable for processing have fruits with the smallest proportion of stone in the mass of the fruit (9–10% on average), i.e. 'Jelena', 'Grienadier', 'Ekzotycznyj' from Ukrainian cultivars (Klimienko, 2004). Among Polish cultivars, the smallest share of the stone in total mass of fruit was found for the 'Florianka' and 'Dublany' cultivars (11%). Cultivars with a large stone can be used to produce oil rich in unsaturated fatty acids, with the highest share of linoleic acid (70–75%) and oleic acid (15–16.7%). Valuable cornelian seed oil can be used as a dietary supplement. Some Polish cultivars are characterised by a large proportion of the stone in the total weight of the fruit (15–16.5%), e.g. 'Kotula', 'Kresowiak', 'Bolestraszycki', 'Paczoski' and 'Kresowiak' (Kucharska et. al, 2011).

The condition for the establishment of intense cornelian cherry orchards is the availability of high-quality planting material. Cornelian cherry can be propagated from seeds, but generative propagation does not ensure the same characteristics as the mother plant (Bosančić, 2009). Moreover, plants harvested this way have a long juvenile period. Individual cornelian cherry cultivars are reproduced exclusively vegetatively. Difficulties with effective rooting of cornelian cherry necessitate the use of more effective methods (Bijelić et al., 2016). One of them is propagation by budding or grafting on cornelian cherry seedlings.

The objective of this experiment was to investigate differences in the growth of selected cultivars and genotypes of cornelian cherry at the end of the technology of production of maiden trees.

MATERIALS AND METHODS

The experiment was carried out in the period 2016–2017 in a private nursery in Dąbrowica (near Lublin) in Poland (22,454 N; 51,270 E).

In both studied years, the mean annual air temperature and annual precipitation were higher than the multi-annual mean/total (Table 1).

Table 1. Mean monthly air temperature and precipitation in the study years against the multi-annual mean/total (1951–2012)

Month	Air temperature (°C)			Precipitation (mm)		
	Monthly mean			Monthly total		
	2016	2017	Multi-annual mean (1951–2012)	2016	2017	Multi-annual total (1951–2012)
April	8.8	7.1	7.4	42.92	54.6	39.0
May	14.4	13.6	13.0	30.49	41.7	60.7
June	18.3	17.7	16.3	58.16	30.2	65.9
July	18.9	18.1	18.0	130.04	87.9	82.0
August	17.8	19.2	17.2	61.97	56.1	70.7
September	15.1	13.6	12.6	11.93	88.4	53.7
October	6.7	8.8	7.6	111.74	91.4	40.1
Mean/total for the year	8.7	8.5	7.3	876.7	819.2	558.9

In the experiment, two hundred two-year-old generative cornelian cherry rootstocks with a diameter of 12–15 mm were used in both seasons of the study. In August 2016, budding was performed by means of the 'T' method. Buds of selected cornelian cherry genotypes: Gruszkowy, Okazały, Roch, Za bankiem S_1, and Za bankiem S_2 were collected from mother plants growing in Dąbrowica. The buds of cultivars 'Paczoski', 'Raciborski', 'Dublany', 'Bolestraszycki', 'Szafer' came from the Arboretum and Institute of Physiography in Bolestraszyce, near Przemyśl, Poland. Buds were taken only from hardwood shoots. Shield pieces containing a bud wood were inserted in a 'T' shaped incision made on the stock, always on the western side. The rootstock and bud were tied together

Figure 1. Measuring the height of Cornelian cherry maiden plant.

with polythene tape to make the entire cutting surface adhere to the place on the rootstock. During attachment, care was taken not to break the bud. At the end of the 2016 season, manual weeding was carried out. At the beginning of the 2017 season, parts of the stock were cut off above the place of budding. The cut was made at a right angle in the place of the upper cut with the budding method in the letter 'T'. The place of cutting was protected with a mixture of emulsion and Funaben Plus 03 PA. Immediately after cutting, nitrogen fertilisation was applied with 'Mocznik' fertiliser. Soil fertilisation with multi-ingredient 'Fructus truskawka' (concentrated, granular multi-component: N 9%, P 9%, K 16%, Mg 2%, s 24,5%) fertiliser was applied on 2 dates, namely 10.05 and 20.06. Sprouting of the buds began in mid-April 2017. Plants were also foliar supplied with the 'Ekolist Standard' fertiliser from the end of June to mid-August, after 2 days, every 2 weeks. A potassium preparation was also used to accelerate the growth of plants. During the season, mechanical weeding and soil tillage were carried out. In August, the formation of maiden trunks was performed through cutting strongly growing three bottom pairs of shoots that could compete with the leader. One of the final treatments was manual defoliation.

Figure 2. Measuring of stem diameter of Cornelian cherry maiden plant.

During the experiment, the height of plants was determined (from the ground surface to the tip of the leader Fig. 1), as well as the diameter of the plant (just above the place of budding Fig. 2), average number of shoots, and number of leaves on selected shoots. The measurements were made at monthly intervals on four dates, namely: 21.06.2017, 27.07.2017, 29.08.2017, and 10.10.2017. The last step was removing plants from the nursery (at the end of October), and determining the number and length of roots (Fig. 3).

Figure 3. Preparation of maiden plants for assessing the quality of the root system.

Data Analysis

Statistical calculations were performed in STATISTICA for Windows Version 5.5A. The obtained results were subject to statistical analysis by means of analysis of variance (ANOVA). The significance of the differences among the characteristics was assessed based on confidence intervals calculated by means of a Tukey's test, with a significance level of 5%.

RESULTS AND DISCUSSION

Because of its very valuable fruit, Cornelian cherry is bred in many countries, including Ukraine, the Czech Republic, Slovakia, Iran, Turkey, Serbia, France, Austria, and Poland. Polish cultivars, bred in the Arboretum in Bolestraszyce near Przemyśl, include: 'Bolestraszycki', 'Dublany', 'Florianka', 'Juliusz', 'Kresowiak', 'Paczoski', 'Podolski', 'Raciborski', 'Słowianin', and 'Szafer'. They differ in terms of fruit ripening, shape, colour, taste, share of the seed in the fruit, as well as chemical properties, e.g. extract, vitamin C, and anthocyanins (Kucharska et al., 2011).

An important factor intensifying production is high quality nursery material. Generative reproduction does not guarantee obtaining high-quality trees, because young plants are very diverse in terms of quality characteristics. Therefore, cornelian cherry should be vegetatively propagated for production purposes. At a small scale, cornelian cherry can be propagated by layering (Ivanicka & Cvopa, 1977; Klimenko, 2004; Fedosova & D'Antuano, 2012). Another way of vegetative propagation is cuttings. Softwood cuttings are very delicate, sensitive, and require the use of mist (Ivanicka, 1989). It is an effective method, but to a large extent dependent on the date of collection of cuttings, and the type of preparation used for rooting (Pirlak, 2000). The ability to root largely depends on genetic traits, but it can be improved by using growth regulators (auxins), as well as proper fertilisation, irrigation, and maintenance of high humidity (Stepanowa et al., 1986; Smykov et al., 1987). Jagła (2011), comparing the reproduction efficiency of several lesser-known fruit species, including cornelian cherry, using cuttings harvested at the end of June and at the turn of July/August, assisted by rooting preparations such Himal and Rhizopon, stated that cornelian cherry was the poorest rooting species. In both dates of obtaining cuttings, the degree of their rooting varied from 0% (cuttings without rooting preparation) to 33% of cuttings treated with rooting preparation Rhizopon. Cornelian cherry can also be propagated *in vitro*. Cornelian cherry is a species relatively easy to replicate, but in tissue cultures, because in the case of cuttings rooting is very poor.

Not many experiments have been conducted comparing the effect of planting material on the growth and yielding of cornelian cherry in orchards. Nonetheless, the assessment of the rate at which fruiting begins suggests that the best method is grafting or budding on *Cornus mas* seedlings (Klimenko, 2004) or *Cornus amomum* seedlings (Ochmian et al., 2019). In addition to repeating the characteristics of the mother plant, it guarantees much faster beginning of fruiting, even compared to plants obtained by cuttings. Approximately 50% of maiden trees flower and yield in the second year after budding, and almost 100% in the third year (Klimenko, 1990). However, plants obtained from seedlings have the first fruit not earlier than after 8 years after planting. Ochmian et al. (2019) found that cornelian cherry cultivars budded on *Cornus amomum* were characterised by weaker tree growth, but the quality of fruit did not decrease compared to trees on their own roots. Fruits of cultivars with dark skin (‘Jolico’, ‘Schönbrunner’ and ‘Shumen’) from budded trees had significantly more anthocyanins. The ‘Jolico’ fruit from budded trees showed a much larger diameter and mass.

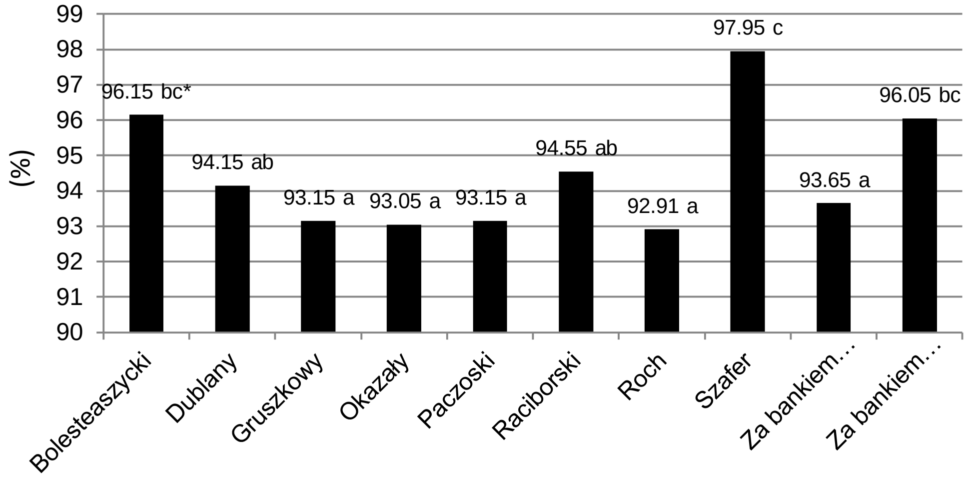

Figure 4. Efficiency of budding (%) of cultivars and genotypes of Cornelian cherry (*Cornus mas* L.).

*Bars with different letters are significantly different at $P < 0.05$.

The rootstock is an important component of the tree, because it determines its strength of growth, adaptation to soil conditions, resistance to disease, size and quality of crops, and tolerance to salinity and drought. In the case of cornelian cherry, a rootstock good in terms of physiological compatibility is cornelian cherry (*Cornus mas* L.). Difficulties with vegetative propagation enforce the use of generative rootstocks, i.e. propagated from seeds. This type of reproduction does not ensure homogeneous quality, although in the case of stone fruits (cherries, cherries, and plums) in the nursery technology, generative rootstocks are also used. The comparative assessment of the method of maiden cornelian cherry production can be referred to the production of two-year-old maiden trees (Sadowski et al., 2006). The root system of such trees is three years old. The trees must be well-grown and branched. The quality features of trees depend on the species. In the case of apple trees, budding trees 20 cm above the ground is most desirable, on dwarf rootstocks with a high-formed crown, at least at a height of 60–80 cm, with not too long lateral shoots (up to 60 cm in length). Based on research by Bielicki et al. (2008) on the quality of obtained young organic trees of apple, pear, cherry, cherry, plum, and quince, the highest number of trees in comparison to budded rootstocks was obtained in the case of apple trees (80.3–91.2%) and pear trees (42.9–87%), and the smallest for sweet cherries (8.4–17.3%). Klimenko (2004) and Fedosova & D’Antuano (2012) reported that the most effective way of propagation of cornelian cherry is budding (efficiency 90–98%) and grafting (efficiency 75–78%). In the present experiment, the efficiency of budding was very high (from 92.1 to 97.95%), suggesting the usefulness of this method for Cornelian cherry propagation (Fig. 4). The quality of rootstocks has considerable influence on the reception of buds. In organic farming, it is difficult to protect against diseases and pests. Due to this, the rootstock is less grown, and the length of cambium activity is reduced. In the absence of monocultures, Cornelian cherry is resistant to diseases and serious pests such as aphids and mites. The growth of maiden trees also to a large extent depends on the time of budding. According to nurseries, the best date for T-budding is the period of intensive division of the cambium. In the conditions of central, south, and east Poland, sour cherry budding should start between the end of July and middle of August (Hołubowicz et al., 1993). In the present experiment, budding was carried out in mid-August, and the high effectiveness of the treatment testifies to good activity of the Cornelian cherry cambium in this period. cv. 'Szafer' was characterised by the highest efficiency of budding (97.95%), while the smallest efficiency of budding concerned genotypes: Roch, Okazały, Gruszkowy, Za bankiem S_1, and cultivar 'Paczoski' (from 92.91 to 93.65%). This confirms results by Bijelić et al. (2016) who in the conditions of Serbia proved that budding in August is a method of reproduction more effective (from 56.67 to 83.62%) than grafting in April (from 21.67 to 30.33%).

The growth of maiden trees in a nursery depends on the genetic characteristics of the species and the variety. Bielicki et al. (2008) found that among the tested species, prune trees were best grown on seedlings, and the variety had a decisive impact on the quality of the obtained maiden trees. Stachowiak & Świerczyński (2004) proved that growth of maiden sweet cherries in the nursery particularly depends on the rootstock, although among the studied cultivars, 'Hardy Giant' and 'Sumit' had the largest trunk diameter and height. According to Jacyna (2004), in the case of pear, the cultivar has a more significant impact on the branching of maiden trees than the rootstock. Jacyna (2004) and Łanczont (2004) evidenced a high correlation between the trunk diameter and number of shoots of maiden trees of apple, pear, and plum trees, which makes the trunk diameter an important quality feature of young trees. Lipecki et al. (2015) found a strong correlation between the diameter of 'Łutówka' maiden trunks and the total length of lateral shoots. The present experiment determined a strong positive correlation of the stem diameter with the height of maiden species and their number of branches (Table 2).

Table 2. Pearson's correlation coefficients (r) between selected measured criteria

Growth parameters	Number of shoots	Tree height	Stem diameter	Number of leaves
Number of shoots	x	0.53*	0.58*	-0.22*
Tree height		x	0.58*	-0.32*
Stem diameter			X	-0.10

Coefficients marked with asterisks (*) are significant at $P < 0.05$.

Meteorological conditions have a strong impact on the quality of nursery material (Baryła, 2005). In the present experiment, maiden trees were damaged by spring frosts in May 2017. Cooling occurred when the plants had 2–3 pairs of leaves (about 10 cm). The frost caused damage to the leaves, manifested in the characteristic scorching of leaf edge, and temporary inhibition of growth. Nonetheless, at the end of the growing season, the height of all dogwood varieties and genotypes exceeded 1,000 mm (Table 3). In the present study, the height of plants was influenced by the cultivar and genotype. The highest values were determined for cv. 'Bolestraszycki', genotype Okazały and cv. 'Dublany' (respectively: 1,493, 1,461, and 1,493 mm), while genotypes Za bankiem S_1

and Roch were the shortest (1,013 and 1,050 mm). The height of maiden trees determined based on Ekaterina (2008) is similar, because in the first year of vegetation maiden trees grow up to 120–150 cm. Bijelić et al. (2016) obtained much lower trees (from 736.7 to 906.8 mm) as a result of budding in August and grafting in April. An important feature that proves the quality of maiden trees is the diameter of the stem. In the present experiment, the stem diameter was similar for more cultivars and genotypes. The largest diameter was reached for cv. 'Dublany' (16.84 mm), and the smallest for cv. 'Raciborski' (12.93 mm). The obtained results confirm the study by Klimenko (1990) in which Cornelian cherry maiden trees had a diameter of 13 to 15 mm, slightly higher than in the case of Bijelić et al. (2016), where the budding genotypes had a diameter of 10 to 13.61 mm.

Table 3. Some above-ground morphometric features of Cornelian cherry cultivars and genotypes

Cultivar/ genotype	Height of plants (mm)	Stem diameter (mm)	Average number of shoots (pieces/plant)	Average number of leaves (pieces/shoot)
Bolestraszycki	1493 c*	16.31 bc	11.4 de	14.8 ab
Dublany	1457 c	16.84 c	11.6 e	14.8 ab
Gruszkowy	1305 bc	15.0 a-c	10.4 b-d	15.8 a-c
Okazały	1461 c	14.71 a-c	9.6 ab	17.7 de
Paczoski	1341 bc	14.75 a-c	11.0 c-e	14.4 a
Raciborski	1182 ab	12.93 a	11.0 c-e	16.3 b-d
Roch	1050.1 a	15.14 a-c	10.0 a-c	18.6 e
Szafer	1103 ab	14.79 a-c	10.4 b-d	15.5 a-c
Za bankiem S_1	1013 a	13.88 ab	9.2 a	17.7 de
Za bankiem S_2	1119 ab	15.11 a-c	10.0 a-c	16.5 cd

*Values in the same column with different letters are significantly different at $P < 0.05$.

The assessment of the effect of the cultivar and genotype on the average number of shoots (Table 3) showed the highest values for cv. ‘Dublany’, ‘Bolestraszycki’, ‘Paczoski’, and ‘Raciborski’ (respectively: 11.6, 11.4, 11.0, and 11.0 pieces/plant), and the lowest values for genotype Za bankiem S_1 – 9.2 pieces/plant. Cultivars and genotypes. differed significantly in the average number of leaves. The highest number of leaves per shoot was determined for genotypes: Roch, Okazały, and Za bankiem S_1 (respectively: 18.6, 17.7, and 17.7 pieces/shoot), and the lowest for cv. ‘Paczoski’ (14.4 pieces/shoot) The results of our study showed that the intense growth of maiden tree height and trunk diameter occurred from June through August, and decreased from September to October (Figs 5, 6). In September and October, intensive formation of leaves on the Cornelian cherry maidens occurred (Fig. 7).

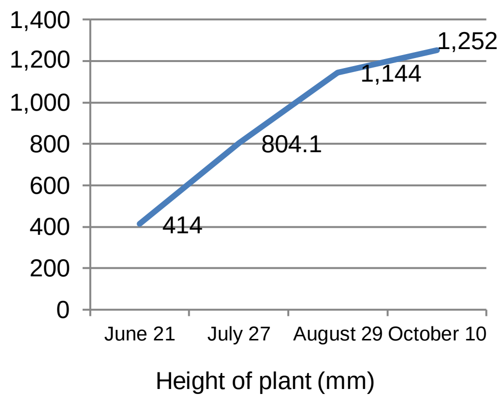

Figure 5. Growth rate in height of Cornelian cherry maiden trees in the growing season.

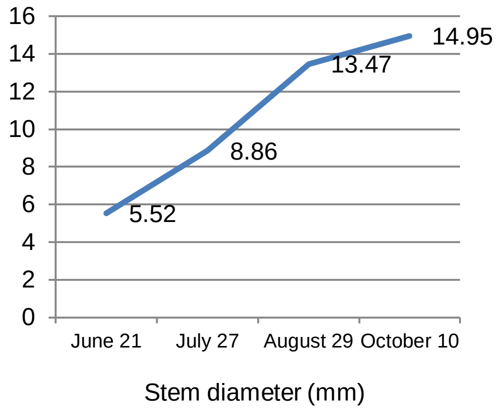

Figure 6. Growth rate in stem diameter of Cornelian cherry maiden trees in the growing season.

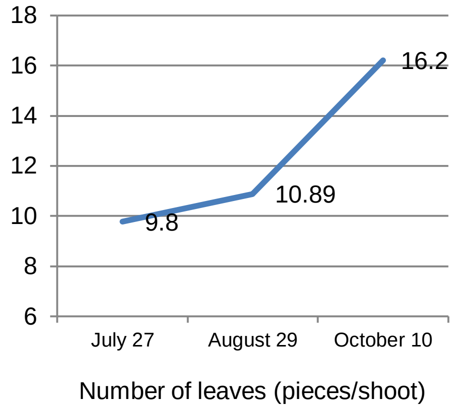

Figure 7. Changes in the number of leaves on maiden shoots in the growing season.

An important element determining correct adoption and commencement of growth on a plantation is a properly developed root system. The Cornelian cherry root system is adapted to grow even in mountainous conditions. It is therefore shallow and very branched, with a high number of very thin roots allowing penetration of the substrate. Cornelian cherry is difficult to root. The number of roots obtained during propagation by cuttings is smaller than during budding. Pirlak et al. (2000) rooting 10–15 cm sections of hardwood shoots recorded from 9.51 to 12.39 roots (depending on the genotype tested), and the root length varied from 4.49 to 5.65 mm. Bijelić et al. (2016) counting roots of Cornelian cherry budded in August recorded from 12 to 19.67 pieces, and their length ranged from 103.34 to 142.38 mm. Klimenko (2004) found a smaller number of maiden roots: from 10.6 to 14.4. In the present study, the most-grown roots were produced in the case of the 'Szafer' cultivar (24.15), and the least-grown ones by genotypes: Roch, Gruszkowy, Za bankien S_1, Za bankiem S_2, and cultivars ‘Dublany’ and ‘Paczoski’ (from 18.10 to 20). Particular cultivars and genotypes substantially differed in the length of roots (Table 4). The longest roots were created by cv. 'Szafer' (160.0 mm), and the shortest by Za bankiem S_1 (127.3 mm).

Table 4. Effect of the cultivar and genotype on selected quality features of roots and efficiency of budding

Cultivar/ genotype	Length of root (mm)	Number of roots (pieces/plant)
Bolestraszycki	149.8 e*	21.00 ab
Dublany	139.0 bc	19.50 a
Gruszkowy	149.6 e	18.10 a
Okazały	142.9 cd	13.15 bc
Paczoski	145.5 de	19.90 a
Raciborski	127.8 a	25.75 c
Roch	144.8 de	18.10 a
Szafer	160.0 f	24.15 c
Za bankiem S_1	127.3 a	20.00 a
Za bankiem S_2	135.3 b	19.20 a

*Values in the same column with different letters are significantly different at $P < 0.05$.

Producing high-quality trees does not guarantee sufficiently rapid growth on a plantation. It is very important not to allow the root system to dry out. In the nursery technology, the method of cultivation needs to be refined: in pots of 2–3 L, or directly in the soil, and then sales with a 'bare' but much better developed root system. Propagation by budding is not the easiest and quickest method. It still requires refinement at many stages of nursery production. A minimum period of 2 years is required to obtain rootstocks. Cornelian cherry seeds obtained from harvested fruits require a rest period. This period can be reduced by stratification or scarification. There are many physical and physiological factors, including the fact that the seeds are covered with thick shells. Seeds are stimulated for germination by means of special treatments. Ensuring the continuity of production of rootstocks, followed by proper budding and transport of trees to a permanent place, however, allows for a quick entry into the fruiting period and high fruit quality.

CONCLUSIONS

Cornelian cherry maidens obtained by budding with dormant bud in August on two-year-old seedlings of Cornelian cherry (*Cornus mas* L.) constitute high quality material suitable for establishing commercial plantations. Maiden trees of individual cultivars and genotypes of Cornelian cherry significantly differ in height, diameter, number of branches and leaves, as well as size of the root system. The diameter of the trunk is a good indicator of the quality of Cornelian cherry maiden, because it is closely positively correlated with the height of plants and the number of shoots. The highest quality trees in terms of their height, stem diameter and number of shoots were obtained in the cv. 'Dublany'. Whereas the weakest growth, the smallest number of shoots and a relatively small diameter of the trunk was characterized by the genotype Za bankiem S_1.

REFERENCES

Baryła, P. 2005. Relationship Between the Climatic Factors and The Growth of Young Cherry Trees in a Nursery. *Acta Agroph*. **6**(1), 31–41.

Bernardi, B., Benalia, S., Fazar, A., Zimbalatti, G., Stillitiano, T. & De Luca, A.I. 2016. Mechanical harvesting traditional olive orchards: oli-picker case study. *Agronomy Research* **14**(3), 683–688.

Bielicki, P., Rozpara, E., Grzyb, Z.S. & Badowska – Czubik, T. 2008. Production of Nursery Material For The Purposes of Ecological Orchards Considering New Techniques of Propagation. Report from basic research conducted in 2008 for ecological agriculture in the scope of horticulture. http://www.insad.pl/files/EKO_SAD/sprawozdania_2008/Szkolka_Eko_2008.pdf

Bijelić, S.M., Gološin, B.R., Cerović, S.B. & Bogdanović, B.V. 2016. A Comparison of Grafting Methods For The Production of Quality Planting Matarial of Promising Cornelian Cherry Selections (*Cornus mas* L.) in Serbia. *J. Agr. Sci. Tech.* **18**, 223–231.

Bijelić, S., Gološin, B., NinićTodorović, J., Cerović, S. & Bogdanović, B. 2012. Promising Cornelian cherry (*Cornus mas* L.) genotypesfrom naturalpopulation in Serbia. *Agriculturae Conspectus Scientificus* **7**(1), 5–10.

Bosančić, B. 2009. Domestification and Morphological Variation in Wild and Cultivated Populations of Cornelian cherry (*Cornus mas* L.) in the Area of the Dvar Valley, Bosnia and Herzegowina). *International Master Programme at the Svedish Biodiversity centre*, CBM Master Thesis No. **69**.

Ekaterina, 2008. Where There Is a Cornelian Cherry, a Doctor is Not Required. Garden, No. http://fermer02.ru/earth/sotok/268-tam-gde-est-kizil-lekar-ne-nuzhen.html.

Fedosova, K. & D'Antuono, L.F. 2012. Cornelian Cherry (Dogwood) in South of Ukraine. *Charczowa Sci and Technol.* **2**(19), 60–64.

Hołubowicz, T., Rebandel, Z. and Ugolik, M. 1993. *Cultivation of Sweet Cherry and Sour cherry PWRiL*, Warszawa, 287 pp. (in Polish).

Ivanicka, J. 1989. Propagation of Unusual Fruit Crops From Softwood Cuttings Under Mist. *Vedecke prace Vyskumneho ustavu ovocnych a okrasnych drevin v Bojniciach* **7**, 163–170.

Ivanicka, J. & Cvopa, J. 1977. Propagation of Dogwood (*Cornus mas* L.) by Softwood and Semi-Hardwood Cuttings. *Gartenbauwissenchaft* **42**(4), 169–171.

Jacyna, T. 2004. The Role of Cultivar and Rootstock in Sylleptic Shoot Formation in Maiden Pear Trees. *J. Fruit Ornam. Plant Res.* **12**, 41–47.

Jagła, J. 2011. Propagation of LittleKnown Horticulture Species. *Sad Nowoczesny* **1**, 20–23.

Klimenko, S. 1990. *Cornelian Cherry in Ukraine*. Academy of Sciences, Kiev, Naukova Dumka, 174 pp.

Klimenko, S. 2004. The Cornelian Cherry (*Cornus mas* L.) Collection, Preservation and Utilization of Genetic Resources. *J. Fruit Ornam. Plant Res*. **12**, 93–98.

Kucharska, A., Sokół-Łętowska, A. & Piórecki, N. 2011. Morphological, Physicochemical, and Antioxidant Characteristics of Fruits of Polish Cultivars of Cornelian Cherry (Cornus mas L.). *Food. Sci. Technol. Quality* **3**(76), 78–89.

Łanczont, M., 2004. Growth Correlations in One-year Occulants of Apple, Pear, and Prune Tree Ms. Thesis. *Univ. Agric. Lublin*, Poland, 21–29.

Lipecki, J., Lipa, T. & Szot, I. 2015. The Growth Relationship in Maiden Trees of Sour Cherry 'Łutówka'. *Acta Agrobot*. **68**(3), 255–260.

Magdhradze, D., Abashidze, E., Bobokashvili, Z., Tchipashvili, R. & Maghlakelidze, E. 2009. Cornelian Cherry in Georgia. *Acta Hortic*. **818**, 65–72.

Ochmian, I., Oszmiański, J., Lachowicz, S. & Krupa – Małkiewicz, M. 2019. Rootstock Effect on Physico-chemical Properties and Content of Bioactive Compounds of Four Cultivars Cornelian Cherry Fruits. *Sci. Hortic*. **256**, 1–12.

Pirlak, L., 2000. Effects of Different Cutting Times and IBA Doses on the Footing Rate of Hardwood Cuttings of Cornelian Cherry (*Cornus mas* L.). *Anadolu J of AARI* **10**(1), 122–134.

Sadowski, A., Lewko, J. & Dziuban, R. 2006. Evaluation of Some Nursery Techniques in the Production of 'Knip boom' Apple Trees. *Latvian J. Agronom*. **9**, 130–134.

Smykov, V.K, Stepanova, A.F. & Lithochenko, N.A. 1987. Effects of β-indolybutyric acid on Root Formation in Fruit Crops. *Hort. Abst*. **57**(2), 1659.

Stachowiak, A. & Świerszczyński, S. 2004. Growth of Six Sweet Cherry Cultivars on Colt and on Mazzard Seedling (*Prunus avium* L.) Rootstocks in a Nursery. *Rocz. AR Pozn. CCCLX, Ogrodn*. **38**, 149–156.

Stepanova, A.F., Litchochenko, N.A. & Smykov, A.V. 1986. Propagating Fruit Crops by Softwood Cuttings. *Hort. Abst*. **56**(11), 8498.

Strizhevskaya, V., Pavlenkova, M., Nemkova, S., Nosachyova, N., Simakova, I. & Wolf, E. 2019. Possibility and prospects of preservation of minor components in technology of fruit raw materials conservation. *Agronomy Research* **17**(5), 2082–2088. https://doi.org/10.15159/AR.19.139

Agronomy Research **18**(S2), 1537–1546, 2020
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Aerobic solid-state fermentation of the solid fraction of pig slurry

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Abstract. Current expansion in the pig number in Russia and their concentration in separate locations contribute to higher environmental risks. One key risk factor is the slurry produced. To utilize it more efficiently, many pig farms introduce its solid-liquid separation. The study objective was to explore the feasibility of accelerated aerobic solid-state fermentation of the solid fraction of pig slurry in closed installations. The stable thermophilic process under the temperature above +55 °C achieves shorter processing time of 3–4 days. To date, however, there is no representative evidence of such an experience. Four options of fermented mixture composition were tested based on two types of the solid fraction of pig slurry: Type 1 fraction coming from a screw separator and Type 2 fraction coming from a decanter centrifuge. The fermenter operating modes were tested in the authors' previous studies associated with processing of the solid fraction of cattle manure and bedding poultry manure. The intensity measure of fermentation was the temperature reached by the processed material in the fermenter. Under the investigated operation modes, the stable temperature was observed for nine days in the mesophilic process: 20 °C to 55 °C; in some cases, the transition to the thermophilic process – above 55 °C was recorded. Adding the catalytic components to the processed material accelerated the substrate self-heating and a higher temperature up to 59 °C was reached. This suggests that the considered operating modes of the fermenter were suitable for the fermentation of the specified substrate.

Key words: solid-state fermentation, aerobic fermentation, pig slurry, slurry handling.

INTRODUCTION

Ensuring food security is one of the top priorities for global economic development. The Baltic Sea Region, which includes Denmark, Estonia, Finland, Germany, Latvia, Lithuania, Poland, Russia (Northwestern Federal District), and Sweden, is one of the most economically developed regions in the world. With a population of above 45 million people (Eurostat, 2018; DYR, 2018), the Baltic Sea Region provides about 30% of European trade (Kivikari & Antola, 2004; Studzieniecki, 2016). Highly developed agriculture is to be in place to supply such a population with the sufficient quantity of food products of proper quality.

As the Northwestern Federal District of the Russian Federation has the cool temperate climate and humus-poor soils, it falls within the zone of risky crop farming thus making the animal husbandry the main agricultural sector. For example, the gross volume of livestock products here is three to four times higher than that of crop products (Briukhanov, 2017).

Pig farming is one of the most dynamic agricultural sectors in the region. In particular, in Russia, from 2010 to 2018, the number of pigs in the Baltic Sea catchment area increased from 463.4 to 1704.6 thousand head, and in the whole country – from 17251.4 to 23726.6 million head (UIISS, 2018). Such significant growth has been underpinned by the production increase and intensification. In the pig industry in 2010, the 20 largest producers accounted for 49.2% of all pork produced in the country. By 2018, this value amounted to 65% (NPFU, 2011; NPFU, 2019).

Higher pig stock and its concentration in particular locations bring, however, the challenge of waste utilization to the next quality level. The current trend to solid-liquid slurry separation on the large-scale pig complexes promotes the search for new, more intensive and environmentally friendly slurry utilisation technologies. Several studies have verified the inverse relationship between the moisture content of the resulting organic fertiliser and its nutritional value and effective transportation distance (Vasilev, 2014; Briukhanov et al., 2017; Uvarov et al., 2018; Sharma et al., 2019).

Aerobic solid-state fermentation has proved to be an efficient method of various organic waste processing including a solid fraction of animal/poultry manure. The closed-type fermenters minimize the impact of the external weather factors, such as precipitation, the outdoor air temperature and humidity, etc. This has a positive effect on the nutrient loss, the end-product quality and the processing time, reducing it to several days (Leach, 2015; Uvarov et al., 2016; Fournel et al., 2019).

Currently, the aerobic solid-state fermentation of pig slurry is less investigated compared to other waste, cattle and poultry manure in particular. The literature survey did not reveal sufficient data on the mesophilic and thermophilic processes of the set intensity in the pig slurry. Therefore, the study objective was to explore the possibility of accelerated processing of the solid fraction of pig slurry in a fermenter.

MATERIALS AND METHODS

The study had two steps. Step 1 explored the possibility of accelerated processing of separated solid fraction of pig slurry. Step 2 identified the composition of the fermentable substrate based on the solid fraction of pig slurry, which provided the required intensity of the aerobic solid-state fermentation. With this aim in view, two types of the solid fraction of pig slurry were supplemented with grain mechanical cleaning waste, water and BIAGUM – an organic fertiliser produced from the bedding poultry manure after the aerobic fermentation. The optimal ratio of substrate components was determined with due account for the restrictive values of the above-considered criteria by the formula (1):

$$X_{i\,a} = \frac{M_I \cdot X_{i\,I} + M_{II} \cdot X_{i\,II} + M_G \cdot X_{i\,G} + M_W \cdot X_{i\,W} + M_B \cdot X_{i\,B}}{M_I + M_{II} + M_G + M_W + M_B} \quad (1)$$

where $X_{i\,a}$ – actual (measured) value of the *i*-th parameter of the substrate (*W, pH, C/N*) with the standard deviation; M_I – calculated mass of Type 1 fraction, kg; $X_{i\,I}$ – actual (measured) value of the *i*-th parameter of Type 1 fraction; M_{II} – calculated mass of Type 2 fraction, kg; $X_{i\,II}$ – actual (measured) value of the *i*-th parameter of Type 2 fraction; M_G – calculated mass of the grain mechanical cleaning waste, kg; $X_{i\,G}$ – actual (measured) value of the *i*-th parameter of the grain mechanical cleaning waste; M_W – calculated mass of water, kg; $X_{i\,W}$ – actual (measured) value of the *i*-th parameter of water; M_B – calculated mass of BIAGUM organic fertiliser, kg; $X_{i\,B}$ – actual (measured) value of the *i*-th parameter of BIAGUM organic fertiliser.

The optimal composition was chosen among the set of Pareto-optimal solutions.

The experiments were carried out in the laboratory of the organic waste bioconversion of IEEP – branch of FSAC VIM, the analyses – in the analytical laboratory of the same institute in February – April 2019. The experiments had three replications.

Two types of the solid fraction of pig slurry were the starting material for the accelerated processing: Type 1 fraction – coming from a screw separator, and Type 2 fraction – coming from a decanter centrifuge. The initial values of physical and chemical composition of the native pig slurry were the same since the considered separation technology variants were applied on the same pig-rearing complex of the full cycle, located in the North-West Russia.

The study subject was the accelerated processing of Type 1 and Type 2 fractions in the express-fermenter. The fermenter had the volume of 0.8 m^3 that provided the minimal critical mass required for the successful process of the solid-state aerobic fermentation. The installation was a closed chamber with the aeration holes in the bottom. The installed mixer simulated the controlled fermentation in the stationary operating mode and the regular mixing mode. The scheme of the laboratory-scale express-fermenter is shown on Fig. 1. The RK-102-10-50 compressor supplied the air.

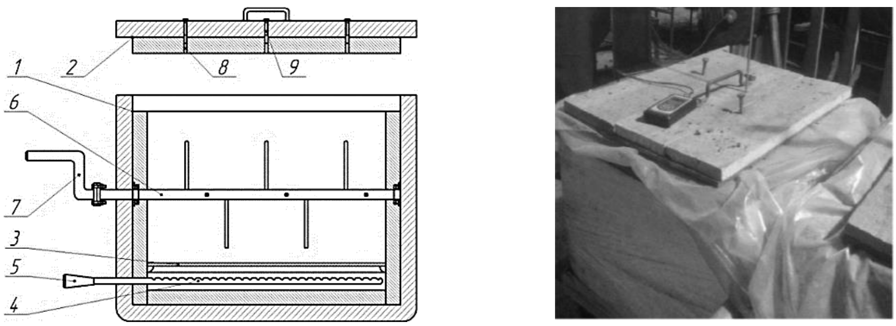

Figure 1. Laboratory-scale express-fermenter: 1 – chamber; 2 – loose cover; 3 – perforated plate; 4 – aeration pipe; 5 – air inlet; 6 – mixer; 7 – free handle; 8 – plugs; 9 – temperature measurement holes.

According to our previous studies, the key factor to successful solid-state aerobic fermentation was to maintain a stable temperature in the range of 55–80 °C that ensured the maximum activity of the thermophilic microorganisms (Manure Composting Manual, 2005).

The calibrated equipment was used in the experiment: the ambient temperature was measured by an inside air thermometer TS-77; the temperature inside the express-fermenter was measured by TCM 9410/M2 thermometer with a thermal probe. The physical and chemical properties of the processed material were determined following the relevant State Standards:

- State Standard GOST 26713-85. ‘Organic fertilisers. Method for determination of moisture and dry residue’;
- State Standard GOST 26714-85. ‘Organic fertilisers. Method for determination of ash content’;
- State Standard GOST 26715-85. ‘Organic fertilisers. Methods for determination of total nitrogen’;
- State Standard GOST 26716-85. ‘Organic fertilisers. Methods for determination of ammonium nitrogen’;
- State Standard GOST 26717-85. ‘Organic fertilisers. Method for determination of total phosphorus’;
- State Standard GOST 26718-85. ‘Organic fertilisers. Method for determination of total potassium’;
- State Standard GOST 27979-88. ‘Organic fertilisers. pH determination method’;
- State Standard GOST 27980-88. ‘Organic fertilisers. Organic substance determination methods’.

The sampling followed the State Standard GOST R 54519-2011. ‘Organic fertilisers. Methods of sampling’.

The express-fermenter operating modes were tested in the previous studies associated with the processing of the solid fraction of cattle manure and the bedding poultry manure – Table 1 (Uvarov et al., 2016; Uvarov et al., 2017a; Uvarov et al., 2017b).

The experimental data was statistically analysed in *StatGraphics Centurion v.16.1* software package.

Table 1. Variation levels of the controlled factors

Parameter	Unit	Mode I	Mode II	Mode III
Aeration time	min h^{-1}	20	13	7
Aeration speed	m s^{-1}	10	7.5	5.5
Mixing interval	h	24	18	12

RESULTS AND DISCUSSION

Step 1

Fermentation process was affected by several factors, such as moisture content, C/N ratio, pH and the physical and chemical properties of the starting materials (Manure Composting Manual, 2005; Takahashi et al., 2017). These indicators were determined by the laboratory analyses – Table 2.

The variation of the material moisture content was below 5%, with the values being in the upper acceptable recommended range. The pH values varied significantly. Type 2 fraction was 4.1 units more alkaline and had a significantly higher ash content (45.4%) than Type 1 fraction with a pH of 7.9and the ash content

Table 2. Physical and chemical properties of the starting materials

Indicator	Unit	Type 1 fraction	Type 2 fraction
Moisture content (*W*)	%	70.59	67.36
pH	-	7.9	12.0
NH_4^+	mg kg^{-1}	464.0	62.9
NO_3^-	mg kg^{-1}	118.1	947.1
K^+	mg kg^{-1}	495.0	479.0
P_{total}	mg kg^{-1}	2,000.0	8,100.0
N_{total}	mg kg^{-1}	5,370.0	8,148.0
Ash content	%	6.4	45.4

of 6.4% since during the on-farm processing of this waste the quicklime (CaO) was added. Thus launched nitrification processes also significantly reduced ammonium nitrogen (NH_4^+) content and increased the nitrate nitrogen (NO_3^-) content.

The starting materials were loaded simultaneously in two laboratory-scale express-fermenters (Fig. 2).

Figure 2. Trial establishment: a) laboratory-scale express-fermenters; b) solid fraction of pig slurry coming from the screw separator (Type 1 fraction); c) solid fraction of pig slurry coming from the decanter centrifuge (Type 2 fraction).

The ambient conditions were the same for the two express-fermenters. The average air temperature inside the laboratory was 12 ± 1 °C. The temperature in the express fermenters was recorded once a day for nine days (Fig. 3) since in the similar studies of other types of organic waste this particular experiment duration and measurement frequency were found optimal (Uvarov et al., 2016; Briukhanov & Uvarov, 2016; Uvarov et al., 2017b).

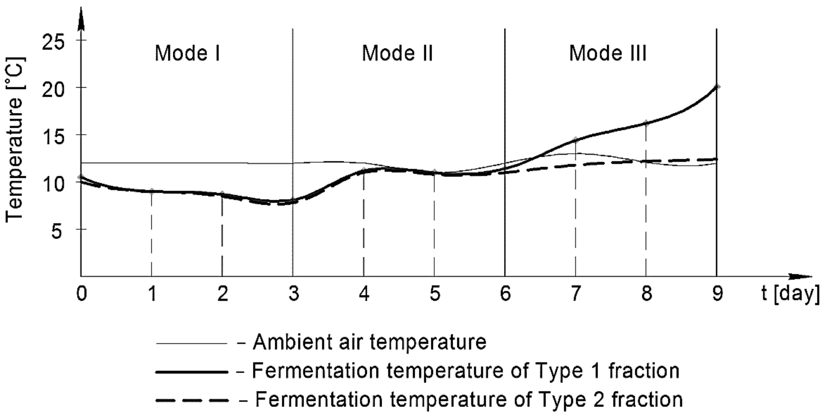

Figure 3. The temperature under the different operating modes of the express-fermenters (Stage 1).

The experiments established that the temperature of 55 °C to 80 °C, required for the stable activity of thermophilic microorganisms, was not reached under the considered operating modes. The fermentation process did not enter the mesophilic phase either, when the decomposition of complex carbohydrates, amino acids, and lignin began. The alkaline environment in Type 2 fraction inhibited the activity of microorganisms and did not allow reaching the fermentation temperature above 12.5 °C, while the maximum value achieved in Type 1 fraction was 20.1 °C.

Analysis of Type 2 fraction revealed its high alkalinity. Considering that the main aim of fermentation is to disinfect the slurry from the pathogenic microflora and weed seeds, it can be assumed that the alkaline medium observed in this material (pH = 12) is not favorable for pathogens and parasites, and does not contribute to weed seeds germination. In case they are not found, this type of organic waste can be dried and used as an organic fertiliser for acidic soils, as it has a potential reclamation effect. This assumption, however, needs further examination.

Since the considered operating modes of the express-fermenters in the previous studies proved to be effective, the need to change the physical and chemical composition of the fermented material was assumed.

The analysis of the physicochemical composition of Type 1 and Type 2 fractions showed the C/N ratio not to correspond to the optimal values of (20…30)/1 (Manure Composting Manual, 2005; MDAIC 1.10.15.02-17, 2017). Following the reported experience (Huang, 2004; Chen, 2005), the carbon-containing components were added to the solid fraction of pig slurry to be processed.

Step 2

Based on Step 1 outcomes, the decision was to change the composition of the processed material by adding the grain mechanical cleaning waste and BIAGUM organic fertiliser, a fermented bedding poultry manure. The physical and chemical properties of the starting components were determined in the laboratory analysis – Table 3.

Table 3. Physical and chemical properties of the starting materials

Indicator	Unit	Type 1 fraction	Type 2 fraction	Grain mechanical cleaning waste	BIAGUM organic fertiliser
Moisture content (*W*)	%	71.26	66.86	13.86	62.2
pH	-	8.0	12.0	6.9	8.5
NH_4^+	mg kg^{-1}	443.0	61.4	167.0	2,770.0
NO_3^-	mg kg^{-1}	117.5	921.5	47.2	890.0
K^+	mg kg^{-1}	492.0	473.0	1,373.0	2,890.0
P_{total}	mg kg^{-1}	2,000.0	8,100.0	3,300.0	4,970.0
N_{total}	mg kg^{-1}	5,320.0	8,128.0	1,036.0	20,500.0
Ash content	%	6.4	45.3	12.5	19.2

The composition of the test samples of the substrate to be fermented was determined by the results of physical and chemical analysis of the starting materials – Table 4.

The samples were prepared and loaded in the express-fermenters simultaneously. The ambient conditions were the same for the two express-fermenters. The average air temperature inside the laboratory was 12 ± 1 °C. The temperature in the express fermenters was recorded once a day for nine days (Fig. 4) as in the experiment on Step 1 of the study.

Table 4. Quantitative composition of the tested samples of the substrate

Component	Sample 1		Sample 2	
	kg	%	kg	%
Type 1 fraction	24.40	45.6	28.403	52.5
Type 2 fraction	14.34	26.8	17.961	33.2
Grain mechanical cleaning waste	3.58	6.7	4.003	7.4
Water	2.78	5.2	3.733	6.9
BIAGUM organic fertiliser	8.35	15.6	-	-
TOTAL	53.5	100	54.1	100

The experiment demonstrated that under the considered operating modes the temperature from 20 °C to 55 °C, required for the stable activity of mesophilic microorganisms, was achieved and remained stable. A periodic transition to the thermophilic phase under the temperature above 55 °C was also observed. A factor limiting the further temperature increase was, presumably, a small mass of the substrate. Testing of the considered operating modes under the bigger mass of the substrate, starting, for example, with 500 kg, might verify this assumption.

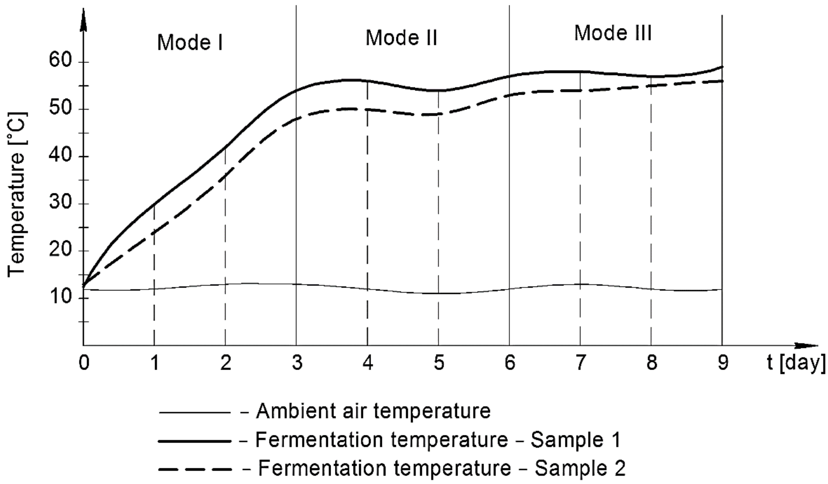

Figure 4. The temperature under different operating modes of the express-fermenters (Stage 2 of the study).

Mixing Type 1 fraction, Type 2 fraction and the grain mechanical cleaning waste had a positive effect on the physical and chemical composition of the processed substrate making it more suitable for the solid-state aerobic fermentation. The addition of BIAGUM organic fertiliser to Sample 1 contributed to a more intense dynamics of temperature increase. Under Mode I, the temperature difference of the substrates was about 6 °C (12–25%) that was primarily associated with the temperature increase; under Mode III, when the temperature in the express fermenters stabilised, this indicator reached 2–4 °C (3.5–7%).

With this in mind, it is acceptable to assert the feasibility of using BIAGUM fertiliser, or similar, as a starting catalyst for the process of solid-state aerobic fermentation.

The study findings support the general viewpoint concerning the feasibility of pig slurry separating into fractions for its more efficient processing (Hjorth, 2010; Hou, 2018). However, aerobic fermentation or composting of the solid fraction is difficult due to its non-optimal chemical composition. Adding lignin-rich substances to the processed substrate can even the C/N balance and contribute to the onset of fermentation processes owing to the activity of mesophilic and thermophilic microorganisms (Uao, 1993; Huang, 2004). With this in mind, it is acceptable to assert the feasibility of using the fermented poultry manure, or similar, as a starting catalyst for the process of solid-state aerobic fermentation (Uvarov, 2016; Onwosi, 2017).

CONCLUSIONS

According to the previously conducted studies, the fermentation of the solid fraction of pig slurry presented certain challenges due to the physical and chemical properties of the processed material. The direction of further research was to identify the quantitative and qualitative composition of the mixture required for the successful fermentation process.

The experiments verified that the mix of the solid fraction of pig slurry coming from the screw separator – Type 1 fraction (45.6–52.5% of the total volume), the solid fraction of pig slurry coming from the decanter centrifuge – Type 2 fraction (26.8–33.2% of the total volume), grain mechanical cleaning waste (6.7–7.4% of the total volume) and water (5.2–6.9% of the total volume) ensured the normal course of the fermentation process.

The catalyzing components, for example, BIAGUM organic fertiliser, produced from the bedding poultry manure, added to the substrate at its preparation stage, accelerated the substrate self-heating and allowed to reach a higher temperature – up to 59°C. However, when the fermentation process passed to the thermophilic phase, this difference was insignificant (2–4 °C).

At this stage of study, it is too early to speak about the advantages of a certain operating mode of the fermenter. For example, Mode I (aeration time – 20 min h^{-1}; aeration speed – 10 m s^{-1}; mixing interval – 24 hours), featured the lowest average temperature inside the express fermenters but the highest dynamics of its increase. Mode II (aeration time – 13 min h^{-1}; aeration speed – 7.5 m s^{-1}; mixing interval – 18 h) and Mode III (aeration time – 7 min h^{-1}; aeration speed – 5.5 m s^{-1}; mixing interval – 12 h) had a similar dynamics of temperature increase – it stabilised in the range of 50 °C to 55 °C but periodically exceeded this range, with the fermentation process passing to the thermophilic phase. Optimisation of the operating modes of fermentation installations when processing the solid fraction of pig slurry is an area of further research.

The study outcomes allow concluding that the solid-state aerobic fermentation is one of the promising options for the utilization of the solid fraction of pig slurry.

REFERENCES

Briukhanov, A. 2017. *How to provide environmental compatibility of livestock and poultry farms. Best Available Techniques*. Institute for Engineering and Environmental Problems in Agricultural Production – IEEP, Saint Petersburg, Russia, 296 pp. (in Russian).

Briukhanov, A.Yu. & Uvarov, R.A. 2016. Mathematical model of accelerated composting technology of farm animal waste in closed type installations. *KGTU News* **41**, 137–147 (in Russian).

Briukhanov, A.Yu., Shalavina, E.V. & Uvarov, R.A. 2017. Logistics model of secondary resources management in agriculture (on example of the Leningrad region). *Economics of Agricultural and Processing Enterprises* **4**, 38–41 (in Russian).

Chen, X., Chen, S., Sun, M. & Yu, Z. 2005. High yield of poly-γ-glutamic acid from Bacillus subtilis by solid-state fermentation using swine manure as the basis of a solid substrate. *Bioresource Technology* **96**(17), 1872–1879. doi: 10.1016/j.biortech.2005.01.033

DYR (Demographic Yearbook of Russia). 2018. Available at: https://gks.ru/bgd/regl/B19_18/Main.pdf (in Russian).

Eurostat. Population. Total (persons). 2018. *https://ec.europa.eu/eurostat/cache/RCI/#?vis=nuts1.population&lang=en.* Accessed 12.12.2019.

Fournel, S., Godbout, S., Ruel, P., Fortin, A., Duquette-Lozeau, K., Létourneau, V., Généreux, M., Lemieux, J., Potvin, D., Côté, C., Duchaine, C. & Pellerin, D. 2019. Production of recycled manure solids for use as bedding in Canadian dairy farms: II. Composting methods. *Journal of Dairy Science* **102**(2), 1847–1865. doi: 10.3168/jds.2018-14967

Hjorth, M., Christensen, K.V., Christensen, M.L. & Sommer, S.G. 2010. Solid-liquid separation of animal slurry in theory and practice. A review. *Agronomy for Sustainable Development* **30**(1), 153–180. doi:10.1051/agro/2009010

Hou, Y., Velthof, G.L., Case, S.D.C., Oelofse, M., Grignani, C., Balsari, P., Zavatta L., Gioelli F., Bernal, M.P., Fangueiro, D., Trindade, H., Jen, L.S. & Oenema, O. 2018. Stakeholder perceptions of manure treatment technologies in Denmark, Italy, the Netherlands and Spain. *Journal of Cleaner Production* **172**, 1620–1630. doi: 10.1016/j.jclepro.2016.10.162

Huang, G.F., Wong, J.W. C., Wu, Q.T. & Nagar, B.B. 2004. Effect of C/N on composting of pig manure with sawdust. *Waste Management* **24**(8), 805–813. doi: 10.1016/j.wasman.2004.03.011

Kivikari, U. & Antola, E. 2004. *Baltic Sea Region – A dynamic third of Europe*. City of Turku, Turku, Finland, 35 pp.

Leach, K.A., Archer, S.C., Breen, J.E., Green, M.J., Ohnstad, I.C., Tuer, S. & Bradley, A.J. 2015. Recycling manure as cow bedding: Potential benefits and risks for UK dairy farms. *The Veterinary Journal* **206**(2). 123–130. doi: 10.1016/j.tvjl.2015.08.013

Manure Composting Manual. 2005. Alberta Agriculture, Food and Rural Development, Edmonton, Canada, 27 pp.

MDAIC 1.10.15.02-17 (Management Directive for Agro-Industrial Complex 1.10.15.02-17 Recommended Practice for Engineering Designing of Animal and Poultry Manure Removal Systems and the Systems of Animal and Poultry Manure Preparation for Application). Moscow, Rosinformagrotech Publishers, 2017. 166 pp.

NPFU (National Pig Farmers Union). 2011. Rating of the largest pork producers in Russia in 2010. Available at: http://www.nssrf.ru/images/statistics/243280_810.pdf (in Russian).

NPFU (National Pig Farmers Union). 2019. Rating of the largest pork producers in Russia in 2018. Available at: http://www.nssrf.ru/images/statistics/243874_810.pdf (in Russian).

Onwosi, C.O., Igbokwe, V.C., Odimba, J.N., Eke, I.E., Nwankwoala, M.O., Iroh, I.N. & Ezeogu, L.I. 2017. Composting technology in waste stabilization: on the methods, challenges and future prospects. *Journal of Environmental Management* **190**, 140–157.

Sharma, B., Vaish, B., Singh, U.K., Singh, P. & Singh, R.P. 2019. Recycling of organic wastes in agriculture: an environmental perspective. *International Journal of Environmental Research* **13**(2), 409–429. doi: 10.1007/s41742-019-00175-y

Studzieniecki, T. 2016. The development of cross-border cooperation in an EU macroregion – a case study of the Baltic Sea Region. *Procedia Economics and Finance* **39**, 235–241. doi: 10.1016/S2212-5671(16)30318-5

Takahashi, N., Mochizuki, S., Masuda, K., Shimada, I., Osada, M. & Fukunaga, H. 2017. Influence of temperature, water content and C/N ratio on the aerobic fermentation rate of woody biomass. *Kagaku Kogaku Ronbunshu* **43**(4), 231-237. doi: 10.1252/kakoronbunshu.43.23

Uao, P.H., Vizcarra, A.T., Chen, A. & Lo, K.V. 1993. Composting of separated solid swine manure. *Journal of Environmental Science and Health. Part A: Environmental Science and Engineering and Toxicology* **28**(9), 1889–1901. doi:10.1080/10934529309375985

UIISS (Unified Interdepartmental Information and Statistical System). 2018. Animal and poultry stock on the farms of all categories. *https://www.fedstat.ru/indicator/31325.* Accessed 15.12.2019 (in Russian).

Uvarov, R., Briukhanov, A. & Shalavina, E. 2016. Study results of mass and nutrient loss in technologies of different composting rate: case of bedding poultry manure. In: *15th International Scientific Conference Engineering for Rural Development.* ERDev, Jelgava, Latvia, pp. 851–857.

Uvarov, R., Briukhanov, A. & Shalavina, E. 2018. Logistic transport model of region-scale distribution of organic fertilizers. In: *17th International Scientific Conference Engineering for Rural Development.* ERDev, Jelgava, Latvia, pp. 270–277. doi: 10.22616/ERDev2018.17.N301

Uvarov, R., Briukhanov, A., Spesivtsev, A. & Spesivtsev, V. 2017a. Mathematical model and operation modes of drum-type biofermenter. In: *16th International Scientific Conference Engineering for Rural Development.* ERDev, Jelgava, Latvia, pp. 1006–1011. doi: 10.22616/ERDev2017.16.N212

Uvarov, R., Briukhanov, A., Subbotin, I. & Shalavina, E. 2017b. Disinfection of solid fraction of cattle manure in drum-type bio-fermenter. *Agronomy Research* **15**(3), 915-920.

Vasilev, E.V. 2014. Basis of the rational radius of the manure transportation. *Dairy Newsletter* **1**(13), 49–55 (in Russian).

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Comparative analysis of performance by cows confined in different typologies of compost barns

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Abstract. The compost barn system was designed to be a sustainable alternative housing system for dairy cows. In order to help producers in the region to choose the best type of facility from the point of view of the milk production of the animals, this study compared the productivity of cows confined in an open composting barn with natural ventilation and in a closed composting barn with negative pressure ventilation and evaporative cooling panels. The temperature and relative humidity of the air were monitored, as well as the milk production of the animals housed in the facilities, and THI (Temperature and Humidity Index) were calculated. During the trials, the maximum daily temperatures of the air reached values around 27 °C and the THI remained within the normal range of up to 70. The average productivity remained in the general pattern described in the literature from 23 to 44 kg of milk cow-^{1} day^{-1}, with lower rates obtained in the closed house. As the variation of the index used to describe the internal environment was not significant, it can be inferred that climatic elements as temperature and air humidity, under the conditions analyzed were not the main factors influencing the productivity rates of dairy cattle. In conclusion, under the analysed conditions the use of a closed barn with negative pressure ventilation is hardly justified as a plant that favoured the productivity of the confined cows.

Key words: compost-bedded pack barn, dairy cows, cattle housing, thermal comfort.

INTRODUCTION

Brazil is an important world producer of milk, equivalent to approximately 35 billion litres in 2018. This activity represents one of the main sources of economic income for the Country (EMBRAPA, 2019).

As Brazil includes tropical and sub-tropical areas, the climatic conditions represent an important factor, which significantly affects milk productivity, also for the reason that the animals with higher yields come from countries with temperate climate. A form of intervention to overcome this type of problem is the adoption of facilities with climate control to alleviate the effect of heat stress on the animals and thus reduce productive losses.

Thus, to maintain high levels of production combined with good conditions of comfort and welfare of animals, studies to improve confinement situation have been intensified in recent years. For dairy production in Brazil, there is still predominance of grazing systems, but in some Brazilian States an increase in the number of producers opting for feedlot systems with greater control of environmental variables has been remarked (Pilatti, 2017).

Compost-bedded pack barns (CBP), generally known as compost dairy barns, are alternative housing systems for dairy cows (Leso et al, 2013; Leso et al., 2020). In these barns, the whole surface of the resting area is covered with a deep-bedded pack that is frequently stirred in order to incorporate fresh manure into the pack and to enhance the evaporation of water. The cows remain in free circulation within a covered shed without any containment partitions, like those present in freestall and tie-stall systems (Eckelkamp et al., 2016). The diffusion of this system is mainly due to its efficiency, since, in addition to provide greater comfort to animals, it also allows to obtain manure of good quality. Studies have indicated that CBP, compared with conventional systems such as freestall barns, have the potential to improve the welfare of dairy cows. CBP housing system may improve longevity of dairy cows, which is reported to be one of the most important motivations for building this kind of housing (Leso et al., 2019).

The construction and materials used for buildings can influence together with technological equipment and system of ventilation the microclimatic conditions inside the sheds (Kic, 2016). The compost barns can be made by applying simple construction techniques, also referred to green-house type building (Leso et al., 2017) and to the use of emerging principle of 'design for deconstruction' extensively (Leso et al., 2018).

The profitability of the composting granary has been studied in several studies. De Oliveira et al. (2019) compared the milk production systems of the composting barn and free stall. They have concluded that the requirements for choosing the most suitable installation should be based on the ease of handling, productive and reproductive performance, animal health, environmental issues and availability of water and bedding in the region.

The Compost Barn system has found a wide spreading in Brazil in last years (Lobeck et al., 2017). However, in Brazil, there is still little information about compost barns and their respective characteristics that interfere with animal thermal comfort, as well as those related to the productive performance of cows confined in different ways. The building typology of the shed, as well as the ventilation system applied in the barn, are of great importance in the efficacy of climatisation. The thermal stress, especially of cows of breeds with high genetic potential for milk production, can be strongly influenced by these factors (Garcia, 2017).

The natural ventilation system is still widely used by most Brazilian farmers. In naturally ventilated Compost Barns, the building should be located in open areas to allow a proper functioning of natural ventilation (Damasceno, 2012). Oliveira et al. (2019), applying the technique of geostatistics, remarked that the thermal environment in compost barns is greatly influenced by the ventilation system adopted. Damasceno et al. (2019) used the technique of geostatistics to evaluate the distribution and spatial dependence of different environmental variables. Spatial distribution maps showed the occurrence of high variability of attributes and content within the animal facility. Thermal environment variables showed alert situations throughout practically the entire facility.

The facility should be located at a slight elevation of the surrounding terrain to prevent the wetting of the bedding during rainy periods and the raising of the relative humidity to undesirable levels (Janni et al., 2007).

However, artificial ventilation systems can be applied to improve the microclimatic conditions inside the barn favouring the removal of air humidity and excessive heat generated by animals. The choice of the climatic control system should consider the ability of the fan to provide good air discharge, its size, drive type, operation costs and purchase (Damasceno, 2012).

Taking into account the growing interest of Brazilian dairy farmers towards the compost barn system, it is necessary to obtain more information about the efficacy of different climatic control systems. The present work aims to compare the productivity during the winter season of dairy cows confined in two different building typologies.

MATERIALS AND METHODS

The trials were carried out in a commercial milk farm with two different solutions of compost barn: a closed-side shed with negative pressure ventilation (CBF) and an open shed with natural ventilation system (CBA). The facilities are located in the municipality of Cajuri, Minas Gerais (Brazil), altitude 670 m, latitude 20°46'41'S and longitude 42°48'57"W. The region has a tropical climate with an average annual temperature of 19 °C, characterized as Cwb (wet temperate climate with dry winter and temperate summer) by Köppen climate classification.

The closed barn has a polyethylene curtain closure, tunnel-style ventilation associated with the evaporative cooling system, composed of panels of porous cellulose material.

The closed barn (CBF) (Fig. 1) has a Northwest-Southeast orientation, 0.8 m eave galvanized steel roofing and it is closed with polyethylene curtains and deflectors. The pillars are made by reinforced concrete, spaced 5.5 m. The ceiling height of the shed is 5.0 m, the height to the ridge is 7.0 m.

The facility is 55 m long and 26.2 m wide, of which 16 m are for the bedding area. Inside, in addition to the bedding area, there is a 3.80 m feeding alley with a concrete floor. Four drinkers are placed in the feeding alley. Two corridors are present, one 4.0 m wide for the circulation of machines and one 2.40 m wide for service.

Figure 1. The closed compost barn (CBF) for dairy cows.

The sides of the barn have a fixed polyethylene curtain, while the southeast face has porous cellulose panels, with a surface area of approximately 11.52 m², which can be moistened for evaporative cooling. The northwest face of the installation has five exhaust fans (BigFan®, 3.5 m diameter, 150,000 m³ h^{-1} air volume and 2.0 HP power) for tunnel-type ventilation. Humidification of the panels occurs when the air temperature is above 21 °C and relative humidity below 75%. The facility has polyethylene curtains and nine deflectors.

In the resting area a mixture of sawdust and coffee husks is used as bedding, about 0.60 m thick. The bedding is cultivated twice a day. Approximately 88 Holstein cows in the lactation phase are housed in this shed, with a surface of 10 m^2 cow^{-1} in the bedding area.

The open barn (Fig. 2) is also oriented in the Northwest-Southeast direction and it is covered with galvanized steel plates, with a 0.5 m eave. The ceiling height is 6.0 m, the ridge height is approximately 7.5 m. The ridge type used was overshot. The facility is 60.0 m long and 20.7 m wide, of which space of 14.3 m is reserved to the bedding area. The barn has a feeding alley 4.25 m wide with slatted concrete floor and a 2.15 m wide corridor for animal handling. The facility has completely open sides. The bedding is made by sawdust and is cultivated twice a day. The height of the bedding is approximately 0.30 m. In this barn, 63 lactating cows are kept, with a surface/head of 13.6 m² in the bedding area and the specific building characteristics favour the natural ventilation.

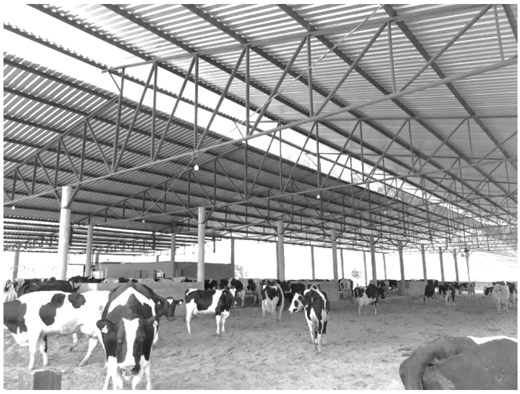
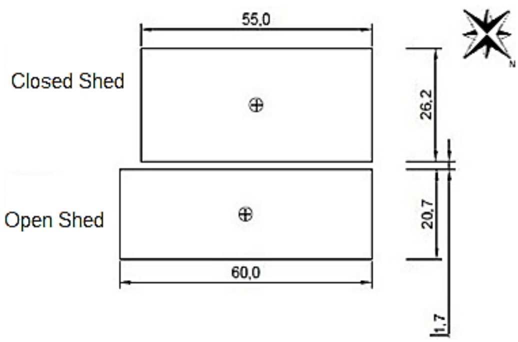


Figure 2. The open compost barn (CBA) for dairy cows.

HOBO® (Data Logger Ux100-003 – Onset) sensors were used to collect data of the temperature and relative humidity of the air inside the barns. They were placed in the central point of the shed, at the height of the centre of mass of the compost barn (Fig. 3). Sensors of the same type were employed to collect air temperature and relative humidity outside the sheds.

A sensor was installed in each barn to ensure that the entire area of both sheds were monitored in real time, so that the final values of the variables were the average representative of the internal thermal environment, 24 hours each day, as per methodology used by Barbeg et al. (2007).



where Tdb – Dry bulb temperature (ºC);
Tdp – Dew Point Temperature (°C).

Figure 3. Schematic representation of the installation points of air temperature and relative humidity sensors (HOBO®) at the centre point of each compost barn.

Thermal data collection took place during winter (14 days of collection, on August 2019) and data were stored every 5 minutes, 24 hours a day, during the mentioned period.

Dry bulb temperature and dew point temperature, obtained by microclimatic data collected, were used to calculate the Temperature and Humidity Index (THI) by the Eq. 1 proposed by Thom (1959).

$$THI = Tdb + 0.36Tdp + 41.5 \quad (1)$$

Based on the THI data, daily graphs over the experimental period were made to analyse the daily thermal conditions and hourly averages and to define the most critical conditions for the animals.

Information regarding the milk production of each animal for each shed were obtained, based on production data collected by the PDPL (Viçosa Dairy Cattle Development Program) team, which monitors milk production of each animal of this unit regularly. The productivity data were referred to the litres of milk produced per animal per day, for groups of cows at first and second lactation (G1), third lactation (G2), fourth lactation (G3) and fifth lactation onwards (G4). During the experimental period for each barn, the average productivity data of each group were correlated with the average, maximum and minimum THI.

The average test was performed to test the interference of the environment represented by the THI on the productivity of each group of animals, and it was presented by tables and graphs. The data were compared using the Student's t test with the support of the R software (5% significance).

RESULTS AND DISCUSSION

Environment

Table 1 shows the values of average and maximum temperatures and daily THI for both types of barns.

The average hourly temperatures of the experimental period are shown in Fig. 4.

The thermal comfort zone for European dairy cattle breeds, where the animal has its optimal physiological performance, is located between -1 °C and 16 °C. Zones ranging from -10 °C to -1 °C and 16 °C to 27 °C are characterized by modest thermal comfort. At temperatures outside the mentioned zones, the animal already has certain thermoregulatory mechanisms for adjusting its body temperature (Baêta & Souza, 2010). Maximum temperatures above the limiting temperature above the zone of modest thermal comfort were observed.

Table 1. Daily maximum temperatures (T_{max}) and daily THI for the two types of compost barns, closed (CBF) and open (CBA)

Days	Closed barn		Open barn	
	T_{max} (ºC)	THI	T_{max} (ºC)	THI
Aug 06	21.82	65.97	21.42	65.68
Aug 07	26.24	65.68	24.63	65.50
Aug 08	22.35	64.17	25.02	64.43
Aug 09	25.75	63.06	27.49	63.65
Aug 10	25.34	64.67	27.31	65.23
Aug 11	26.95	65.05	29.90	66.07
Aug 12	25.31	62.33	27.27	63.00
Aug 13	26.02	64.52	28.77	64.95
Aug 14	23.12	65.97	21.20	65.11
Aug 15	21.63	61.14	20.85	60.74
Aug 16	22.16	58.22	23.31	58.42
Aug 17	22.02	57.07	23.31	57.08
Aug 18	25.24	60.49	26.92	61.00
Aug 19	31.49	63.79	30.96	64.27

Therefore, environmental influences may occur on the milk production of the cows.

The critical upper temperature established for lactating Holstein cows is 25 °C (Garcia, 2017). In the trials, temperatures above the maximum critical value for daytime comfort were observed, which may be responsible for behavioural and dietary changes in the animals during the studied season.

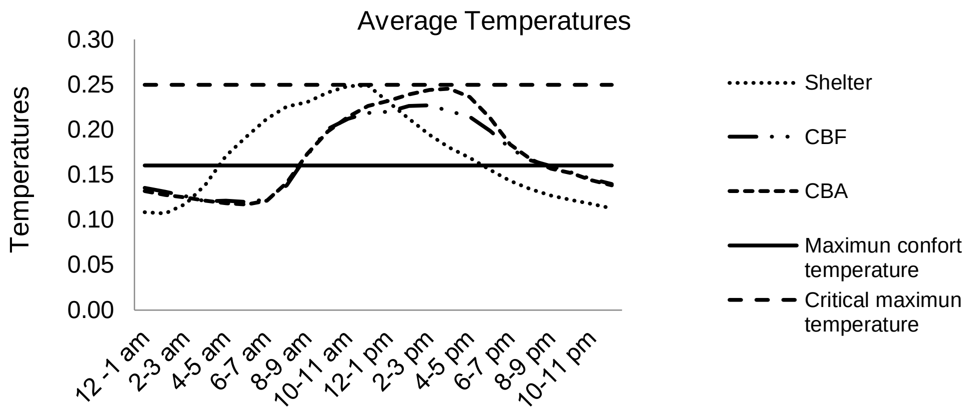


Figure 4. Average hourly temperatures in the two compost barns (closed, CBF; open, CBA) compared to the maximum comfort temperature and critical maximum temperature for dairy cows.

Regarding THI, ranges are used to determine and analyse animal comfort (THI less than or equal to 70: normal situation; THI between 72 and 78: warning situation; THI between 78 and 82: danger situation; THI above 82: need for immediate intervention) (Pires & Campos, 2004).

During the trials the THI values remained within the normal range, assuring comfort conditions for the cows. Fig. 5 shows THI during the experimental period allowing a visual comparative analysis on the thermal environment of the two types of barns.

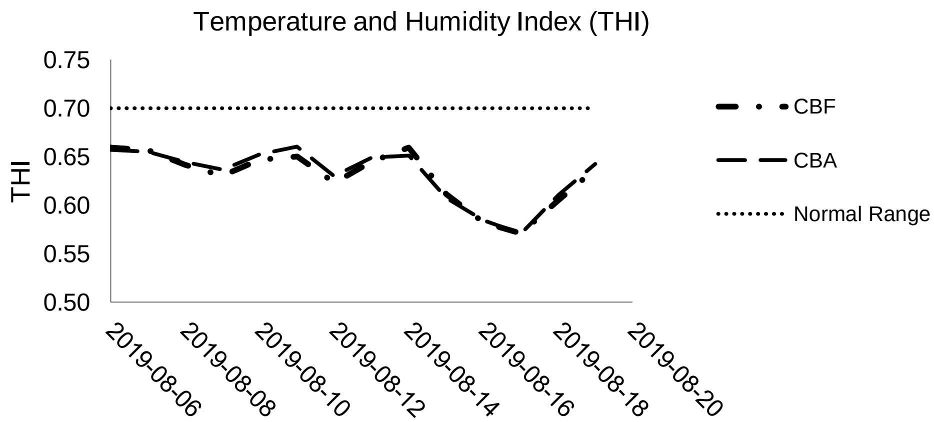


Figure 5. THI in the experimental period in the closed (CBF) and open (CBA) compost barns.

Productivity

Holstein cows at third and fourth lactation have higher milk yield compared to second lactation cows. From the fifth lactation a production fall is registered (Souza et al., 2010).

In order to exclude that the milk production could vary for the lactation order, the animals were divided into lots or groups of different lactations, being the group G1 for cows in first and second lactations, G2 for cows in third lactation, G3 for cows in fourth lactation and G4 for cows in fifth lactation onwards. Productivity analysis of cows housed in both facilities was performed and the results are shown in Tables 2, 3.

Table 2. Productivity average and standard deviation of the cows housed in the closed barn (CBF) for distinct groups classified by lactation orders

Group	Productivity (L head^{-1}day^{-1})	Standard deviation	Coefficient of variation
G1	26.55	9.64	36.32
G2	21.58	6.43	29.80
G3	27.79	8.13	29.24
G4	22.67	3.42	15.09

Table 3. Productivity average and standard deviation of the cows housed in the open barn (CBA) for distinct groups classified by lactation orders

Group	Productivity (L head^{-1}day^{-1})	Standard deviation	Coefficient of variation
G1	25.95	5.44	20.98
G2	33.60	7.03	20.91
G3	34.50	0.71	2.05
G4	27.00	4.24	15.71

Studies show that yield for Dutch Friesian cows ranges from 23 to 44 kg of milk cow^{-1} day^{-1} (Deitos et al., 2010). Thus, the animals in the experimental groups, with the exception of lot 2 (G2) of the closed shed, produce according to standards described in the literature. In general, the most expressive values of milk production are presented for the animals confined in the open shed.

The average values of productivity per lot of animals are showed in Table 4, compared with the maximum, minimum and average indices of the experimental period.

Table 4. Average productivity data of the cows housed in both types of barns in relation to maximum, minimum and average values of THI

Group	Closed Barn (CBF)			Open Barn (CBA)		
	Productivity (L head^{-1}day^{-1})	THI (total)		Productivity (L head^{-1}day^{-1})	THI (total)	
G1	26.55a	Maximum	65.97	25.95a	Maximum	66.07
G2	21.58b	Medium	63.01	33.60a	Medium	63.22
G3	27.79a			34.50a		
G4	22.67a	Minimum	57.07	27.00a	Minimum	57.08

*averages followed by the same letter on the line do not differ statistically by the t-student test.

There was no statistically significant difference ($P > 0.05$) in relation to the average milk production between lots 1, 3 and 4 when the two facilities were compared. For lot 2, there was a statistically significant difference ($P < 0.05$), suggesting that the average production was higher for the open facility. In the studied conditions, during the winter, the closed barn did not obtain satisfactory results to justify its use, because both in construction and maintenance, this typology presents higher costs.

CONCLUSIONS

The thermal environment inside the two compost barns, based on THI, was comfortable for the cows during the experimental period.

The thermal conditions inside the two different barns during the trials carried out in the winter season did not represent a factor influencing the productivity rates of dairy cows. The productive performance of animals housed in the open compost barn was slightly higher than that observed for animals housed in the closed compost barns.

In conclusion, the use of a closed barn with negative pressure ventilation is hardly justified as a system that favoured the productivity of the confined cows under the analysed conditions.

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REFERENCES

Baêta, F.C. & Souza, C.F. 2010. *Ambience in rural buildings: animal comfort*. 2. ed., Viçosa, MG: Ed. UFV, 269 pp. (in Portuguese).

Damasceno, F.A. 2012. *Compost bedded pack barns system* and computational simulation of airflow through naturally ventilated reduced model. Thesis, Universidade Federal de Viçosa, Brazil, 404 pp.

Damasceno, F.A., Oliveira, C.E.A., Ferraz, G.A.S., Nascimento, J.A.C., Barbari, M. & Ferraz, P.F.P. 2019. Spatial distribution of thermal variables, acoustics and lighting in compost dairy barn with climate control system. *Agronomy Research* **17**(2), 385–395. 2019https://doi.org/10.15159/AR.19.115

Deitos, A.C., Maggioni, D. & Romero, E.A. 2010. Milk production and quality of cows of different genetic groups. *Campo Digital Magazine*. pp. 26–33, Campo Mourão (in Portuguese).

De Oliveira Silva, G.R., Lopes, M.A., Lima, A.L.R., Da Costa, G.M., Damasceno, F.A., Barros, V.P. & Barbari, M. 2019. Profitability analysis of compost barn and free stall milk-production systems: a comparison. *Semina. Ciências Agrárias* **40**, 1165–1183.

Eckelkamp, E.A., Taraba, J.L., Akers, K.A., Harmon, R.J. & Bewley, J.M. 2016. Understanding compost bedded pack barns: Interactions among environmental factors, bedding characteristics, and udder health. *Livestock Science* **190**, 35–42.

Embrapa. Yearbook 2019. *http://embrapa.br/gado-de-leite*. Accessed 11.8.2019 (in Portuguese).

Garcia, P.R. 2017. *Low-Profile Cross-Ventilated freestall: thermal, zootechnical and animal welfare performance*. Thesis, Universidade de São Paulo, Brazil, 150 pp. (in Portuguese).

Janni, K.A., Endres, M.I., Reneau, J.K. & Schoper, W.W. 2007. Compost dairy barn layout and management recommendations. *Appl. Eng. Agric.* **23**, 97–102.

Kic, P. 2017. Effect of construction shape and materials on indoor microclimatic conditions inside the cowsheds in dairy farms. *Agronomy Research* **15**(2), 426–434.

Leso, L., Uberti, M., Morshed, W. & Barbari, M. 2013. A survey of Italian compost dairy barns. *J. Agr. Eng.* **44**(3), 120–124.

Leso, L., Morshed, W., Conti, L. & Barbari, M. 2017. Evaluating thermal performance of experimental building solutions designed for livestock housing: The effect of greenery systems. *Agronomy Research* **15**(1), 239–248.

Leso, L., Conti, L., Rossi, G. & Barbari, M. 2018. Criteria of design for deconstruction applied to dairy cows housing: a case study in Italy. *Agronomy Research* **16**(3), 794–805. https://doi.org/10.15159/AR.18.085

Leso, L., Pellegrini, P. & Barbari, M. 2019. Effect of two housing systems on performance and longevity of dairy cows in Northern Italy. *Agronomy Research* **17**(2), 574–581. https://doi.org/10.15159/AR.19.107

Leso, L., Barbari, M., Lopes, M.A., Damasceno, F.A., Galama, P., Taraba, J.L. & Kuipers, A. 2020. Invited review: Compost-bedded pack barns for dairy cows. *Journal Dairy Science* **103**(2), 1072–1099.

Lobeck, K.M., Endres, M.I., Janni, K.A., Mota, V.C., Campos, A.T., Damasceno, F.A., de Melo Resende, E.A., do Amaral Rezende, C.P., de Abreu, L.R. & Vareiro, T. 2017. Feedlot for dairy cattle: history and characteristics. *PUBVET* **11**, 424–537 (in Portuguese).

Oliveira, C.E.A., Damasceno, F.A., Ferraz, P.F.P., Nascimento, J.A.C., Ferraz, G.A.S. & Barbari, M. 2019. Geostatistics applied to evaluation of thermal conditions and noise in compost dairy barns with different ventilation systems. *Agronomy Research* **17**(3), 783–796. https://doi.org/10.15159/AR.19.116

Pilatti, J.A. *Daytime behavior and welfare of cows in a Compost Barn feedlot syste*m. 2017. Thesis, Federal Technological University of Paraná. (In Portuguese).

Pires, M.D.F.A. & de Campos, A.T. 2004. Environmental modifications to reduce stress calorie in dairy cattle. *Embrapa Gado de Leite-Comunicado Técnico (INFOTECA-E)*. Juiz de Fora/MG. 6 pp. (in Portuguese).

Souza, R.D., dos Santos, G.T., Valloto, A.A., dos Santos, A.L., Gasparino, E., da Silva, D.C. & dos Santos, W.B.R. 2010. Milk production and quality of Holstein cows in function of the season and calving order. *Revista Brasileira de Saúde e Produção Animal* **11**, 484–495 (in Portuguese).

Thom, E.C. 1959. The discomfort index. *Weatherwise* **12**, 57–61.

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https://doi.org/10.15159/AR.20.018

Restricting the eligible maintenance practices of permanent grassland – a realistic way towards more active farming?

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Abstract. As a result of agricultural, land and ownership reforms coupled with liberal agricultural policy during the transition, agricultural land use in Estonia became more fragmented. A significant portion of agricultural land users are now considered passive farmers who maintain their agricultural land (often permanent grasslands) in good agricultural and environmental conditions and are therefore eligible for single area and greening payment. The maintenance of permanent grassland is one of the objectives of the EU Common Agricultural Policy (CAP), which contributes to the overall climate and biodiversity objectives of the EU. Until 2014, in Estonia, the minimum eligible activity for the maintenance of permanent grassland was to cut the grass and leave it on the ground. In 2015 and 2016, the area on which the cut grass could be left on the ground was restricted in order to increase incentives for more active agricultural land use. This paper analyses the likely effects of such restriction on the use and maintenance of permanent grasslands. The results of the study show that in the case of restrictions on the eligible practices of permanent grassland maintenance, passive land users as well as crop and mixed crop-livestock farms are likely to reduce the area of permanent grasslands (*shrinking farms*). At the same time, grazing livestock farms (*expanding farms*) would be willing to expand their permanent grassland area. More than 70% of the permanent grasslands of *shrinking farms* are located within 1 km and more than 90% within 2 km of *expanding farms*. However, in some regions it is likely that the maintenance of permanent grasslands is stopped as a result of the restrictions. It is argued that if permanent grasslands are to be maintained, it is necessary to introduce supports for grazing livestock farms, targeted supports for passive land users for their maintenance or more comprehensive land use policy that takes the climate change mitigation requirements into account.

Key words: permanent grasslands, passive farming, greening, Common Agricultural Policy, direct payments.

INTRODUCTION

Changes in agricultural policy and land use

Following the restoration of independence in 1991, ownership and land reforms were undertaken in Estonia (Alanen, 1999; Swinnen, 1999) that resulted in the fragmentation of agricultural land (Rudbeck Jepsen et al., 2015; Jürgenson, 2016; Arslan et al., 2019). The restructuring of agriculture resulted in the polarisation of commercially oriented farms, as well as small lifestyle or environmental stewardship oriented farms.

The shift from the planned economy of the Soviet Union to liberal agricultural policy (Unwin, 1997) caused a shock in Estonian agriculture. Due to a marked decline in producer support estimate (OECD, 1996), the utilised agricultural area (UAA) in Estonia declined by 49% from 1,374,000 ha in 1992 to 698,200 ha in 2002 (FAOSTAT, 2018).

With its accession to the European Union (EU) in 2004, Estonia implemented the EU Common Agricultural Policy (CAP). This resulted in higher prices for agricultural commodities, higher direct payments, agri-environmental payments and investment subsidies. One of the new policy instruments was the decoupled single area payment (SAP), which required beneficiaries to maintain their agricultural land in good agricultural and environmental conditions (GAEC).

The introduction of the SAP resulted in a new type of agricultural land use – permanent grassland temporarily not used for production purposes. In Estonia, this land use type mainly characterises those agricultural land users who became land owners as a result of restitution but are not producing agricultural products for market (any more), along with those who have bought agricultural land as a real estate investment.

Since 2015, the maintenance of permanent grasslands became one of the mandates related to the SAP and greening payment (Regulation (EU) No 1307/2013; Commission Delegated Regulation (EU) No 639/2014). It contributes to several of the CAP goals (and also climate policy): 1) the avoidance of abandonment of agricultural land; 2) the avoidance of ploughing of permanent grasslands for the cultivation of annual crops, and; 3) resultant contribution to carbon sequestration. If one considers these as primary goals of the SAP and greening payment, the question of whether permanent grasslands are maintained in GAEC by active or passive farmers[1] becomes a false problem, as suggested by Pupo D'Andrea & Romeo Lironcurti (2017).

While the effects of agri-environmental payments on the extensification of agricultural production in less favoured areas are considered positive (e.g. by Jones et al., 2016), views on the effects of SAP on the subsidised passive use of agricultural land are opposing. Passive farming is seen as an obstacle to structural change in remote areas (Brady et al., 2015; 2017), as the largest share of it takes place in regions with low soil fertility and is characteristic of relatively small, unprofitable farms (Trubins, 2013). Passive land use prevents the grazing livestock farms in these regions from expanding and increasing their production efficiency, resulting in them becoming marginalised and ultimately passive land holders themselves (Swinnen et al., 2013; Brady et al., 2015). It has been shown that passive farming reduces the ability of the SAP and basic payment scheme of the CAP to support the incomes of tenant farmers (Di Corato & Brady, 2019). On the other hand, subsidies for land maintained in GAEC are necessary for maintaining the agricultural land of high nature and cultural value, but of low fertility and in remote

[1] Regulation (EU) No 1307/2013 states that no direct payments shall be granted to natural or legal persons (passive farmers), whose agricultural areas are mainly areas naturally kept in a state suitable for grazing or cultivation and who do not carry out on those areas the minimum activity defined by Member States. According to Brady et al. (2017), passive farming occurs when landowners maintain their agricultural area to collect payments without producing commodities.

regions, from abandonment (Renwick et al., 2013; Abolina & Luzadis, 2015; Barnes et al., 2016; Lasanta et al., 2017).

Maintenance of permanent grasslands

The CAP has favoured a recovery of Estonian agricultural land use and production. From 2004–2019, the UAA in Estonia increased by 25% from 792,409 ha to 987,614 ha (Statistics Estonia, 2019). By 2015, the area of permanent grassland temporarily not used for production purposes increased to 125,053 ha and accounted for 13% of the UAA. This was the highest proportion of such land among the EU member states (Eurostat, 2018). The increase in the area of permanent grassland temporarily not used for production purposes by 103,099 ha (by 5.7 times) during the period of 2004–2015 comprised 51% of the total increase in the UAA (Statistics Estonia, 2019).

In Estonia, the appearance of this phenomena since 2004 and its persistence has fuelled discussion and disputes over the stimulating effects of the CAP, namely the SAP and greening payment, on passive ways of agricultural land use, as well as its effects on the price of agricultural land and the position of agricultural producers (active farmers) on the land market.

The cheapest practice for permanent grassland maintenance is to cut the grass biomass once a year and leave the residues on the ground. In Estonia, this practice was mainly used by arable farmers and passive land users (Viira et al., 2016). From 2004–2014, the land owners who carried out this practice for the maintenance of permanent grasslands in GAEC were eligible for the SAP (Regulation 11 of Agricultural minister, 30.07.2012). In order to provide incentives for utilising (more active) agricultural practices (such as grass or hay harvesting, or grazing) for permanent grassland maintenance, in 2015 and 2016 restrictions were set on using biomass cutting as a permanent grassland maintenance practice that is eligible for the SAP and greening payment (Regulation 32 of Agricultural minister, 25.04.2015; 25.04.2016). In 2017, the restrictions were eased (Regulation 32 of Agricultural minister, 28.04.2017) and the eligibility of the grass biomass cutting practice was restored.

Such restrictions on the eligibility of certain practices may have several outcomes. According to Van Herck & Vranken (2013), the increase of land rents has a negative effect on land mobility and, therefore, indirectly a negative effect on farm restructuring, because new farmers face a higher initial investment cost and existing farmers face a higher cost of expansion. Since the restrictions increase the cost of the maintenance of permanent grassland for those farmers who do not have grazing animals, they may reduce the rental or selling price of permanent grasslands and stimulate the transfer of permanent grassland from passive to active farmers. However, it also may result in the abandonment of permanent grassland, which is contrary to the aims of the CAP. The land use decline in Estonia in the 1990s demonstrates that the abandonment of less fertile agricultural land is not a theoretical option (Prishchepov et al., 2013; Terres et al., 2015). The possible behavioural responses of active farmers and passive land owners were analysed by Ariva et al. (2017) based on a survey of farmers in 2016 (Viira et al., 2016), and by Viira & Ariva (2019) based on the data of agricultural registers.

If the goal of the restricting certain management practice is to achieve a transfer of permanent grassland from passive to active farmers, the precondition for this is that the land parcels that passive farmers would want to sell or rent are located within a reasonable distance of active farmers. Therefore, the objective of this paper is to determine to what extent the permanent grassland parcels of active farmers who could potentially be interested in expanding their permanent grassland area, and passive farmers who could potentially give up (sell or rent) some of the permanent grassland, are in a reasonable distance of each other. If these two groups are not located near each other, the actual change in active and passive land use as a result of the restriction on eligible permanent grassland maintenance practices would be limited.

In order to achieve the objective, the paper seeks answers to three research questions: 1) what kinds of farms are likely to reduce or expand their permanent grassland area; 2) what proportion of the permanent grassland parcels of those farms that are likely to reduce their permanent grassland area are located in proximity to those farms that are likely to expand their permanent grasslands; 3) what could be the results of restricting the eligible practices of permanent grasslands on permanent grassland maintenance at regional level? The next chapter of the paper gives an overview about the data and methods that were used to fulfil the objective and answer the research questions. After that, the results are presented and discussed. Final chapter of the paper summarises the main conclusions.

MATERIALS AND METHODS

Data

Information about the actual land use of the beneficiaries of the SAP and greening payment, as well as the number of their agricultural animals, was obtained from the Estonian Agricultural Registers and Information Board (ARIB). In 2015, there were 17,322 beneficiaries of the SAP and greening payment.

At the end of 2016, a survey was conducted among the beneficiaries of the SAP and greening payment. A sample of 6,811 beneficiaries was drawn from the total number of beneficiaries of the SAP and greening payment. In total there were 1,858 respondents (response rate 27.3%). The respondents were asked about their likely intentions (expansion, no changes, or reduction) regarding their permanent grassland use if the practice of cutting the grass and leaving biomass on the ground would become ineligible for the SAP and greening payment in the future (Viira et al., 2016).

Since the land use changes are related to changes in the structure of farm types and size (Stokstad & Krøgli, 2015; Pilgaard Kristensen et al., 2016; Van der Sluis et al., 2016), a farm type was assigned for each beneficiary of the SAP and greening payment, using the FADN typology (Commission Regulation (EC) No 1242/2008; Commission Regulation (EC) No 867/2009; Agricultural Research Centre, 2016). The SAP and greening payment beneficiaries who did not have field crops, permanent crops, fallow or agricultural animals were considered to belong to an additional farm type called *passive land user*, which is not present in the FADN typology.

Classification of expanding and shrinking farms

Probit regression was used to estimate how farm type, farm size, share of permanent grassland in total land use, and livestock density affect the likelihood that farms will increase, decrease or not change their permanent grassland area. For the regression analysis, the survey data, information on the land use of the SAP and greening payment beneficiaries, the number of their agricultural animals and information about their farm type were combined. Those SAP and greening payment beneficiaries who did not respond or provided an ambiguous response about their likely intentions regarding their permanent grassland use (expansion, no changes, reduction) if cutting the grass and leaving biomass on the ground would become restricted in the future were excluded from the regression analysis. After merging the data from various sources, information on 629 beneficiaries of the SAP and greening payment remained valid for the regression analysis.

Table 1. The descriptive statistics of variables for probit regression models (N = 629)

Variable	Definition	Scale/ measurement	Average	St.Dev	Source
Dependent variables					
Land_dec	Land use will decrease	Yes = 1; No = 0	0.66	0.47	Survey
Land_inc	Land use will increase	Yes = 1; No = 0	0.14	0.35	Survey
Land_stable	Land use will not change	Yes = 1; No = 0	0.20	0.40	Survey
Explanatory variables					
Field_crops	Farm is specialised in field crops	Yes = 1; No = 0	0.27	0.44	ARIB/FADN
Horticulture	Farm is specialised in horticulture	Yes = 1; No = 0	0.00	0.06	ARIB/FADN
Perm_crops	Farm is specialised in permanent crops	Yes = 1; No = 0	0.02	0.14	ARIB/FADN
Gr_livestock	Farm is specialised in grazing livestock	Yes = 1; No = 0	0.40	0.49	ARIB/FADN
Granivores	Farm is specialised in granivores	Yes = 1; No = 0	0.00	0.04	ARIB/FADN
Mixed_crops	Farm is specialised in mixed cropping	Yes = 1; No = 0	0.01	0.11	ARIB/FADN
Mixed_livestock	Farm is specialised in mixed livestock holdings	Yes = 1; No = 0	0.00	0.06	ARIB/FADN
Crops_livestock	Farm is specialised in mixed crops-livestock	Yes = 1; No = 0	0.10	0.29	ARIB/FADN
Passive	Beneficiary is passive land user	Yes = 1; No = 0	0.20	0.40	ARIB/FADN
Area	Agricultural area	Ha	84.5	234.4	ARIB/FADN
Grassland_share	Share of permanent grassland in agricultural area	Share	0.63	0.39	ARIB
LU	Livestock units	LU	24.1	128.0	ARIB
LU_density	Livestock density	LU/ha	0.21	0.47	ARIB
Private_person	Beneficiary is private person	Yes = 1; No = 0	0.39	0.49	ARIB

Source: authors' calculations.

Table 1 provides descriptive statistics of the dataset used for estimating the parameters for 42 one-variable models and five models with interactions of explanatory variables using the probit regression, as shown by equation (1).

$$\Pr(Y = 1|X) = \Phi(X^T\beta) \quad (1)$$

In general, the probit model employed in this study estimates the probability (Pr) of the respondents' intentions regarding their land use in the future. The respondents' intentions to increase, decrease or not change their permanent grassland area were considered as three different binary dependent variables *Y* which were obtained from the survey conducted in 2016. The vector of *T* explanatory variables *X* was assumed to influence the future land use change *Y*. The *ß* denotes the respective parameters to be estimated. For estimation of the model parameters, the R programme (version 3.5.1) was used.

The results of the regression analyses were used as an input for the logical classification, which divided the beneficiaries of SAP and greening payment into three groups: 1) farms that would likely reduce their permanent grasslands use; 2) farms that would likely expand their permanent grasslands; 3) farms that probably would not change their land use.

Based on this classification, all 17,322 beneficiaries of the SAP and greening payment in 2015 were assigned with a variable showing whether their use of permanent grasslands is likely to decrease, increase or not change in the future. The composition of all three groups according to the farm and land user types is given in Annex I. In the further analysis, farms that are likely to increase their permanent grassland area are referred to as *expanding farms*, and the farms and passive land users that intended to decrease their area of permanent grasslands are further referred as *shrinking farms*.

In order to analyse the proximity of the permanent grassland parcels of *expanding* and *shrinking* farms, the ARIB data on all the eligible parcels for SAP and greening payments was used. In 2015, there were 170,516 eligible land parcels for the SAP and greening payment.

Spatial analysis

The ArcGIS software was used for analysis of the spatial relations between the permanent grassland parcels of *expanding* and *shrinking farms*. For the purpose of spatial analysis, the data about the investigated farms and land users and the map of ARIB field parcels (hereafter parcels) were merged into one dataset. Two methodical approaches were used for the assessment of the distances between the permanent grassland parcels of *shrinking* and *expanding farms.*

At first, the buffer zones of 1 km, 2 km, 3 km and 4 km were generated around the parcels of *shrinking farms* and the corresponding number (1–4) was assigned to those zones. The maximum radius of zones was set to 4 km. See left panel of Fig. 1 as an example.

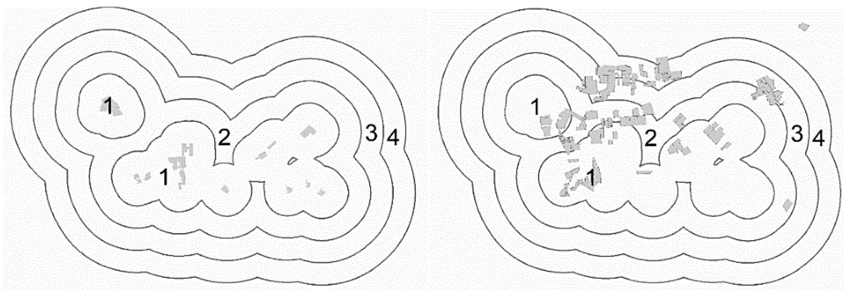

Figure 1. Buffer zones around permanent grassland parcels (left panel) of *shrinking farms* and location of permanent grassland parcels of *expanding farms* in those buffer zones (right panel).

Then, the overlay procedure of the buffer zone layer and the layer of the parcels of *expanding farms* was performed in ArcGIS. See the right panel of Fig. 1. The result of this overlay procedure is that all parcels of the layer of *expanding farms* received the appropriate zone number. This zone number shows the distance interval from the parcels of *shrinking farms* to the nearest parcels of *expanding farms*. For example, zone number 1 means that this distance is between zero and one kilometre, number 2 means that this distance is between one and two kilometres, and so on. The total number of *expanding farms'* permanent grassland parcels and the total area of those parcels in the buffer zones is the measure that characterises the potential to use the permanent grassland parcels of *shrinking farms.*

The second methodical approach was the calculation of distances from the permanent grassland parcels of *expanding farms* to the nearest permanent grassland parcel of *shrinking farms.* ArcGIS was also used for that purpose.

Finally, the heat maps were composed for illustration of the spatial distribution of the permanent grassland parcels of *shrinking farms* and location of the permanent grassland parcels of *expanding farms*.

RESULTS AND DISCUSSION

Likely changes in the use of permanent grasslands

It appears that 66% of the respondents of the survey indicated a willingness to decrease their land use due to restrictions in the eligible permanent grassland maintenance practices in the future, while 14% of the respondents said they would increase their land use and 20% said their land use would not change. The average agricultural area of the respondents was 84.5 ha. 27% of the respondents were specialist field crops farms, 40% were specialist grazing livestock farms and 20% were passive land users. The average share of permanent grassland was 63.2% which is markedly higher than the total Estonian average. On average, the respondents had 24.1 LU with an average livestock density of 0.21 LU ha^{-1}. 39% of the respondents were private persons who were not registered as private limited companies or sole proprietorships.

Table 2 summarises the results of the probit estimations of 42 one-variable models and 5 models with integrations of explanatory variables. Specialist field crops, mixed crop-livestock farms and passive land users were found to be more likely to decrease their permanent grassland use if the restrictions on cutting the grass and leaving the residues on the ground come into force. This could be explained by these farm types having no use for the grass biomass. Therefore, if leaving the cut biomass on the ground is restricted, these farm types have fewer incentives to maintain their permanent grasslands in the GAEC. On the contrary, grazing livestock farms were found more likely to maintain or increase their permanent grassland use. Farms with more LU and higher livestock density had a higher probability of increasing their permanent grassland use. As suggested also by Swinnen et al. (2013) and Brady et al. (2015), in the case of grazing livestock farms, additional permanent grassland parcels could potentially help in farm expansion or in the reduction of risks related to forage quantity. In addition, agricultural area (farm size) positively affected the likelihood that the farmer will expand permanent grassland use and it reduced the probability of a decrease in permanent grassland use. This is in line with previous results from Estonia that indicate that larger farms are more likely to expand their size (Viira et al., 2013). Farms and land users with the higher share of permanent grassland in their agricultural area were found more likely to reduce their permanent grassland area. Beneficiaries of the SAP and greening payment who were private persons and not registered as business enterprises were more likely to decrease and less likely to increase their permanent grassland area. This suggests that if the eligible practices of maintaining permanent grasslands in the GAEC are restricted, passive land users are more likely to decrease their use of permanent grasslands, which is in accordance with the aim of the restrictions. This, in turn, could create opportunities for these grazing livestock farmers who are interested in farm expansion to expand their permanent grassland area.

Table 2. The summary of the probit regression results

Explanatory variables	*Dependent variables*					
	Permanent grassland use will increase		Permanent grassland use will not change		Permanent grassland use will decrease	
	Intercept	*ß*	Intercept	*ß*	Intercept	*ß*
Field_crops	-0.983***	-0.362*	-0.790***	-0.252’	0.312***	0.400**
Horticulture	-1.065***	-3.755	-0.850***	-3.970	0.409***	4.410
Perm_crops	-1.061***	-0.322	-0.850***	-0.118	0.407***	0.267
Gr_livestock	-1.546***	0.921***	-1.178***	0.692***	0.914***	-1.114***
Granivores	-1.066***	-3.754	-0.851***	-3.968	0.410***	4.201
Mixed_crops	-1.066***	-0.085	-0.843***	-4.368	0.404***	0.746
Mixed_livestock	-1.065***	-3.755	-0.850***	-3.970	0.409***	4.410
Crops_livestock	-1.016***	-0.826**	-0.845***	-0.069	0.375***	0.421*
Passive	-0.954***	-0.891***	-0.735***	-0.846***	0.250***	1.094***
Area	-1.129***	0.001**	-0.872***	0.000	0.468***	-0.001**
Grassland_share	-0.963***	-0.168	-0.748***	-0.167	0.267**	0.231’
LU	-1.089***	0.001’	-0.858***	0.000	0.430***	-0.001
LU_density	-1.214***	0.590***	-0.908***	0.234’	0.548***	-0.640***
Private_person	-0.888***	-0.780***	-0.697***	-0.448***	0.141*	0.779***
Gr_livestock LU_density*	-1.288***	0.673***	-	-	-	-
*Gr_livestock*Area*	-1.108***	0.001*	-	-	-	-
*Gr_livestock*LU*	-1.104***	0.001*	-	-	-	-
Gr_livestock grassland_share*	-1.397***	0.888***				
Crops_livestock Grassland_share*	-	-	-	-	0.380***	0.616*

’significant at 0.1 level; *significant at 0.05 level; **significant at 0.01 level; ***significant at 0.001 level
-model was not estimated.

Since specialist grazing farms were found likely to expand their permanent grassland area, in this farm type the parameters of additional models with interactions between explanatory variables were estimated. The results indicated that specialist grazing farms with higher livestock density, a larger agricultural area, a larger number of LU, and a higher share of permanent grasslands in their land use were more likely to expand their permanent grassland area.

The parameters of models with interactions of explanatory variables were additionally estimated in the case of mixed crop-livestock farms. In this farm type, the share of permanent grasslands in total agricultural land had a positive effect on the probability of a reduction in permanent grassland area.

Based on the results of the regression analyses, the following logical classification was used to divide the beneficiaries of SAP and greening payment into three groups:

1) Area of permanent grasslands was considered likely to decline in the case of:
   - all specialised field crops farms;
   - those mixed crop-livestock farms in which the share of permanent grasslands exceeded the median (73.2%) for this farm type;
   - all passive land users.
2) Area of permanent grassland was considered likely to increase in the case of those specialised grazing livestock farms in which:
   - livestock density exceeded the median (0.417 LU/ha) for this farm type, or;
   - the share of permanent grasslands exceeded the median (89.5%) for this farm type, or;
   - the agricultural area exceeded the median (24 ha) for this farm type, or;
   - the total number of LU exceeded the median (4.8 LU) for this farm type.
3) Area of permanent grassland was considered likely to remain unchanged in all farms that did not belong to groups 1 or 2.

Proximity of permanent grassland parcels of *expanding* and *shrinking farms*

For a farm, one of the preconditions for expanding its agricultural land use is the availability of land that is for sale or for rent within reasonable proximity to its current boundaries. Fig. 1 demonstrates the situation where the permanent grassland parcels of several *expanding farms* are situated within a distance of 1–4 kilometres from the permanent grassland parcels of *shrinking farms*. In this case, it is apparent that the permanent grassland parcels of *expanding farms* are located around and between the permanent grassland parcels of potentially *shrinking farms*, as if in the pieces of a puzzle. These *expanding farms* would primarily be interested in buying or renting the permanent grassland parcels of neighbouring *shrinking farms*.

Table 3 shows that the number of *shrinking farms* exceeded the number of *expanding farms* by 2.7 times. However, the area of permanent grassland used by the *shrinking farms* comprised 53% of the area of the permanent grassland of *expanding farms*. The average *shrinking farm* was characterised by the smaller average number of parcels (3.23), the smaller average size of a permanent grassland parcel (3.35 ha) and the smaller average area of permanent grassland parcels (10.83 ha) compared to the respective values of the average *expanding farm* (10.58, 5.23 ha and 55.31 ha). This is consistent with the results from previous research (Viira et al., 2013), which determined that smaller farms have a higher probability of giving up agricultural production, while larger farms are more likely to expand their land use and production volume. However, it also suggests that the permanent grassland parcels of *shrinking farms* had less desirable characteristics (smaller size) compared to the parcels of *expanding farms*. Therefore, it is likely that *expanding farms* might not be interested in all the permanent grassland parcels of *shrinking farms*.

Table 3. A general description of the investigated farms and their permanent grassland parcels

Groups of farms	Number of investigated farms	Total number of parcels	Total area of parcels, ha	Average number of parcels per farm	Average area of parcels per farm, ha	Average parcel size, ha
Shrinking farms	7,998	25,829	86,579	3.23	10.83	3.35
Expanding farms	2,953	31,230	163,327	10.58	55.31	5.23
Total	10,951	57,059	249,906	5.21	22.82	4.38

Source: authors' calculations.

The number and area of the permanent grassland parcels of *shrinking farms* that were located in the buffer zones around the permanent grassland parcels of *expanding farms* was measured. 72% of the permanent grassland area of the *shrinking farms* were located within a 1 km buffer zone (Table 4), i.e. for 72% of the permanent grassland area of *shrinking farms*, there were potential *expanding farms* within a distance of less than 1 km. Respective figures for a 2 km buffer zone is 91%, 97% for a 3 km buffer zone and 99% for a 4 km buffer zone. Only 1% of the permanent grassland area of *shrinking farms* was located more than 4 km from the permanent grassland parcels of *expanding farms*. This suggests that the majority of the permanent grassland parcels of *shrinking farms* are located in sufficiently close proximity to *expanding farms*. Therefore, the main question mark over the active use of these permanent grassland parcels is not their location with respect to active farmers but rather the willingness of the passive land owners to sell or rent this land, as well as the financial capacity of active farmers to buy or lease this land.

Table 4. The amount and area of permanent grassland parcels of *shrinking farms* in the zones surrounding the permanent grassland parcels of *expanding farms*

The radius of buffer zones surrounding parcels of *expanding farms*, km	The number of parcels of *shrinking* farms in the zones	The total area of the parcels in the zones, ha	The proportion of parcels in the zones, %	
			by the number of parcels	by the area of parcels
1	18,327	62,690	70.9	72.4
2	5,163	16,472	20.0	19.0
3	1,569	4,746	6.1	5.5
4	525	1,651	2.0	1.9
Not in buffer zones	245	1,020	1.0	1.2
Total	25,829	86,579	100.0	100.0

Source: authors' calculations.

The measurement of the distance from the permanent grassland parcels of *expanding farms* to the nearest permanent grassland parcel of *shrinking farms* (Table 5) also confirmed that the bulk of the permanent grassland parcels of *expanding farms* (83% by the area) was located less than 1 km from the parcels of *shrinking farms*. 96% of the number and area of permanent grassland parcels of *expanding farms* were within a distance of 2 km from the locations of *shrinking farms*. Therefore, if any of the *shrinking farms* decide to sell or rent their permanent grassland, there is a high probability they will find an interested *expanding farm* within a radius of 1–2 kilometres.

Table 5. The distribution of the permanent grassland parcels of *expanding farms* to the nearest permanent grassland parcel of *shrinking farms* by distances

The distance from the parcels of *expanding farms* to the nearest parcel of *shrinking farms*, km	The number of parcels of *expanding farms* in the group	The total area of the parcels, ha	The proportion of parcels in the group, %	
			by the number of parcels	by the area of parcels
Less than 1.0	25,716	135,826	82.4	83.1
1.1 to 2.0	4,111	20,232	13.2	12.4
2.1 to 3.0	951	4,847	3.0	3.0
3.1 to 4.0	253	965	0.8	0.6
More than 4.0	199	1,457	0.6	0.9
Total	31,230	163,327	100.0	100.0

Source: authors' calculations.

The results in Tables 4 and 5 suggest that if the practices of maintenance of permanent grasslands are restricted in a way that *shrinking farms* are willing to sell or let their permanent grassland parcels to *expanding farms*, it is likely that the share of permanent grassland that would not be maintained in the GAEC would be modest[2].

Regional effects

Heat maps (Figs 2–5) illustrate the spatial distribution of the permanent grassland parcels of *shrinking farms* and *expanding farms*. The darker the colour on the map the higher the concentration of the phenomenon or objects in the space. It should be noted that the colour differences on the map show the relative differences in concentration. Thus, each map must be interpreted independently, as a comparison of colours on the different maps would lead to incorrect conclusions.

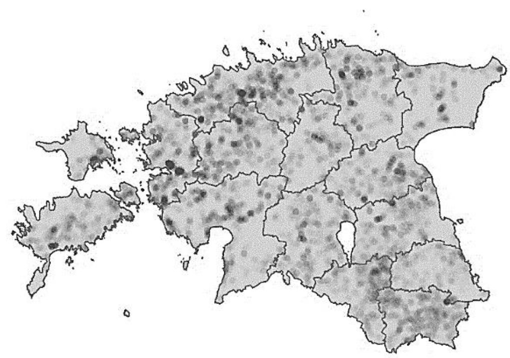

Figure 2. Concentration of permanent grassland parcels of *shrinking farms*.

The heat map on Fig. 2 shows the concentration of the permanent grassland of all *shrinking farms* across the whole of Estonia. Relatively higher concentration areas (darker colour) can be observed in the northern part of the country, around the region of the capital (Tallinn, in the north), in the coastal regions of the West-Estonian lowlands and in the southeastern part of Estonia with its hilly topography. This suggests that restrictions on the maintenance practices of permanent grassland that are eligible for SAP and greening payment would more severely affect passive land users, crop and mixed crop-livestock farms around the region of the capital, in coastal lowlands and in hilly regions that are less favourable for agricultural production due to their natural conditions. The last finding is concurrent with Brady et al. (2017) in that the passive management of

[2] However, this presumes well-functioning land and financial markets to facilitate the transactions. The functioning of land and financial markets, and land owners' willingness to sell or rent their land, falls outside of the scope of this paper due to the lack of such information.

agricultural land derives from low productivity. A higher concentration of passive farming around the region of the capital could be explained by a more active labour market and better options for higher remuneration outside of the agricultural sector.

Fig. 3 shows the concentration of permanent grassland parcels of *shrinking farms* locating at a distance of more than 1 km from the nearest *expanding farms*. This indicates the relatively higher risk that the permanent grasslands will not be maintained in the GAEC if the eligible maintenance practices are restricted. It appears that the risk is higher around the two largest cities – the capital Tallinn (in the north), and Tartu (in the southeast). This is dueto the lack of *expanding farms* that would potentially be interested in buying or renting permanent grassland parcels in these regions (Fig. 4).

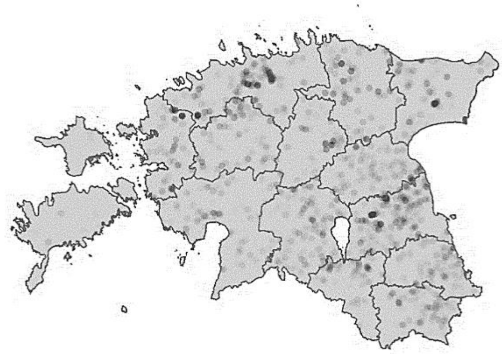

Figure 3. Concentration of permanent grassland parcels of *shrinking farms* located more than 1 km from permanent grassland parcels of *expanding farms*.

The permanent grasslands of *expanding farms* are concentrated on the islands and coastline of Western Estonia, Central Estonia and Southern Estonia (Fig. 4). This coincides with the regional concentration of cattle, sheep and goat farms (Kaasik et al., 2012) as well as the regional concentration of permanent grasslands in Estonia. It is likely that in these regions the restrictions on the eligible maintenance practices of permanent grasslands will not cause a decline in the area of permanent grasslands that are not maintained in the GAEC.

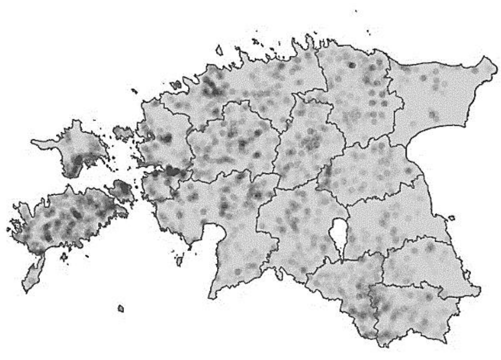

Figure 4. Concentration of permanent grassland parcels of *expanding farms*.

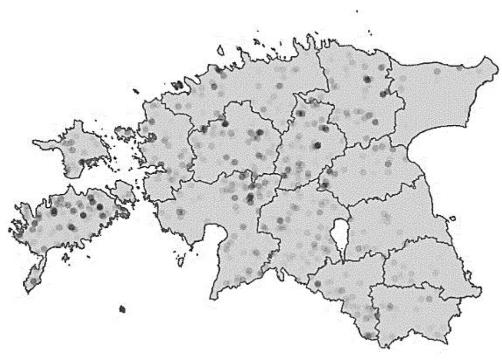

Figure 5. Concentration of permanent grassland parcels of *expanding farms* located more than 1 km from permanent grassland parcels of *shrinking farms*.

Fig. 5 presents the concentration of permanent grassland parcels of *expanding farms* located at a distance of more than 1 km from the nearest permanent grassland parcel of *shrinking farms*. It appears that in central Estonia and on the island of Saaremaa there is some concentration of *expanding farms* that do not have permanent grasslands parcels of *shrinking farms* in the vicinity of less than 1 km. Central Estonia is the main region for intensive and larger scale dairy farms (Kaasik et al., 2012), which is why there are not many passive land users, crop and mixed crop-livestock farms that would maintain their permanent grasslands in the GAEC using the restricted grass biomass cutting practice. On Saaremaa, the concentration of *expanding farms* is higher than that of *shrinking farms*. Therefore, it is likely that the potential demand for permanent grassland parcels associated with the restriction on eligible permanent grasslands maintenance practices exceeds the potential supply.

Policy implications

The previous results show that the policy change in the form of the restriction of eligible practices in permanent grassland maintenance could only be partly successful in promoting more active use of permanent grasslands. It is likely, as a result, that the maintenance activities of permanent grasslands would be stopped in some parts of the country. Brady et al. (2017) conclude that elimination of the SPS would result in less land in production, specifically low-productive land, because production, rather than passive farming, is the least-cost alternative to meet the maintenance obligation for substantial areas of land. Therefore, if the maintenance of permanent grasslands via active farming practices, i.e. grazing or forage production (Mõtte et al., 2019), is the desired policy objective, then extensive grazing should be supported with respective subsidies or otherwise promoted. However, dairy and beef production comprise a significant effect on agriculture related greenhouse emissions (GHG) (Lesschen et al., 2011, Lenerts et al., 2019). Therefore, expanding grazing livestock systems is a questionable policy goal in the context of the urgent need to reduce GHG emissions in the EU.

This suggests that a subsidised passive farming type of maintenance is necessary for some low-productivity permanent grasslands if these permanent grasslands are to be maintained in the GAEC according to the aims of the CAP. This is in line with the conclusion of van der Zanden et al. (2017) that a discussion on agricultural land abandonment should consider the spatial diversity and help to develop context-dependent, nuanced management strategies. Furthermore, the maintenance of permanent grasslands should be considered within a wider context of land use and bioeconomy policies. In some cases, afforestation could be a viable alternative for permanent grassland maintenance, in other cases the collected biomass could be used for bioenergy purposes (Nurmet et al., 2019). However, in each case, the local context (soil quality, landscape, biodiversity, farm structure) should be considered before making any decision.

CONCLUSIONS

The results of the study show that more than 70% of the permanent grassland parcels of shrinking farms are located less than 1 km away from the permanent grassland parcels of expanding farms. Therefore, the distance from the active *expanding farms* is not the main obstacle to permanent grassland maintenance. The main reasons are most likely related to the willingness of passive land users to sell or rent their permanent grasslands to active farmers and the financial capacity of active farmers to buy or lease the respective land. If the eligible maintenance practices of permanent grasslands are restricted, then passive land users, crop and mixed crop-livestock farms are more likely to reduce their use of permanent grasslands, while grazing livestock farms are likely to increase their use of permanent grasslands. Also, there are some regions (around two largest cities – the region of the capital Tallinn (in the north), and Tartu (in the southeast)) where there are not enough *expanding farms* close to *shrinking farms*, which suggests that in such regions permanent grassland maintenance might stop as a result of the restriction of eligible maintenance practices. Therefore, a policy change on the form of restriction of eligible practices could only be partly successful in promoting the more active use of permanent grasslands.

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REFERENCES

Abolina, E. & Luzadis, V.A. 2015. Abandoned agricultural land and its potential for short rotation woody crops in Latvia. *Land Use Policy* **49**, 435–445. https://doi.org/10.1016/j.landusepol.2015.08.022

Agricultural Research Centre. 2016. Calculator of economic size and farm type. *http://maainfo.ee/data/so_calc/*.

Alanen, I. 1999. Agricultural Policy and the Struggle over the Destiny of Collective Farms in Estonia. *Sociologia Ruralis* **39**(3), 431–458.

Ariva, J., Kall, K., Oper, L. & Viira, A.-H. 2017. Effects of the Restrictions of Practices Used for the Maintenance of Permanent Grasslands. In: Raupelienė, A (ed.) *Proceedings of the 8th International Scientific Conference Rural Development 2017,* Aleksandras Stulginskis University, Kaunas, Lithuania, pp. 1–7. http://doi.org/10.15544/RD.2017.163

Arslan, F., Değirmenci, H., Rasva, M. & Jürgenson, E. 2019. Finding least fragmented holdings with factor analysis and a new methodology: a case study of kargılı land consolidation project from Turkey. *Agronomy Research* **17**(3), 683–693. https://doi.org/10.15159/AR.19.052

Barnes, A., Sutherland, L.-A., Toma, L., Matthews, K. & Thomson, S. 2016. The effect of the Common Agricultural Policy reforms on intentions towards food production: Evidence from livestock farmers. *Land Use Policy* **50**, 548–558. https://doi.org/10.1016/j.landusepol.2015.10.017

Brady, M.V., Hristov, J., Sahrbacher, C., Söderberg, T. & Wilhelmsson, F. 2015. Passive Farming: Hindering Agricultural Development or Preserving Valuable Landscapes? Paper prepared for presentation at the 147th EAAE Seminar *'CAP Impact on Economic Growth and Sustainability of Agriculture and Rural Areas'*, Sofia, Bulgaria, October 7–8, 2015.

Brady, M.V., Hristov, J., Sahrbacher, C., Söderberg, T. & Wilhelmsson, F. 2017. Is Passive Farming A Problem for Agriculture in the EU? *Journal of Agricultural Economics* **68**(3), 632–650.

Commission Delegated Regulation (EU) No 639/2014 of 11 March 2014 supplementing Regulation (EU) No 1307/2013 of the European Parliament and of the Council establishing rules for direct payments to farmers under support schemes within the framework of the common agricultural policy and amending Annex X to that Regulation. *Publications Office of the European Union.* http://data.europa.eu/eli/reg_del/2014/639/oj. Accessed 29.10.2018.

Commission Regulation (EC) No 1242/2008 of 8 December 2008 establishing a Community typology for agricultural holdings. *Official Journal of the European Union*. L335/3.

Commission Regulation (EC) No 867/2009 of 21 September 2009 amending and correcting Regulation (EC) No 1242/2008 establishing a Community typology for agricultural holdings. *Official Journal of the European Union*. L248/17.

Di Corato, L. & Brady, M.V. 2019. Passive farming and land development: A real options approach. *Land Use Policy* **80**, 32–46. https://doi.org/10.1016/j.landusepol.2018.09.029

Eurostat. 2018. Online database. https://ec.europa.eu/eurostat/data/database. Accessed 25.12.2019

FAOSTAT. 2018. Online database. *http://www.fao.org/faostat/en/.* Accessed 11.03.2018.

Jones, N., Duarte, F., Rodrigo, I., van Doorn, A. & Graaff, J. 2016. The role of EU agri-environmental measures preserving extensive grazing in two less-favoured areas in Portugal. *Land Use Policy* **54**, 77–187. https://doi.org/10.1016/j.landusepol.2016.01.014

Jürgenson, E. 2016. Land reform, land fragmentation and perspectives for future land consolidation in Estonia. *Land Use Policy* **57**, 34–43. https://doi.org/10.1016/j.landusepol.2016.04.030

Kaasik, A., Karp, K., Keres, I., Kosk, A., Lauringson, E., Leming, R., Reintam, E., Roasto, M., Selge, A., Sepp, K., Vahejõe, K., Viira, A.-H., Vooremäe, A., Värnik, R., Timmusk, T. & Sinijärv, L. 2012. Põllumajanduse poolt loodud avalike hüvede hindamine Eestis. Lõpparuanne. Eesti Maaülikool (in Estonian). *https://www.agri.ee/sites/default/files/public/Avalike_Hyvede_l6pparuanne_2012.pdf*

Lasanta, T., Arnáez, J., Pascual, N. Ruiz-Flaño, P., Errea, M.P. & Lana-Renault, N. 2017. Space-time process and drivers of land abandonment in Europe. *CATENA* **149**(3), 810–823. https://doi.org/10.1016/j.catena.2016.02.024

Lenerts, A., Popluga, D. & Naglis-Liepa, K. 2019. Benchmarking the GHG emissions intensities of crop and livestock–derived agricultural commodities produced in Latvia. *Agronomy Research* **17**(5), 1942–1952, 2019. https://doi.org/10.15159/AR.19.148

Lesschen, J.P., van den Berg, M., Westhoek, H.J., Witzke, H.P. & Oenema, O. 2011. *Greenhouse gas emission profiles of European livestock sectors. Animal Feed Science and Technology* **166–167**, 16–28. https://doi.org/10.1016/j.anifeedsci.2011.04.058

Mõtte, M., Lillemets, J. & Värnik, R. 2019. A systematic approach to exploring the role of primary sector in the development of Estonian bioeconomy. *Agronomy Research* **17**(1), 220–233, 2019. https://doi.org/10.15159/AR.19.068

Nurmet, M., Mõtte, M., Lemsalu, K. & Lehtsaar, J. 2019. Bioenergy in agricultural companies: financial performance assessment. *Agronomy Research* **17**(3), 771–782, https://doi.org/10.15159/AR.19.131

OECD. 1996. *Review of Agricultural Policies: Estonia*. OECD Publishing, Paris.

Pilgaard Kristensen, S.B., Gravsholt Busck, A., van der Sluis, T. & Gaube, V. 2016. Patterns and drivers of farm-level land use change in selected European rural landscapes. *Land Use Policy* **57**, 786–799. https://doi.org/10.1016/j.landusepol.2015.07.014

Prishchepov, A.V., Müller, D., Dubinin, M., Baumann, M. & Radeloff, V.C. 2013. Determinants of agricultural land abandonment in post-Soviet European Russia. *Land Use Policy* **30**(1), 873–884. https://doi.org/10.1016/j.landusepol.2012.06.011

Pupo D'Andrea, M.R. & Romeo Lironcurti, S. 2017. Is the question of the 'active farmer' a false problem? B*io-based and Applied Economics* **6**(3), 295–313.

Regulation (EU) No 1307/2013 of the European Parliament and of the Council of 17 December 2013 establishing rules for direct payments to farmers under support schemes within the framework of the common agricultural policy and repealing Council Regulation (EC) No 637/2008 and Council Regulation (EC) No 73/2009. *Official Journal of the European Union*. L347/608. http://data.europa.eu/eli/reg/2013/1307/oj. Accessed 29.10.2018.

Renwick, A., Jansson, T., Verburg, P.H., Revoredo-Giha, C., Britz, W., Gocht, A. & McCracken, D. 2013. Policy reform and agricultural land abandonment in the EU. *Land Use Policy* **30**(1), 446–457.

Regulation 32 of Agricultural minister, 25.04.2015. Otsetoetuste saamise üldised nõuded, ühtne pindalatoetus, kliima- ja keskkonnatoetus ning noore põllumajandustootja toetus. Maaeluministri määrus nr 32. https://www.riigiteataja.ee/akt/122042015027. Accessed 24.8.2016 (in Estonian).

Regulation 32 of Agricultural minister, 25.04.2016. Otsetoetuste saamise üldised nõuded, ühtne pindalatoetus, kliima- ja keskkonnatoetus ning noore põllumajandustootja toetus. Maaeluministri määrus nr 32. *https://www.riigiteataja.ee/akt/122042016004*. Accessed 24.8.2016 (in Estonian).

Regulation 32 of Agricultural minister, 28.04.2017. Otsetoetuste saamise üldised nõuded, ühtne pindalatoetus, kliima- ja keskkonnatoetus ning noore põllumajandustootja toetus. Maaeluministri määrus nr 32. *https://www.riigiteataja.ee/akt/125042017014*. Accessed 24.2.2018 (in Estonian).

Regulation 11 of Agricultural minister, 30.07.2012. Head põllumajandus- ja keskkonnatingimused, püsirohumaa pindala säilitamise kohustuse täitmise täpsem kord, püsirohumaa pindala säilitamise kohustuse üleandmise alused ja kord ning püsirohumaa säilitamiseks vajalike abinõude rakendamise täpsem kord. *https://www.riigiteataja.ee/akt/127072012011*. Accessed 24.8.2016 (in Estonian).

Rudbeck Jepsen, M., Kuemmerle, T., Müller, D., Erb, K., Verburg, P.H., Haberl, H., Vesterager, J.P., Andrič, M., Antrop, M., Austrheim, G., Björn, I., Bondeau, A., Bürgi, M., Bryson, J., Caspar, G., Cassar, L.F., Conrad, E., Chromý, P., Daugirdas, V., Van Eetvelde, V., Elena-Rosselló, R., Gimmi, U., Izakovicova, Z., Jančák, V., Jansson, U., Kladnik, D., Kozak, J., Konkoly-Gyuró, E., Krausmann, F., Mander, Ü., McDonagh, J., Pärn, J., Niedertscheider, M., Nikodemus, O., Ostapowicz, K., Pérez-Soba, M., Pinto-Correia, T., Ribokas, G., Rounsevell, M., Schistou, D., Schmit, C., Terkenli, T.S., Tretvik, A.M., Trzepacz, P., Vadineanu, A., Walz, A., Zhllima, E. & Reenberga, A. 2015. Transitions in European land-management regimes between 1800 and 2010. *Land Use Policy* **49**, 53–64. https://doi.org/10.1016/j.landusepol.2015.07.003

Statistics Estonia. 2019. Online database. *http://www.stat.ee*. Accessed 25.12.2019.

Stokstad, G. & Krøgli, S.O. 2015. Owned or rented—does it matter? Agricultural land use change within farm properties, case studies from Norway. *Land Use Policy* **48**, 505–514. https://doi.org/10.1016/j.landusepol.2015.06.019

Swinnen, J.M. 1999. The Political Economy of Land Reform Choices in Central and Eastern Europe. *Economics of Transition* **7**(3), 637–664.

Swinnen, J., Ciaian, P., Kancs, d'A., Van Herck, K. & Vranken, L. 2013. Possible effects on EU land markets of new CAP direct payments. Study. European Parliament. *http://www.europarl.europa.eu/RegData/etudes/STUD/2013/495866/IPOL-AGRI_ET%282013%29495866_EN.pdf*. Accessed 8.11.2018

Terres, J.-M., Nisini Scacchi, L., Wania, A., Ambar, M., Anguiano, E., Buckwell, A., Coppola, A., Gocht, A., Nordström Källström, H., Pointereau, P., Strijker, D., Visek, L., Vranken, L. & Zobena, A. 2015. Farmland abandonment in Europe: Identification of drivers and indicators, and development of a composite indicator of risk. *Land Use Policy* **49**, 20–34. https://doi.org/10.1016/j.landusepol.2015.06.009

Trubins, R. 2013. Land-use change in southern Sweden: Before and after decoupling. *Land Use Policy* **33**, 161–169.

Unwin, T. 1997. Agricultural Restructuring and Integrated Rural Development in Estonia. *Journal of Rural Studies* **13**(1), 93–112.
Van Herck, K. & Vranken, L. 2013. Direct Payments and Land Rents. Evidence from New Member States. In: *Centre for European Policy Studies Factor Markets Working Paper No.* **62**. http://dx.doi.org/10.2139/ssrn.2329931
Van der Sluis, T., Pedroli, B., Kristensen, S.B.P., Lavinia Cosor, G. & Pavlis, E. 2016. Changing land use intensity in Europe – Recent processes in selected case studies. *Land Use Policy* **57**, 777–785. https://doi.org/10.1016/j.landusepol.2014.12.005
van der Zanden, E.H., Verburg, P.H., Schulp, C.J.E. & Verkerk, P.J. 2017. Trade-offs of European agricultural abandonment. *Land Use Policy* **62**, 290–301. https://doi.org/10.1016/j.landusepol.2017.01.003
Viira, A.-H. & Ariva, J. 2019. Maintenance of permanent grasslands – agri-environmental protection, passive land use or constraint for the structural development? Paper prepared for presentation at the 172nd EAAE Seminar *'Agricultural policy for the environment or environmental policy for agriculture?* May 28-29, 2019. Brussels.
Viira, A.-H., Ariva, J., Kall, K. & Oper, L. 2016. Põllumajanduslike otsetoetuste raames minimaalsete hooldustööde nõuete rakendamine aastatel 2013–2016. Research report. Institute of Economics and Social Sciences, Estonian University of Life Sciences, Tartu, Estonia. Available at *http://www.pikk.ee/upload/files/Otsetoetuste_minimaalsed_hooldustoode_nouded_aruanne_2_.pdf* . Accessed on 25.01.2018 (in Estonian).
Viira, A.-H., Põder, A. & Värnik, R. 2013. The Determinants of Farm Growth, Decline and Exit in Estonia. *https://www.researchgate.net/publication/270590153_The_Determinants_of_Farm_Growth_Decline_and_Exit_in_Estonia*

Annex I

Descriptive statistics of farms and passive land users that are likely to decrease, increase or not change their use of permanent grasslands

Characteristic	Permanent grassland use will decrease	Permanent grassland use will increase	Permanent grassland use will not change
Agricultural area, ha	39.6	115.3	60.6
Share of specialist field crops farms	31.8%	0.0%	0.0%
Share of specialist horticulture farms	0.0%	0.0%	0.8%
Share of specialist permanent crops farms	0.0%	0.0%	5.3%
Share of specialist grazing livestock farms	0.0%	100.0%	38.8%
Share of specialist granivores farms	0.0%	0.0%	3.3%
Share of mixed cropping farms	0.0%	0.0%	2.7%
Share of mixed livestock farms	0.0%	0.0%	0.9%
Share of mixed crops – livestock farms	16.2%	0.0%	47.0%
Share of non-classified holdings	0.0%	0.0%	1.2%
Share of passive land users	52.0%	0.0%	0.0%
Share of permanent grassland in agricultural area	71.5%	73.9%	27.3%
Livestock Units, LU	0.283	57.486	30.904
Livestock density, LU ha^{-1}	0.006	0.669	0.246
Share of private persons	64.2%	29.8%	60.5%

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Effects of the interaction between slurry, soil conditioners, and mineral NPK fertilizers on selected nutritional parameters of *Festulolium braunii* (K. Richt.) A. Camus

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Abstract. The research was aimed at assessing the biomass yield of *Festulolium braunii* and its content of raw protein and crude ash after application of slurry, both on its own and together with soil conditioners (UGmax and Humus Active), and mineral fertilizers. The studies were conducted on the basis of a two-year field experiment. The interaction between slurry and soil conditioners and between slurry and mineral fertilizers was studied on the Sulino variety of *Festulolium braunii*, a hybrid between *Lolium multiflorum* and *Festuca pratensis*.
Compared with plants treated with liquid manure on its own, slurry applied with soil conditioners and mineral fertilizer did not significantly increase the biomass yield of the grass. However, there was higher protein content in *Festulolium braunii*, even if statistically insignificant, as a response to slurry supplemented with mineral fertilizer than in plants treated with slurry only. Various forms of treatment did not differentiate crude ash content in plant dry matter in a statistically significant way.

Key words: forage grass, fertilization, nutritional value.

INTRODUCTION

Festulolium braunii (K. Richt.) A. Camus is a hybrid between *Lolium multiflorum* and *Festuca pratensis*. According to Borowiecki (2002a, 2002b), it produces higher yields of better nutritional value than meadow fescue. For this reason the grass is grown in arable fields to produce forage, but it is also used to overseed permanent grassland (Wolski et al., 2006). This species has high yield potential and provides good quality forage. In his research Sosnowski (2012) found that it produced a high yield of both fresh and dry matter (11–15 t ha^{-1} DM) with a favourable chemical composition, especially regarding protein content.

An organic fertilizer commonly applied to grassland, slurry is a very valuable source of organic matter and plant nutrients. According to Mazur & Mokra (2009), cow manure contains more dry matter and nutrients than pig manure. As reported by Christensen (2009), its available nitrogen accounts for 70% of its total nitrogen. According to Oenema et al. (2008), slurry nitrogen losses resulting from its release into the atmosphere range from 10% to over 30%. Grzebisz et al. (2007) recorded a reduction in nitrogen losses when aqueous chalk solution was added to slurry. Similarly, Kall et al. (2016) proved that the fertilizer value of liquid manure was largely dependent on application technology.

In the present research, an attempt was made to use biological soil amendments together with slurry in order to reduce its nitrogen losses and thus to increase nitrogen use efficiency. Moreover, slurry was supplemented with soil conditioners in order to study how it would affect the amount of biomass and the content of protein and ash in plants. The experiment was also to assess whether the addition of biological amendments to liquid manure could reduce the use of complementary mineral fertilizers.

The purpose of the studies was to assess the biomass yield of *Festulolium braunii* and the content of total protein and crude ash as an effect of slurry applied on its own but also together with soil conditioners (UGmax and Humus Active) and NPK mineral fertilisers.

MATERIAL AND METHODS

The studies were conducted on the basis of a two-year (2016–2017) field experiment established at the experimental facility of the University of Natural Sciences and Humanities in Siedlce (52°10′N, 22°17′E) in three replications, random layouts, and on plots of 4.5 m^2 (1.5×3.0 m). The main experimental factor tested in the research was slurry used on its own and supplemented with NPK mineral fertilizers and soil conditioners with the commercial names of UGmax and Humus Active. The experiment consisted of the following research units: (1) control (no treatment); (2) slurry; (3) slurry + UGmax; (4) slurry + Humus Active; (5) slurry + NPK.

The interaction between slurry and soil conditioners or mineral fertilizers was tested on forage grass of the *Festulolium braunii* species, the Sulino variety, sown in autumn 2015 at the sowing standard rate of 25 kg ha^{-1}. Liquid manure used in the experiment had been produced by dairy cows. Each year it was used at a total dose of 30 m^3 ha^{-1} divided into three parts applied before each growth cycle. The slurry had dry matter concentration of 10%, a narrow ratio of C:N (8:1), and the concentration of selected macronutrients as follows (g kg^{-1} DM): N-33, P_2O_5-16, K_2O-16, MgO-10, and Ca-21.

According to the Institute of Soil Science and Plant Cultivation in Puławy, Poland, the soil conditioners used in the experiment improve soil properties. Their composition is presented in Table 1.

Table 1. Composition of soil conditioners

Soil conditioner	Macronutrients (g kg^{-1})						Micronutrients (mg kg^{-1})				Microorganism and others
	N	P	K	Ca	Mg	Na	Mn	Fe	Zn	Cu	
UGmax	1.2	0.2	2.9	-	0.1	0.2	0.3	-	-	-	Lactic acid bacteria, photosynthetic bacteria, Azotobacter, Pseudomonas, yeast, actinomycetes
Humus Active	0.2	1.3	4.6	3.0	0.5	-	15	500	3	1	Permanent active humus with beneficial microorganisms

Soil conditioners were applied each year in the spring at doses recommended by the manufacturer: UGmax at 0.6 L ha^{-1} and Humus Active at 50 L ha^{-1}. Mineral nitrogen-phosphorus-potassium fertilizers (NPK) were used as follows: N – 100 kg ha^{-1}, P (P_2O_5) – 80 kg ha^{-1}, and K (K_2O) – 120 kg ha^{-1}. Mineral nitrogen was in the form of ammonium nitrate (NH_4NO_3), phosphorus was used as granular triple superphosphate ($Ca(H_2PO_4)_2$), and potassium as potassium chloride (KCl). Phosphorus was applied once a year in the spring whereas nitrogen and potassium doses were divided into three equal parts: the first before the start of vegetation. the second and third before the second and third grass growth cycle.

The experiment was set up on the soil with granulometric composition of loamy sand, the order of anthropogenic soils, the type of culture earth soils, and the subtype of hortisole (Polish Soil Classification, 2011). According to chemical analysis of the soil, carbon concentration in organic compounds (C_{org}) was 14.50 g kg^{-1} DM, with total nitrogen of 1.36 g kg^{-1} DM. The ratio of C:N was 10.6 : 1, and the pH value was 6.7. The concentration of absorbable forms of phosphorus (170 mg kg^{-1} DM) and magnesium (84 mg kg^{-1} DM) was high, with moderate concentration of potassium (114 mg kg^{-1} DM).

Hydrothermal conditions were determined on the basis of meteorological data from the Hydrological and Meteorological Station in Siedlce. In order to measure temporal variability of weather conditions and their effects on plant growth and development Sielianinov's hydrothermal coefficient (Table 2) was determined (Bac et al., 1993). It was calculated using the monthly sum of atmospheric precipitation (P) and the monthly sum of average daily air temperatures (Σt), applying the formula: $K = P/0.1\ \Sigma t$ (Skowera & Puła, 2004).

Table 2. The value of Sielianinov's hydrothermal coefficient (K) in the growing seasons

Year	Month						
	April	May	June	July	August	September	October
2015	1.36 (o)	1.87 (mw)	1.64 (mw)	0.59 (sd)	1.92 (mw)	0.64 (sd)	0.12 (ed)
2016	1.22 (md)	2.63 (sw)	0.87 (d)	1.08 (md)	0.18 (ed)	1.46 (o)	1.94 (mw)
2017	2.88 (sw)	1.15 (md)	1.08 (md)	0.45 (sd)	0.96 (d)	1.92 (mw)	1.90 (mw)

$K \leq 0.4$ extremely dry (ed); $0.4 \leq K \leq 0.7$ severely dry (sd); $0.7 \leq K \leq 1.0$ dry (d); $1.0 \leq K \leq 1.3$ moderately dry (md); $1.3 \leq K \leq 1.6$ optimal (o); $1.6 \leq K \leq 2.0$ moderately wet (mw); $2.0 \leq K \leq 2.5$ wet (w); $2.5 \leq K \leq 3.0$ severely wet (sw); $K > 3.0$ extremely wet (ew).

In the first year (2016) the optimum thermal and humidity conditions were only in September, with severely wet May, while June, July, and August, the most important months for plant growth and development, were dry, moderately dry, and extremely dry. In the second year of the experiment the period from May to August ranged from moderately dry to severely dry, and the optimum conditions were not recorded during any month of the growing season.

During the two-year experiment in each growing season three harvests of grass were collected. Immediately after each harvest fresh matter was weighed, and a sample of 0.5 kg was collected to determine the yield of dry matter and to perform chemical analyses. The yield of dry matter was determined by the drying method. Total protein and crude ash content was measured by the near-infrared spectroscopy method (NIRS), using the NIRFlex N-500 with the ready-to-use INGOT calibration for dry forage. The latter method is described in detail in the Polish standard of PN-EN ISO 12099:2010 and in the literature (Burns et al., 2010; Reddersen et al., 2012).

Analysis of variance for a three-factor experiment was used to process the results statistically. The statistical program of Statistica 6.0-2001 was used to evaluate forage parameters. The significance of differences was verified by Tukey's test at the level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

The yield of *Festulolium braunii* dry matter (Table 3) significantly varied depending on the treatment and the harvest. In the first and second years of the experiment the greatest amount of biomass was on plots treated with slurry and from those where liquid manure was applied together with NPK, with the yield of 14.40 and 14.00 Mg ha^{-1}, respectively. In the second year the equally high level of the yield (14.00 Mg ha^{-1}) was recorded on plots where the Humus Active soil conditioner was applied together with slurry.

Table 3. Dry matter yield of *Festulolium braunii* (Mg ha^{-1} DM)

Growing season (B)	Harvest (C)	Treatment (A)					Means
		*O	S	S+UGmax	S+HA	S+NPK	
2016	I	3.40	5.10	5.30	5.50	5.60	4.98 (0.81)
	II	3.50	4.00	3.90	4.60	4.70	4.14 (0.45)
	III	3.30	4.30	3.80	4.10	4.10	3.92 (0.35)
	Total	10.20	13.90	13.00	14.20	14.40	13.00
2017	I	3.20	5.30	5.50	5.20	5.70	4.98 (0.91)
	II	2.70	3.80	4.10	4.50	4.30	3.88 (0.64)
	III	2.80	4.40	3.80	4.30	4.00	3.86 (0.57)
	Total	8.70	13.50	13.40	14.00	14.00	12.72
Means across growing seasons		9.45 ***(0.75)	13.70 (0.20)	13.20 (0.20)	14.10 (0.10)	14.20 (0.20)	12,9 (0.16)

$HSD_{0.05}$ for: A = 0.57; B = **NS; C = 0.37; A/B = 0.42; B/A = 0.28; A/C = 0.52; C/A = 0.43; B/C = NS; C/B = NS

*O – control object (without fertilization); S – slurry; S +UGmax – slurry + UGmax soil conditioner; S + HA – slurry + Humus Active soil conditioner; S + NPK – slurry + mineral NPK fertilizers. **NS – not significant; ***(…) – standard deviation.

The lowest yield was on the control plot, with 10.20 Mg ha^{-1} in the first year and 8.70 Mg ha^{-1} in the second. Compared with plants treated with slurry on its own, the addition of soil conditioners or mineral fertilizer did not significantly increase plant biomass. In both years of research, compared with plants treated with slurry on its own, there was a decrease in the total amount of biomass on plots with liquid manure applied together with the UGmax soil conditioner. As an average of all treatments, the highest yield of grass in both years was in the first harvest (the same amount of 4.98 Mg ha^{-1} in both years), with the smallest in the third one (3.92 and 3.86 Mg ha^{-1}).

As an average for both growing seasons, *Festulolium braunii* (Fig. 1) produced a significantly higher yield on plots where plants were treated with slurry together with mineral fertilizers and on those with slurry supplemented with Humus Active than on plots treated with slurry on its own.

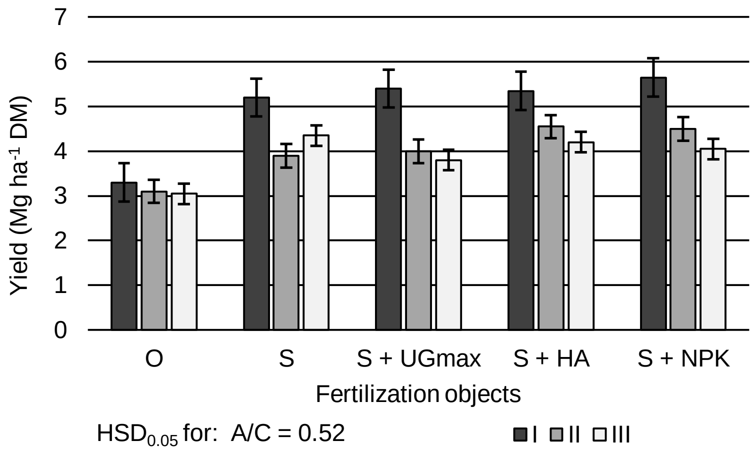

Figure 1. The effect of treatment on average yields of *Festulolium braunii* (Mg ha^{-1} DM) across harvests.

*O – control object (without fertilization); S – slurry; S +UGmax – slurry + UGmax soil conditioner; S + HA – slurry + Humus Active soil conditioner; S + NPK – slurry + mineral NPK fertilizers; I – standard deviation.

Festulolium braunii is a grass species that can be used on temporary grassland only for a few years. This is also confirmed by the studies of Borowiecki (2002a, 2002b) and Staniak (2005, 2010). According to their reports the level of *Festulolium braunii* yields ranges from 11 to 15 Mg ha^{-1}. This grass belongs to the species strongly reacting to nitrogen fertilizer. In the research conducted by Adamovies et. al. (2019) nitrogen fertilizer increased the amount of biomass even by 80% compared to control. Additionally, Obraztsov et al. (2018) recorded a significant effect of nitrogen fertilizer on the seed yield of this grass.

In the present experiment the best results with the increased *Festulolium braunii* annual yield of 14.20 Mg ha^{-1} (an average from all harvests) were recorded on plots where slurry was supplemented with mineral fertilizer. According to Olszewska (2008) crop yields are the basic criterion for assessing the effectiveness of fertilizer treatments. A yield increase as a response to natural fertilizer treatment in the cultivation of grasses was also confirmed by Barszczewski et al. (2011). Additionally, many other authors (Sosnowski & Jankowski, 2010) observed that the yield of the first harvest was the highest. In the present experiment the highest yield of grass dry matter, as an average response to the treatments, was also in the first harvest (4.98 Mg ha^{-1}).

In addition to fertilizer treatments, meteorological conditions, in particular the average air temperature and the volume of atmospheric precipitation, also affected forage grass yields. With regard to the level of yields (on average 13.04 in the first and 12.72 Mg ha^{-1} DM in the second year), it turned out that *Festulolium braunii*, with a low sensitivity to water shortages, was resistant to very adverse weather conditions prevailing in both years of research.

The ineffectiveness of the interaction between slurry and soil conditioners undoubtedly resulted from adverse meteorological conditions. In the first year (2016) the optimum thermal and humidity conditions were only in September, with severely wet May, while June, July and August, the most significant months for the growth and development of plants, were dry, moderately dry, and extremely dry. In the second year of the experiment (2017), the period from May to August ranged from dry to very dry, and the optimum conditions were not recorded during any month of the growing season.

Total protein content in plant dry matter (Table 4) was significantly differentiated by the treatments; there were also differences across harvests and growing seasons.

Table 4. Protein concentration in *Festulolium braunii* dry matter (g kg^{-1} DM)

Growing season (B)	Harvest (C)	Treatment (A)					Means
		*O	S	S+UGmax	S+HA	S+NPK	
2016	I	128.9	153.4	155.1	154.7	165.9	151.6 (13.6)
	II	113.8	149.4	146.2	157.3	158.3	145.0 (18.2)
	III	120.5	145.6	138.7	140.2	154.2	139.8 (12.4)
	Means	121.1	149.5	146.7	150.7	159.5	145.6
2017	I	128.9	165.2	155.6	159.5	170.1	155.9 (16.0)
	II	129.7	164.8	142.0	168.0	165.4	154.0 (17.1)
	III	124.2	159.3	157.0	152.9	156.3	149.9 (14.6)
	Means	127.6	163.1	151.5	160.1	163.9	153.3
Means across growing seasons		124.4	156.3	149.1	155.5	161.7	149.3
		***(6.26)	(8,14)	(7.83)	(9,13)	(6.30)	(6.00)

$HSD_{0.05}$ for: A = 9.09; B = 5.96; C = 4.02; A/B = **NS; B/A = NS; A/C = NS; C/A = NS; B/C = NS; C/B = NS

*O – control object (without fertilization); S – slurry; S + UGmax – slurry + UGmax soil conditioner; S + HA – slurry + Humus Active soil conditioner; S + NPK – slurry + mineral NPK fertilizers. **NS – not significant; ***(…) – standard deviation.

The largest protein concentration, average for all harvests, was noted in plants treated with slurry supplemented with mineral NPK, with 159.5 g kg^{-1} DM in the first year and 163.9 g kg^{-1} DM in the second. The smallest average protein concentration in *Festulolium braunii* was recorded on control plots: 121.1 g kg^{-1} in the first year and 127.6 g kg^{-1} in the second year of the experiment. Combined application of slurry and soil conditioners did not have a favourable effect compared with plants treated with slurry applied on its own. Mean protein content across growing seasons and treatments decreased with consecutive harvests (Fig. 2), and it was the largest in the first one and the smallest in the third.

A very important parameter determining the nutritional value of forage grasses is total protein content. According to Grygierzec (2012) the minimum concentration of this nutrient in forage should range from 150 to 170 g kg^{-1} DM. This amount of protein is necessary for the proper functioning of the digestive tract of dairy cows. According to Jankowska-Huflejt et al. (2011) protein content of grasses is a species trait. *Festulolium braunii* contains approximately 130 g kg^{-1} DM, which is less than in some other grasses. Many authors (Kotlarz et al., 2010; Szkutnik et al., 2012) indicate that plant protein content increases as nitrogen doses increase. In the present experiment it was found that the largest protein content in *Festulolium braunii* biomass was obtained with slurry supplied with NPK, but this was not significantly greater than in plants treated with slurry applied on its own. Moreover, the addition of soil conditioners to slurry did not significantly increase the yield of the grass. This is confirmed by the study of Ciepiela (2004), Jankowska et al. (2008), and Szkutnik et al. (2012), who point out that the content of total protein is not always increased proportionally to the nitrogen applied. Ciepiela (2004) claims that this phenomenon may be due to the dilution of the content of this ingredient in a higher amount of biomass. In the present experiment slurry supplemented with soil conditioners did not increase protein content in plants, which might have been caused by nitrogen uptake from slurry as a result of the bacteria activity in the conditioners.

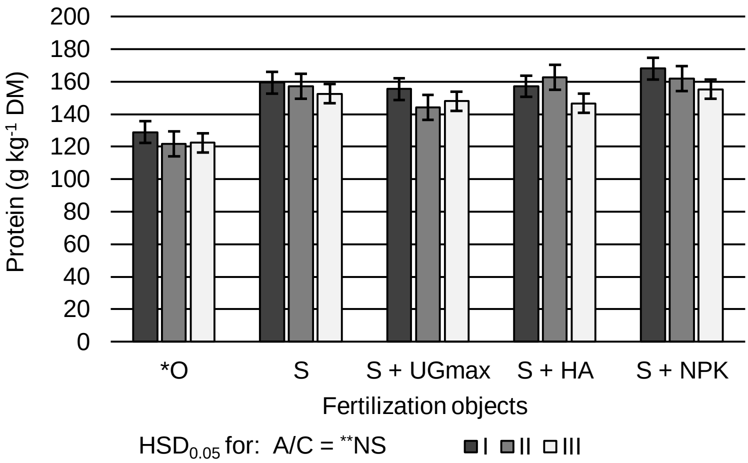

Figure 2. The effect of treatment on total protein concentration in *Festulolium braunii* (g kg^{-1} DM) across harvests.

*O – control object (without fertilization); S – slurry; S + UGmax – slurry + UGmax soil conditioner; S + HA – slurry + Humus Active soil conditioner; S + NPK – slurry + mineral NPK fertilizers. **NS – not significant; I – standard deviation.

Average protein content across years and treatments decreased in subsequent harvests. In the first one it was the largest and in the third the smallest. The impact of the harvest on total protein content in the dry matter of grass is confirmed by Tonn et al. (2013).

Another parameter used to assess plant nutritional value is crude ash content. This content in the dry mass of *Festulolium braunii* (Table 5) did not vary significantly as a response to different forms of treatment.

In the first year the largest amount of ash (112.6 g kg^{-1}) was in plants treated with slurry supplemented with the Humus Active soil conditioner. In the second year the highest average concentration of ash was recorded on plots where slurry was applied on its own (121.1 g kg^{-1}). In the first year the smallest crude ash concentration was noted in plants treated with slurry supplemented with the UGmax soil conditioner (106.6 g kg^{-1}), and in the second on the control plot (97.4 g kg^{-1}). There were also differences between the harvests. In the first year the greatest ash concentration was recorded in the biomass of the third harvest (114.1 g kg^{-1}) and in the second year in the first harvest (111.8 g kg^{-1}).

The lowest concentration was in the first harvest of the first year (105.4 g kg^{-1}) and in the second and third ones in the second year (108.4 g kg^{-1}).

Table 5. Crude ash concentration in *Festulolium braunii* dry matter (g kg^{-1} DM)

Growing season (B)	Harvest (C)	Treatment (A)					Means
		*O	S	S+UGmax	S+HA	S+NPK	
2016	I	104.3	103.5	103.6	111.2	104.3	105.4 (3.27)
	II	115.3	113.8	103.4	110.3	102.8	109.1 (5.79)
	III	109.2	110.6	112.8	116.2	121.6	114.1 (4.96)
	Means	109.6	109.3	106.6	112.6	109.6	109.5
2017	I	92.8	131.4	123.4	118.9	126.9	118.7 (15.7)
	II	99.8	123.6	102.9	102.8	112.8	108.4 (9.82)
	III	99.7	108.4	97.9	115.6	120.6	108.4 (9.83)
	Means	97.4	121.1	108.1	112.4	120.1	111.8
Means across growing seasons		103.5 ***(7.94)	115.2 (10.4)	107.4 (9.23)	112.5 (5.74)	114.9 (9.84)	110.7 (4.79)

$HSD_{0.05}$ for: A = **NS; B = NS; C = NS; A/B = 15.9; B/A 10.8; A/C = NS; C/A =NS; B/C = 8.27; C/B = 10.2

*O – control object (without fertilization); S – slurry; S + UGmax – slurry + UGmax soil conditioner; S + HA – slurry + Humus Active soil conditioner; S + NPK – slurry + mineral NPK fertilizers. **NS – not significant; ***(…) – standard deviation.

Overall, crude ash content across growing seasons (Fig. 3) was the smallest on the control plot and the largest in plants treated with slurry only.

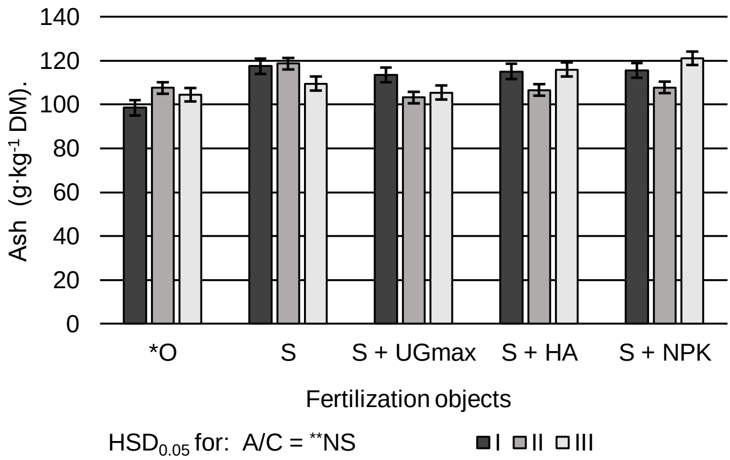

Figure 3. The effect of treatment on crude ash concentration in *Festulolium braunii* (g kg^{-1} DM) across harvests.

*O – control object (without fertilization); S – slurry; S +UGmax – slurry + UGmax soil conditioner; S + HA – slurry + Humus Active soil conditioner; S + NPK – slurry + mineral NPK fertilizers. **NS – not significant; I – standard deviation.

Another ingredient in the assessment of grass nutritional value is crude ash. Its adequate quantity in feed is necessary for the normal growth and development of animal organisms. According to Nazaruk et al. (2009) when crude ash concentration is too high, above 150 g kg^{-1} DM, it may mean that plant material is contaminated with soil. The results of the present experiment indicate that crude ash content of *Festulolium braunii* (the average of the treatments) was 109.5 g kg^{-1} in the first year and 111.8 g kg^{-1} in the second and did not exceed the above limit, which shows the purity of the material analysed.

In the present experiment the analysis of the content of this parameter did not demonstrate significant interaction between slurry and soil conditioners and slurry and mineral fertilizer, compared to the effect of slurry applied on its own or to control plots. This could be due to the fairly high content of absorbable forms of minerals in the soil.

Fairly high content of assimilable forms of P, K, and Mg in the soil might account for a lack of effect of fertilizers on the content of raw ash in the biomass. In addition, the content of these macronutrients constituted a significant share in raw ash. Contrary to that, Jankowska-Huflejt & Wróbel (2010), Barszczewski et al. (2010) as well as Wróbel et al. (2013) recorded an increase in raw ash content of meadow grass as a result of natural fertilizer application. Sosnowski (2012) found that crude ash concentration of *Festulolium braunii* was 109.6 g kg^{-1} DM, and the use of the UGmax soil conditioner did not affect this value significantly. However, mineral fertilizer significantly increased the content of this ingredient (up to 120.8 g kg^{-1} DM). In the present experiment *Festulolium braunii* crude ash content increased in successive harvests of the first year, just like in the studies carried out by Barszczewski et al. (2010). In the second year of this research there was a reverse trend.

CONCLUSIONS

1. The dry mass yield of *Festulolium braunii* was significantly dependent on the treatment applied and on the harvest. Compared with plants treated with slurry only, its addition to soil conditioners or to mineral fertilizer did not significantly increase the biomass of the grass.

2. Total protein content in grass dry matter was significantly differentiated by the treatments applied, the years of research, and the harvest. Compared to the effect of slurry applied on its own, its interaction with soil conditioners contributed to a reduction in protein content in the biomass. Compared to plots where slurry was applied on its own, a non-statistically significant increase in protein content was recorded in plants treated with liquid manure supplemented with NPK.

3. Crude ash content in the dry matter of *Festulolium braunii* showed a significant variation depending on the treatment. The lack of a clear trend was probably due to the relatively high concentration of absorbable forms of basic minerals in the soil.

4. Compared to plants treated with slurry on its own, the combined use of biological soil conditioners and slurry did not result in an expected increase in the yield of *Festulolium braunii* or in its protein content. At the same time, it was found that the use of slurry with biological soil conditioners did not increase the yield and protein content more than combine application of slurry with NPK mineral fertilizers.

REFERENCES

Adamovies, A, Platace, R., Ivanovs, S. & Gulbe, I. 2019. The efficiency of nitrogen fertilizer on the matter yield of tall fescue and festulolium grown as feedstock for combustion. *Agronomy Research* **17**(6), 2146–2157.

Bac, S., Koźmiński, C. & Rojek, M. 1993. *Agrometeorology*. Warsaw, 32–33 (in Polish).

Barszczewski, J., Wróbel, B. & Jankowska-Huflejt, H. 2011. Economic effect of permanent meadow spreading with the meadow limb. *Water-Environment-Rural Areas* **11**(3), 21–37 (in Polish).

Barszczewski, J., Wróbel, B., Jankowska-Huflejt, H. & Mendra, M. 2010. Impact of various fertilization methods on meadow sward and the quality of silage obtained. *Scientific Notebooks of the College of Agribusiness in Łomża* **46**, 7–16 (in Polish).

Borowiecki, J. 2002a. Productivity of papilionaceous plants and their mixtures with grasses, *Puławski Diary* **130**, 57–63 (in Polish).

Borowiecki, J. 2002b. Yielding of *Festulolium* cultivar Felopa in single-species sowing and mixtures with cocksfoot. *Puławski Diary* **131**, 49–58 (in Polish).

Burns, G.A., Gilliland, T.J., McGilloway, D.A., O'Donovan, M., Lewis, E., Blount, N. & O'Kely, P. 2010. Using NIRS to predict composition characteristics of *Lolium perenne* L. cultivars. *Advanc. Anim. Biosci.* **1**, 321–321.

Christensen, M.L., Hjorth, M. & Keiding, K. 2009. Characterization of pig slurry with reference to flocculation and separation. *Water Research* **43**, 773–783.

Ciepiela, A.G. 2004. Reaction of selected grass species to nitrogen fertilization used in urea solution and in ammonium nitrate. *Scientific Dissertation*, ed. AP Siedlce **76**, pp. (in Polish).

Grygierzec, B. 2012. Content of basic nutrients and fiber fractions in hay from extensively used communities of *Alopecuretum pratensis* and *Holcetum lanati*. *Grassland in Poland* **15**, 53–65 (in Polish).

Grzebisz, W., Cyna, K. & Nestorowicz, B. 2007. Wpływ dodatku wodnej zawiesiny kredy do gnojowicy na straty azotu. *Probl. Agric. Engin.* **1**, 101–105.

Jankowska, J., Ciepiela, A.G. Kolczarek, R. & Jankowski, K. 2008. Impact of the type of mineral fertilizer and nitrogen dose on yield and nutritional value of permanent meadow sward. *Puławski Diary* **147**, 125–138 (in Polish).

Jankowska-Huflejt, H. & Wróbel, B. 2010. Assessment of the impact of manure fertilization on the nutritional value of meadow sward and its suitability for ensiling. *J. Res and Appl. in Agric. Engin.* **55**(3), 133–136 (in Polish).

Jankowska-Huflejt, H., Wróbel, B., Barszczewski, J. & Domański, P.J. 2011. Mowing use of meadows on organic farms. (ed.): *Guide of the Organic Farmer*. Grodziec Śląski, 70–110 (in Polish).

Kall, K., Roosmaa, Ü. & Viiralt, R. 2016. Assesment of the economic value of cattle slurry and biogas ddigestate used on grassland. *Agronomy Research* **14**(1), 54–66.

Kotlarz, A., Stankiewicz, S. & Biel, W. 2010. Botanical and chemical composition of hay from a semi-natural meadow and its nutritional value for horses. *Acta Sci. Pol., Zootechnica* **9**(4), 119–128 (in Polish).

Mazur, Z. & Mokra, O. 2009. The content of macronutrients in natural fertilizers in Poland in the years 2003-2005. *Probem Notebooks of Progress in Agricultural Sciences* **537**, 243–247.

Nazaruk, M., Jankowska-Huflejt, H. & Wróbel, B. 2009. Evaluation of feed nutritional value from permanent pasture on the ecological farms studied. *Water - Environment - Rural Areas* **9**(25), 61–76 (in Polish).

Obraztsov, V., Shchedrina, D. & Kadyrov, S. 2018. *Festulolium* seed production dependence on fertilizer application system. *Agronomy Research* **16**(3), 846–853.

Oenema, O., Bannink, A., Sommer, S.G. & Velthof, G. 2001. Gaseous nitrogen emission from livestock farming system. Nitrogen in the Environment: Sources, *Problems and Management*, Elsevier Science B.V., Amsterdam. 255−289.
Olszewska, M. 2008. Leaf greenness (SPAD) and yield of *Festulolium braunii* (K. Richt.) A. Camus grown in mixtures with legumes depending on multiple nitrogen rates. *Pol. J. Natur. Sci.* **23**(2), 310−325.
Reddersen, B., Fricke, T. & Wachendorf, M. 2012. Influence of NIRS – method on the calibration of N-, ash- and NDF-content of grassland hay and silage. *Grassland Sciences in Europe* **17**, 385–387.
Skowera, B. & Puła, J. 2004. Extreme pluvirothermic conditions in spring in Poland in 1971−2000. *Acta Agroph.* **3**(1), 171−177 (in Polish).
Sosnowski, J. 2012. The value of production, energy and food of *Festulolium braunii* (K. Richt.) A. Camus microbiologically and mineral suppled. *Fragm. Agron.* **29**(2), 115−122.
Sosnowski, J. & Jankowski, K. 2010. The effect of soil fertilizer on the floristic composition and yielding of mixtures of *Festulolium braunii* with meadow limb and hybrid alfalfa. *Grassland in Poland*. **13**, 157−166 (in Polish).
Staniak, M. 2010. Productivity of *Festulolium* mixtures with the red limb under various nitrogen fertilization conditions. *Fragm. Agron.* **27**(1), 151−159 (in Polish).
Staniak, M. 2005. Preliminary research on the yield and chemical composition of *Festulolium braunii* variety Felopa depending on the mowing frequency. *Fragm. Agron.* **88**(4), 116−130 (in Polish).
Systematics of Polish soils. 2011. *Ann. Soil Sci.* **62**(3), pp. 1−193 (in Polish).
Szkutnik, J., Kacorzyk, P. & Szewczyk, W. 2012. Change in total protein content and raw fiber depending on the level of fertilization and the development phase of grasses. *Grassland in Poland* **15**, 185–191 (in Polish).
Tonn, B., Bienvenu, C. & Isselstein, J. 2013. Assessing quantity and quality of grazed forage on multi-species swards. The role of grasslands in a green future. EGF. *Grassland Sciences in Europe* **18**, 82−84.
Wolski, K., Bartmański, A. & Gawęcki, J. 2006. Impact of various methods of meadow renovation using *Festulolium* on botanical composition and sward yield. *Grassland in Poland* **9**, 245−251 (in Polish).
Wróbel, B., Zielińska, K.J. & Fabiszewska, A.U. 2013. Impact of bovine manure fertilization on the quality of sward and its suitability for ensilage. *Probl. Agric. Engin.* **2**(80), 151−164 (in Polish).

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The spatio-temporal trend of rapeseed yields in Ukraine as a marker of agro-economic factors influence

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Abstract. The paper demonstrates the applicability of several statistical methods to the analysis and interpretation of the average rapeseed yield data. It proves our hypothesis that the general trend of rapeseed yield variations in Ukraine during 1991–2017 occurred due to agro-economic and agro-technological factors, which are the determinants of the revealed general trend. The temporal trend of rapeseed yield in most administrative districts can be described by a fourth-degree polynomial, namely, its characteristic points enabled us to describe and interpret the dynamics of rapeseed yields. The absolute term of the polynomial shows the initial conditions of the process, and its mapping allows us to identify the areas with the most favorable soil-climatic conditions for the rapeseed cultivation. Indicators of the maximum rate of growth and decrease of yields are the markers of stability of agro-ecosystems to the external influences. Therefore, the mapping of the maximum rate of decline and increase of yields reveal areas in which yields respond rapidly (increasing / falling) to the changes in agro-economic and agro-technological conditions, as well as areas where yields are more stable and change gradually. Thus, the form of the yield trend is determined by the influence of agro-technological and agro-economic factors, whose contribution to the fluctuation in rapeseed yields varies from 53% to 90%.

Key words: yield, rapeseed, trend, dynamics, variability, agro-economic factors, agro-technological factors.

INTRODUCTION

Rapeseed (*Brassica napus L.*) is an important crop due to its use as a source of vegetable oil for food purposes, as a fuel for biodiesel production and a high-protein animal feed (Takashima et al., 2013). It ranks third by production among oilseeds in the world (Rondanini et al., 2012). Currently, rapeseed is grown in 30 countries on an area of 30 million hectares, with total production up to 6 million tones (Cherevko, 2016).

Improved agronomic practices and advances in breeding led to increasing of rapeseed yields in European countries (Gardner, 1994; Habekotte, 1997). Thus, through the optimization of agronomic measures such as sowing rate, sowing date, irrigation, nutrition, pest and disease control, as well as the congruous cultivar, it was possible to achieve the rape seed yield potential (Diepenbrock, 2000).

Ukraine has favorable soil and climatic conditions for growing winter and spring rapeseeds. In particular, appropriate soil fertility, relevant water and air permeability of soil, optimal rainfall and temperature make it possible to produce yields of up to 4 t ha^{-1} due to the adequate cultivation technology (Babiy, 2015; Harbar et al., 2016). Nevertheless, at present, the average rapeseed yields in Ukraine are below the European ones and little is known about the limiting factors of the productive potential of this crop in the country.

Although global rapeseed yields increase, it is accompanied by a significant spatio-temporal variation (Rondanini et al., 2012). For example, in Europe, more stable yields are characteristic for regions with low average yields (Brown et al., 2019). Low temporal variability of crop yields is advantageous for many reasons including reduced income risk and stability of supplies potentially leading to less instability in food prices (Osborne & Wheeler, 2013). Nevertheless, the crop yields variations in space and time are the reality of agriculture. Annual fluctuation in yields may be caused by differences in factors such as seasonal weather conditions, weed, pest and disease pressures, and relevance of management decisions (Lauzon et al., 2005; Nowosad et al., 2016; Brown et al., 2019). Hence, understanding the mechanisms that determine not only the yield potential but also the yield dynamics is critical to global food security.

Therefore, the crop yields spatio-temporal variability is caused by a large number of factors, which are divided into two groups (Rauner, 1981). The first group includes agro-economic and agro-technological factors: the achievement of genetics and breeding, technology of soil cultivation, provision of fertilizers, plant protection, land reclamation etc. The second group combines environmental factors, which determine significant fluctuations of the average yield in some years (Polevoy et al., 2011). Among the environmental factors, weather and climate are the most influential factors of crop productivity. Thus, it has been shown that the latest trends in climate change can significantly affect crop yields, despite the advancement of agronomic practices (Iizumi & Ramankutty, 2016; Lesk et al., 2016; Leng & Huang, 2017). In particular, extreme changes in temperature and precipitation have a profound effect on oilseed rape yield (Brown et al 2019).

However, in practice, there is often a need to separately assess the degree of impact on the yield of both agro-economic (agro-technological) and ecological factors (Polevoy et al., 2011). This assessment based on the idea of decomposing the yields time series into two components: regular (stationary) and irregular (random) (Rauner, 1981; Chen, 2018). The stationary component determines the general trend of yield change in the analyzed period. The trend quite precisely characterizes the average level of yield, due to the level of technology, economic and natural conditions of the area.

In the paper we advance two hypotheses. Hypothesis 1 is that the time series trend of the rapeseed production in Ukraine during 1991–2017 may be an effect of agro-economic and agro-technological factors. Hypothesis 2 is that the residuals of the time series regression model may be an outcome of agro-ecological factors.

In this study we focus on three interconnected research questions: 1) what was the trend of rapeseed yield in Ukraine during 1991–2017; 2) whether the trend can be considered as a marker of the impact of agro-economic factors; 3) whether the agro-economic component is spatially dependent.

MATERIALS AND METHODS

Data of rapeseed yield

Crop data were obtained from State statistics service of Ukraine (http://www.ukrstat.gov.ua/) and its territorial offices. The time series datasets include average crop yields in 267 administrative districts of the annual yield of rapeseed of 10 regions of Ukraine over 27 years, i.e. 1991–2017. The research area is located in two natural vegetation and climatic zones: the Forest (Polissya) and Forest-steppe zone. The research territory includes 10 administrative regions (Cherkasy, Chernihiv, Khmel'nyts'kyy, Kyiv, L'viv, Rivne, Ternopil', Vinnytsya, Volyn, Zhytomyr) (Fig. 1). Information about the annual yields of rapeseed in Ukraine was obtained from FAO (FAOSTAT, 2018).

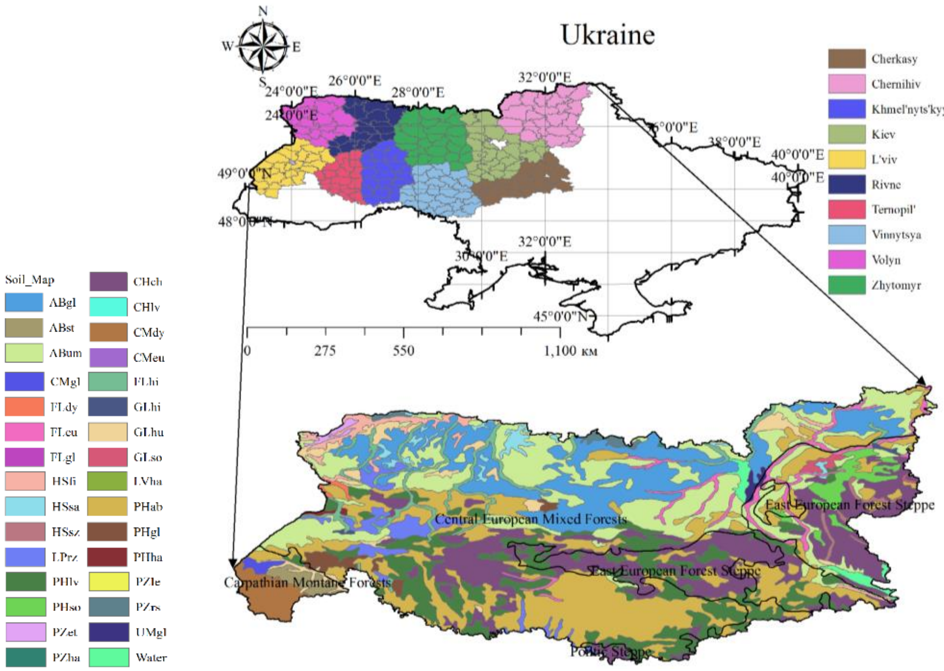

Figure 1. Map of 10 administrative regions in Ukraine, Ecoregions and soil map.

Legend: Soil classification according to World Reference Base for Soil Resources: ABgl – Albeluvisols Gleyic; ABst – Albeluvisols Stagnic; ABum – Albeluvisols Umbric; CHch – Chernozems Chernic; CHlv – Chernozems Luvic; CMdy – Cambisols Dystric; CMeu – Cambisols Eutric; CMgl – Cambisols Gleyic; FLdy – Fluvisols Dystric; FLeu – Fluvisols Eutric; FLgl – Gleyic Fluvisols; FLhi – Fluvisols Histic; GLhi – Gleysols Histic; GLhu – Gleysols Humic; GLso – Gleysols Sodic; HSfi – Histosols Fibric; HSsa – Histosols Sapric; HSsz – Histosols Salic; LPrz – Leptosols Rendzic; LVha – Haplic Luvisols; PHab – Phaeozems Albic; PHgl – Phaeozems Gleyic; PHha – Phaeozems Haplic; PHlv – Phaeozems Luvic; PHso – Phaeozems Sodic; PZet – Podzols Entic; PZha – Podzols Haplic; PZle – Leptic Podzols; PZrs – Podzols Rustic.

Choosing the statistical model that best represents yield trends

As an analytic form of the trend we chose between polynomials of different degree (Ray et al., 2012, Zymaroieva et al., 2019b). Based on the chosen model parameters, crop yield trends may be classified into four main categories (Chen, 2018): increasing, stagnating, collapsed, and never improved. These categories may be considered as a qualitative property of the yield trends.

The Akaike Information Criterion (AIC) developed by Akaike (1974) was used to estimate the likelihood of a statistical model to the observed crop yield data. A good model is the one that has minimum AIC in comparison with all the other models and was chosen as the best representation of the yield trend for a given administrative district. All calculations and data analyses were performed using R v 3.0.2 (R Development Core Team, 2013).

The yield trend within the investigated area can best be described by a fourth-degree polynomial:

$$Y_x = b + a_1x + a_2x^2 + a_3x^3 + a_4x^4 \quad (1)$$

where Y_x – crop yield at the time moment x; b, a_1, a_2, a_3, a_4 – coefficients.

Therefore, in the following analysis phase for the quantitative comparison, the productivity trends in all administrative districts were described by the fourth- degree polynomials. Consequently, we selected the characteristic points of the fourth- degree polynomials: constant, the maximum rate of yields decreases in the range between the first maximum and minimum, the maximum rate of yields increases in the range between minimum and the second maximum (Fig. 2). To quantitatively estimate the special points values, the following calculations are performed.

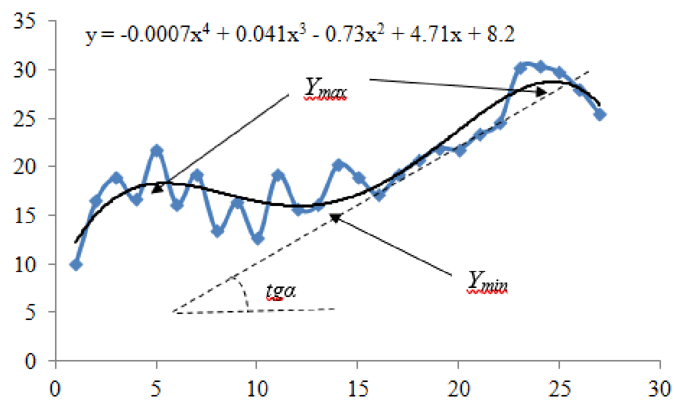

Figure 2. The typical rapeseed yields dynamics during 1991–2017 and approximation of the trend by the fourth-degree polynomial.

Legend: axis of abscissas – time (1–1991, 27–2017); ordinate axis – yields, dt ha^{-1}; b is an absolute term of polynomial equation; YMin – the value of the polynomial at the point of the local minimum; YMax – the value of the polynomial at the points of local maxima; tgα – the maximum rate of increasing crop yields at the time between minimum and maximum, the tangent of the angle of inclination of the tangent to the curve of the polynomial at the inflection point (similar to the maximum rate of yields decline in the downstream branch).

Differentiating the fourth-degree polynomial enables to establish the rate of yields change over time:

$$\mathrm{Y}_x' = a_1 + 2a_2x + 3a_3^2 + 4a_4^3 \quad (2)$$

Points of inflection of the function are where the second derivative is zero:

$$\mathrm{Y}_x'' = 2a_2 + 6a_3 + 12a_4^2 = 0 \quad (3)$$

The corresponding quadratic equation has two roots:

$$x_{1,2} = \frac{-6a_3 \pm \sqrt{36a_3^2 - 96a_4a_2}}{24a_4} \quad (4)$$

Substituting the solutions of Eq. (2) into Eq. (3) we obtain the maximum rates of the decrease and increase of the yields within the study period. These indicators are characteristic of a fourth-degree polynomial. Moreover, the slope of linear regression shows the convergence rate of the approximate trend in the respective statistical model (Marchev et al., 2015). This permits also exploring efficiency and comparison of different statistical models.

A spatial regularity of the crop yields and trend parameters variation were investigated by *I*-Moran statistics (Moran, 1950). The global Moran's statistics were calculated using Geoda095i (http://www.geoda.uiuc.edu/) (Anselin et al., 2005). Spatial database was created in ArcGIS 10.2. The statistical analysis was performed by Statistica 10 software.

RESULTS AND DISCUSSION

Average rapeseed yields in the studied region ranged from 6.9 to 21.9 dt ha^{-1} (Fig. 3, A). The northeastern and southeastern regions are characterized by the lowest yields, and the southeastern regions of the study area have the highest values of yields. The highest values of the rapeseed yield coefficient of variation (45.4–74.3%) are in the northern regions, and the lowest (27.2–39.8%) are in the eastern and southern regions of the study area (Fig. 3, B). The mean of rape yields and its coefficient of variation are spatially structured (Moran *I* statistic 0.51; $p < 0.001$ and 0.28; $p < 0.001$, respectively). There is a negative dependence between the mean of rapeseed yields and the coefficient of variation ($R = -0.77$; $p < 0.001$). In general, the following pattern is observed – areas with a higher rapeseed yield usually have a lower coefficient of variation.

According to FAO data, the average annual rapeseed yield varied from 6.6 (in 1999) to 27.9 dt ha^{-1} (in 2017), with a mean of 15.0 dt ha^{-1} and standard deviation 6.1 during the 27-year period between 1991 and 2017. According to our data, rapeseed yield in 10 regions of the Polissya and Forest-Steppe zones ranged from 9.1 (1996) to 26.48 dt ha^{-1} (2014) during the period of 1991–2017, with the mean 15.6 dt ha^{-1} and standard deviation 5.8. A statistically significant correlation ($r = 0.95$; $p < 0.001$) is observed between the average rapeseed yield in Ukraine and the yield in the research region. It indicates that the temporal dynamics of rapeseed yield variation has the universal pattern for Ukraine.

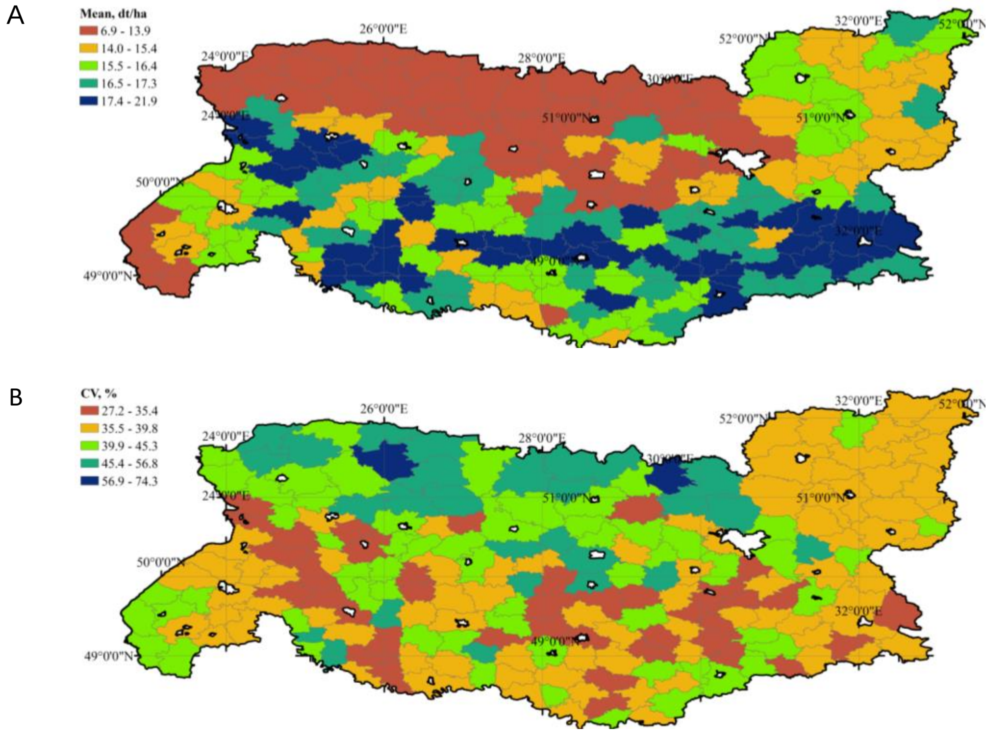

Figure 3. Spatial variation of the mean of rapeseed yield in the studied region of Ukraine (A) and rapeseed yield coefficient of variation (B).

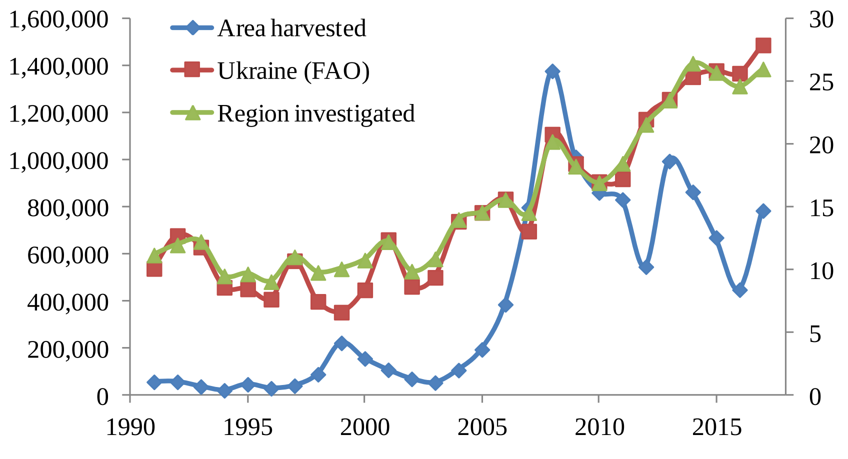

Figure 4. The rapeseed area harvested (ha, left *y*-axis) and yields dynamics during the period from 1991 to 2017 in Ukraine and in region investigated (dt·ha^{-1}, right *y*-axis).

The rapeseed harvested area remained almost unchanged from the early 90s to the middle of 2000s (Fig. 4). It is known that the revival of rapeseed cultivation in Ukraine began only in 1980 and the largest areas under rapeseed were in 1986–1990 (Haidash, 2002; Overchenko & Mishenko, 2007). From our data it is evident that in 2,000 there was a slight increase in the rapeseed harvested area up to 0.22 million hectares (Fig. 4). This tendency is also characteristic for the entire Ukrainian territory, since in 2000 it was planned to plant rapeseed on an area of 0.5 million hectares with the prospect of further expansion to 1.2–1.5 million hectares. However, the growth of acreage in Ukraine in 1990–2000 was much lower than the planned one due mainly to the lack of processing plants and the decrease in the demand for seeds (Haidash, 2002). However, since 2005, there has been a rapid expansion of areas under rapeseed, up to a maximum of 1.4 billion hectares in 2008 (Fig. 4). After 2008, the rapeseed cultivation area has declined slightly, but yields continue to increase, indicating Ukraine's transition to intensive agricultural technologies.

We used the Akaike information criterion to evaluate the suitability of different mathematical models for the explanation of rapeseed yield dynamics in 206 administrative regions of Ukraine. It was found that the overall trend of rapeseed yield in most areas is best described by the cubic function (Fig. 5). Thus, the rapeseed yield has been increasing since the early 2000s, but at present, it has reached its maximum point and started to decline.

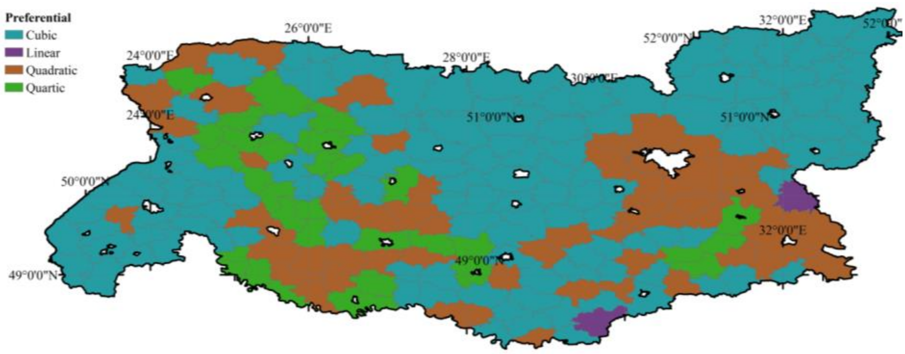

Figure 5. Spatial variations of the yield trend types.

Nevertheless, in this study, we are trying to identify the economic determinants of rapeseed yields. And for this purpose, the fourth-degree polynomial is best suited, since its form is completely in line with the economic cycle that Ukraine has undergone since its independence (Zymaroieva et al., 2019a). Thus, we assume that the rapeseed yield trend is determined by agro-economic factors. Moreover, the fourth-degree polynomial has greater explanatory power and has characteristic points that can be used for meaningful interpretation of rapeseed yield dynamics (Fig. 2).

The trend of rapeseed yields is characterized by two local maximums (Y_{max}) and one local minimum (Y_{min}) (Fig. 2). The local minimum reflects the lowest yields of rapeseed for the entire study period and, in our case, occurred in 2003 and is the result of a protracted socio-economic crisis caused by the collapse of the Soviet Union. The collapse of the Soviet Union caused massive socio-economic and institutional changes, which led to a significant decline in agriculture in Ukraine (Swinnen et al., 2017). The agricultural sectors of the former USSR countries were suddenly faced with increasing international competition, while the spending on agriculture was sharply reduced (Lerman et al., 2004). The rural population left the countryside en masse, the use of fertilizers declined significantly, and agricultural productivity decreased. These events led to a significant decrease in land use and average yield of major crops in the first years of Ukraine's independence (Schaffartzik et al., 2014; Swinnen et al., 2017). Nevertheless, agrosystems have some ‘inertia’ in the face of changing economic conditions, so even after the collapse of the Soviet Union in 1991, for a few more years the yields of main crops will remain almost unchanged, which is related to a previously well-planned agricultural system. Since the mid-1990s the economic crisis has reached Ukraine's agriculture and led to a rapid decline in yields to a minimum in Early 00s (Zymaroieva et al., 2019b).

The local maximums took place in 1993 and 2015 and correspond to the highest rapeseed productivity over the study period. The existence of a local maximum in 1993 was due to a sharp decline in the coming years, and the local maximum in 2015 is explained only by the fact that it is unclear how the rapeseed yield will behave in the coming years, whether there will be a plateau or whether there will be further growth. Since the local maximums are located in points, which close to the edges of the study period range, their exact determination seems doubtful. In many cases, the real maximums are beyond the study period. Therefore, we do not use the value of the function in local maxima as characteristic indicators of rapeseed yield dynamics.

The constant term of polynomial equation (constant *b*) indicates the productivity of rapeseed in the starting period. The constant *b* reflects the yield potential at the initial period of research and it is an independent parameter of the temporary dynamics of rapeseed yields variability in time (Zymaroieva et al., 2019b). The starting level of rapeseed yield in the research region ranged from 5.2 to 20.1 dt ha^{-1} (Fig. 6).

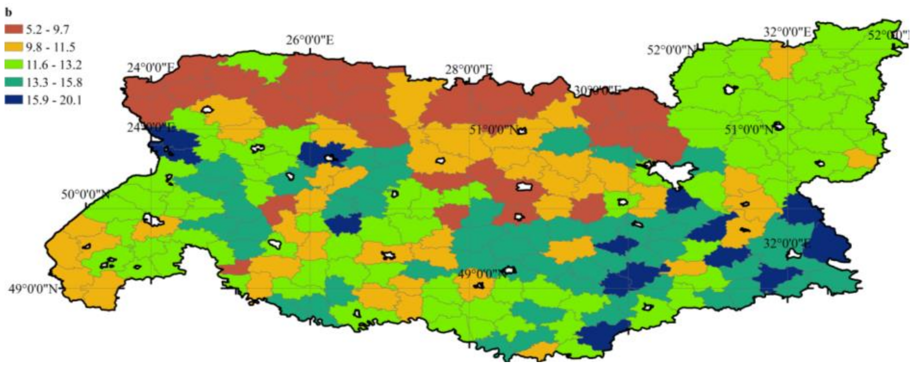

Figure 6. Spatial variations in the level of rapeseed yield in the initial period of research (constant *b* of the regression equation).

Areas with low values of the starting levels are situated in the north and with high values are in the southeast of the study area. The mean yield for the study period and the initial yield (coefficient b of the polynomial equation) are significantly positively correlated ($r = 0.98$; $p < 0.001$). This explains the fact that the variation of the coefficient b values (Fig. 6) is spatially dependent, that was confirmed by Moran test (Moran's *I*-statistic 0.34; $p < 0.001$). The values of this indicator identify the areas with the most favorable conditions for rapeseed cultivation.

From the agro-ecological point of view, Ukrainian Polissya and the Forest-Steppe zone have the most favorable soil and climatic conditions for growing winter and spring rapeseed (Barankova, 2007). According to our research, the highest potential for rapeseed cultivation have Chernihiv, Cherkasy and Vinnytsia regions, which is confirmed by indicators of the starting and average yields.

Indicators of the maximum rate of yield decline and the maximum rate of yield increase can be used as markers of the agro-ecosystem stability to external factors. The rate of rapeseed yield decline (Fig. 7) was not spatially dependent (*I*-Moran statistic 0.02; $p = 0.27$). The initial yield level correlates with the maximum rate of rapeseed yields decline in the first phase of research ($r = –0.14$; $p = 0.03$). Consequently, a higher rate of the starting yield corresponds to a more stable crop yield under adverse conditions.

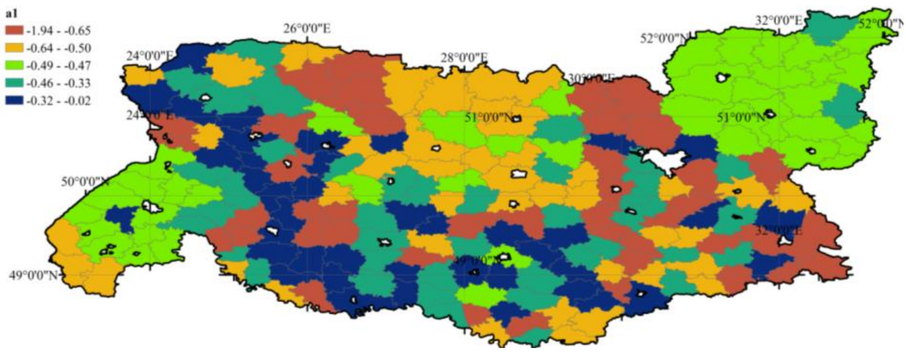

Figure 7. Spatial variation of the maximum rate of yields decline.

The rate of yield recovery was proportional to the intensity of the previous yield decline ($r = 0.25$; $p < 0.001$), indicating that these parameters are interrelated. The intensity of yield growth (Fig. 8) is spatially dependent (Moran *I*-statistic 0.21; $p < 0.001$).

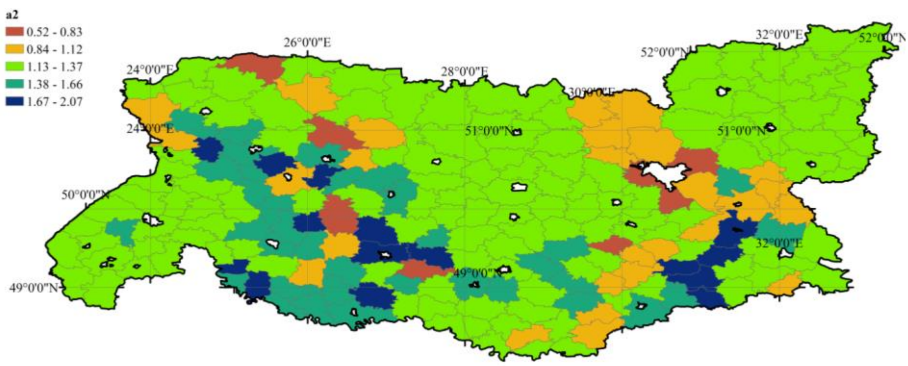

Figure 8. Spatial variations of the maximum rate of yields increase.

The presence of a trend in rapeseed yields, the mathematical form of which is unchanged within different regions of Ukraine, indicates the presence of constant external factors that affected the yield dynamics. The only factor that simultaneously influences the yields of rapeseed on the entire territory of Ukraine is of economic nature, which determines the development of agro-technology (breeding) and, as a result, affects the yields. Moreover, the fourth-degree polynomial describes the yield dynamics of other crops (soybean, rye, grain) in Ukraine at the same period (Kunah et al., 2018, Zymaroieva et al., 2019a), which confirms our theory of the universality of the trend determinant and its economic origin.

The coefficient of determination indicates the level of compliance of the chosen trend with the actual data. Since the yield trend mostly has an agro-economic origin, the determination coefficient can be interpreted as an indicator of the contribution of the agro-technological and agro-economic factors to the rapeseed yield dynamics. The values of the determination coefficient indicate that these factors explain 59–97% of the temporal variation in rapeseed yields (Fig. 9). Thus, the impact of these factors on the productivity of rapeseed in some areas is crucial.

The coefficient of variation is spatially determined (Moran *I*-statistic 0.36; $p < 0.001$), which indicates that the impact of agro-economic (agro-technological) factors has regional differences.

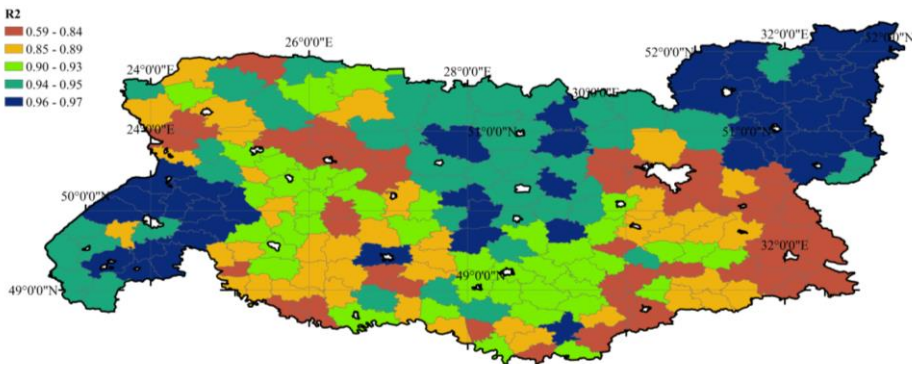

Figure 9. Spatial variation of the determination coefficient of the forth-degree regression model.

Agro-economic factors of the yield trend formation are complex. Differences in both technological investment and agro-technology, such as plant protection, sowing and fertilizer application, can lead to differences in yield as well (Annicchiarico & Iannucci, 2008). A large number of studies have analyzed the extent of crop yield spatial variation according to the types of cultivation, nitrogen fertilization, and soil type and fertility (Aydinalp & Cresser, 2008; Chen et al., 2014; Chen, 2018). However, no such studies have been conducted in Ukraine. Unfortunately, it is not possible to determine what specific agro-economic and agro-technological factors caused the rapeseed yield(s) fluctuation, since accurate accounting of economic conditions was not carried out because Ukraine was in deep crisis during 1990–2000. We have tested and proved the hypothesis that due to the yield trend, we can separate the economic component of yield variation from environmental ones.

Ukraine is considered to belong to countries with relatively large untapped yield potential of many crops (Schierhorn et al., 2013; Ryabchenko & Nonhebel, 2016), including rapeseed (Schaffartzik et al., 2014). In particular, Deppermann et al., 2017 found that oilseeds (rapeseed, soybean, and sunflower) total production could increase by 84% to 50 million tons in Ukraine. However, the reasons for the rapeseed yield gap in Ukraine are uncertain.

Studies focused on the spatial and temporal variability of crop yields are quite important both in terms of forecasting (simulation) models and in terms of farmland management. Therefore, the implications of the research should be reflected in the national agricultural policy, as well as in regional crop management. We consider that agro-economic factors were the major determinants of spatial variability of rapeseed yield in 1991–2017. At the current stage of development of agricultural production in Ukraine, environmental factors, such as climate change, may have a greater impact on rapeseed yield. Investigation of the environmental factors influence on the rapeseed yield variations will be the aim of our further research.

CONCLUSIONS

Our study shows that the trend of rapeseed yield within the study area is best described by a fourth-degree polynomial. The existence of this type of trend is due to the influence of agro-economic factors, whose contribution to the overall variation in yield ranges from 59 to 97%. The impact of agro-economic (agro-technological) factors has regional differences. We also indicated the areas with favorable soil and climate conditions for rapeseed cultivation, which spatially coincide with the regions with the highest rapeseed yields. Indicators of the maximum rate of yield decline and maximum rate of yield increase are the markers of agro-ecosystems resilience to the external factors, in particular, agro-technological and agro-economic ones. Regions with higher levels of yields in the initial period are more stable in the transitional economic period. Besides, the yields in areas with favorable soil and climatic conditions for rape cultivation are less dependent on the influence of agro-economic and agro-technological factors. Therefore, Ukraine has all the prerequisites for increasing yield potential of rapeseed, especially in the areas where the impact of agro-technology and breeding is crucial.

REFERENCES

Annicchiarico, P. & Iannucci, A. 2008. Breeding Strategy for Faba Bean in Southern Europe based on Cultivar Responses across Climatically Contrasting Environments. *Crop Science* **48**(3), 983–991. doi: 10.2135/cropsci2007.09.0501

Anselin, L., Ibnu, S. & Youngihn, Kh. 2005. GeoDa: An Introduction to Spatial Data Analysis. *Geographical Analysis* **38**(1), 5–22. doi: https://doi.org/10.1111/j.0016-7363.2005.00671.x

Aydinalp, C. & Cresser, M.S. 2008. The effects of global climate change on agriculture. American-Eurasian *Journal of Agricultural and Environmental Sciences* **3**, 672–676.

Babiy, S. 2015. Key aspects of the rape selection in the present. *Agribusiness Today* **14**(309), 34–37 (in Ukrainian).

Barankova, N. 2007. Rapeseed cultivation in Ukraine and ways of its development. *Siverianskyi litopys* **6**, 171–174 (in Ukrainian).

Brown, J., Beeby, R. & Penfield, S. 2019. Yield instability of winter oilseed rape modulated by early winter temperature. *Scientific Reports* **9**. doi: 10.1038/s41598-019-43461-7

Chen, H., Yamagishi, J. & Kishino, H. 2014. Bayesian Inference of baseline fertility and treatment effects via a crop yield-fertility model. *PLoS ONE* **9**, e112785.10.1371/journal.pone.0112785

Chen, H. 2018 The spatial patterns in long-term temporal trends of three major crops' yields in Japan. *Plant Production Science* **21**(3), 177–85. doi: https://doi.org/10.1080/1343943X.2018.1459752

Cherevko, G. 2016. Rapeseed Growing for Energy Purposes in Ukraine. *Roczniki Naukowe Stowarzyszenia Ekonomistów Rolnictwa i Agrobiznesu* **18**(3), 29–34.

Deppermann, A., Balkovic, J., Bundle, S.C., Di Fulvio, F., Havlik, P., Leclere, D., Lesiv, M., Prishchepov, A.V. & Schepaschenko, D. 2018. Increasing crop production in Russia and Ukraine-regional and global impacts from intensification and recultivation. *Environmental Research Letters* **13**(2), 025008. doi: 10.1088/1748-9326/aaa4a4

Diepenbrock, W. 2000. Yield analysis of winter oilseed rape (*Brassica napus L.*): A review. *Field Crops Research* **67**, 35–49. doi: 10.1016/S0378-4290(00)00082-4

FAOSTAT. 2018. Food and Agriculture Organization of the United Nations. FAOSTAT. www.fao.org [Accessed 5 December 2018].

Gardner, B. 1994. *EU oilseeds policy, production and the world market 1994-2000*. Agra Europe Special Report No. **76**. London, AGRA Europe, 97 pp.

Iizumi, T. & Ramankutty, N. 2016. Changes in yield variability of major crops for 1981-2010 explained by climate change. *Environmental Research Letters* **11(**3), 034003. doi: 10.1088/1748-9326/11/3/034003

Habekotte´, B. 1997. Evaluation of seed yield determining factors of winter oilseed rape *(Brassica napus L.)* by means of crop growth modelling. *Field Crops Research* **54**(2–3), 137–151.

Haidash, V. 2002. Rape: its current state and prospects in Ukraine. *Propozytsiia* **8–9**, 50–51 (in Ukrainian).

Harbar, L.A., Antal, T.V. & Romanov, S.M. 2016. Winter rapeseed productivity under the influence of foliar fertilizers. *Visnyk ZhNAEU* **2**(56), 113–119 (in Ukrainian).

Kunah, O.M., Pakhomov, O.Y., Zymaroieva, A.A., Demchuk, N.I., Skupskyi, R.M., Bezuhla, L.S. & Vladyka, Y.P. 2018. Agroeconomic and agroecological aspects of spatial variation of rye (Secale cereale) yields within Polesia and the Forest-Steppe zone of Ukraine: The usage of geographically weighted principal components analysis. *Biosystems Diversity* **26**(4), 276–285. doi: https://doi.org/10.15421/011842

Lauzon, J.D., Fallow, D.J., O'Halloran, O.P., Gregory, S.D.L. & Bertoldi, A.P. 2005. Assessing the temporal stability of spatial patterns in crop yields using combine yield monitor data. *Canadian Journal of Soil Science* **85**(3), 439–451, https://doi.org/10.4141/S04-067

Leng, G & Huang, M. 2017. Crop yield response to climate change varies with crop spatial distribution pattern. *Scientific Reports* **7**, 1463 10.1038/s41598-017-01599-2

Lerman, Z, Csaki, C. & Feder, G. 2004. *Agriculture in Transition: Land Policies and Evolving Farm Structures in Post-Soviet Countries*. Lexington Books, Oxford, 176 pp.

Lesk, C., Rowhani, P. & Ramakutty, N. 2016. Influence of extreme weather disasters on global crop production. *Nature* **529**, 84–87. doi: 10.1038/nature16467

Marchev, A., Malechkova, A., Milev, M., Kabaivanov, S., Markovska, V. & Nikolova, Kr. 2015. A Step Beyond the Monte Carlo Method in Economics. In: Application of *Multivariate Normal Distribution, AIP Conference Proceedings*, 8-13 June 2015, vol. **1690**, 65–73.

Nowosad, K., Liersch, A., Poplawska, W. & Bocianowski, J. 2016. Genotype by environment interaction for seed yield in rapeseed (*Brassica napus* L.) using additive main effects and multiplicative interaction model. Euphytica 208, 187–194. doi: 10.1007/s10681-015-1620-z

Osborne, T.M. & Wheeler, T.R. 2013. Evidence for a climate signal in trends of global crop yield variability over the past 50 years. *Environ. Res. Lett*. **8**, 1–9. doi:10.1088/1748-9326/8/2/024001

Overchenko, B.P. & Mishchenko, N.M. 2007. Prospects for development of rapeseed farming and problems of biodiesel production in Ukraine. *Economics and forecasting* **3**, 75–98 (in Ukrainian).

Polevoy, A., Bozko, L. & Dronova, E. 2011. The area spatio temporal variability of winter wheat harvests in Ukraine. *Ukrainian Hydrometeorological Journal* **8**, 84–91 (in Ukrainian).
Rauner, Y.L. 1981. *Climate and grain yield*. Moskov, Nauka, 163 pp. (in Russian).
Ray, D.K., Ramankutty, N., Mueller, N.D., West, P.C. & Foley, J.A. 2012. Recent patterns of crop yield growth and stagnation. *Nature Communications* **3**, 1293. doi: https://doi.org/10.1038/ncomms2296
Rondanini, D., Gómez, N., Agostib, M. & Miralles, D. 2012. Global trends of rapeseed grain yield stability and rapeseed-to-wheat yield ratio in the last four decades. *European Journal of Agronomy* **37**(1), 56–65. doi: 10.1016/j.eja.2011.10.005
Ryabchenko, O. & Nonhebel, S. 2016. Assessing wheat production futures in the Ukraine. *Outlook on Agriculture* **45**(3), 165–172. https://doi.org/10.1177/0030727016664159
Schaffartzik, A., Plank, C. & Brad, A. 2014. Ukraine and the great biofuel potential? A political material flow analysis. *Ecological Economics* **104**, 12–21.
Schierhorn, F., Muller, D., Beringer, T., Prishchepov, A.V., Kuemmerle, T. & Balmann, A. 2013. Post-Soviet cropland abandonment and carbon sequestration in European Russia, Ukraine, and Belarus. *Global Biogeochemical Cycles* **27**, 1175–85.
Swinnen, J., Burkitbayeva, S., Schierhorn, F., Prishchepov, AV. & Muller, D. 2017. Production potential in the "bread baskets" of Eastern Europe and Central Asia. *Global Food Security-Agriculture Policy Economics and Environment* **14**, 38–53. doi: 10.1016/j.gfs.2017.03.005
Takashima, N., Rondanini, D., Puhl, L. & Miralles, D. 2013. Environmental factors affecting yield variability in spring and winter rapeseed genotypes cultivated in the southeastern Argentine Pampas. *European Journal of Agronomy* **48**, 88–100. 10.1016/j.eja.2013.01.008
Zymaroieva, A., Zhukov, O., Fedonyuk, T. & Pinkin, A. 2019a. Application of geographically weighted principal components analysis based on soybean yield spatial variation for agro-ecological zoning of the territory. *Agronomy Research* **17**(6), 2460–2473. https://doi.org/10.15159/AR.19.208
Zymaroieva, A., Zhukov O., Romanchuck, L. & Pinkin, A. 2019b. Spatiotemporal dynamics of cereals grains and grain legumes yield in Ukraine. *Bulgarian Journal of Agricultural Science* **25**(6), 1107–1113.

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