

# Agronomy Research

Established in 2003 by the Faculty of Agronomy, Estonian Agricultural University

## **Aims and Scope:**

*Agronomy Research* is a peer-reviewed international Journal intended for publication of broad-spectrum original articles, reviews and short communications on actual problems of modern biosystems engineering incl. crop and animal science, genetics, economics, farm- and production engineering, environmental aspects, agro-ecology, renewable energy and bioenergy etc. in the temperate regions of the world.

## **Copyright:**

Copyright 2009 by Estonian University of Life Sciences, Latvia University of Life Sciences and Technologies, Aleksandras Stulginskis University, Lithuanian Research Centre for Agriculture and Forestry. No part of this publication may be reproduced or transmitted in any form, or by any means, electronic or mechanical, incl. photocopying, electronic recording, or otherwise without the prior written permission from the Estonian University of Life Sciences, Latvia University of Life Sciences and Technologies, Aleksandras Stulginskis University, Lithuanian Research Centre for Agriculture and Forestry.

## ***Agronomy Research* online:**

*Agronomy Research* is available online at: <http://agronomy.emu.ee/>

## **Acknowledgement to Referees:**

The Editors of *Agronomy Research* would like to thank the many scientists who gave so generously of their time and expertise to referee papers submitted to the Journal.

## **Abstracted and indexed:**

SCOPUS, EBSCO, CABI Full Paper and Thompson Scientific database: (Zoological Records, Biological Abstracts and Biosis Previews, AGRIS, ISPI, CAB Abstracts, AGRICOLA (NAL; USA), VINITI, INIST-PASCAL.)

## **Subscription information:**

Institute of Technology, EULS  
St. Kreutzwaldi 56, 51014 Tartu, ESTONIA  
E-mail: [timo.kikas@emu.ee](mailto:timo.kikas@emu.ee)

## **Journal Policies:**

Estonian University of Life Sciences, Estonian Research Institute of Agriculture, Latvia University of Life Sciences and Technologies, Aleksandras Stulginskis University, Lithuanian Institute of Agriculture and Lithuanian Institute of Horticulture and Editors of *Agronomy Research* assume no responsibility for views, statements and opinions expressed by contributors. Any reference to a pesticide, fertiliser, cultivar or other commercial or proprietary product does not constitute a recommendation or an endorsement of its use by the author(s), their institution or any person connected with preparation, publication or distribution of this Journal.

**ISSN 1406-894X**

# CONTENTS

## **A. Aboltins, J. Tomsu and P. Kic**

Red clover drying coefficient dependences on air velocity at constant drying temperature .....313

## **R.R. Andrade, I.F.F. Tinôco, C.F. Souza, K.P. Oliveira, M. Barbari, V.M.F. Cruz, F.J.F. Baptista, M.O. Vilela, L. Conti and G. Rossi**

Effect of thermal environment on body temperature of early-stage laying hens .....320

## **E. Aplocina**

The role of field beans in nutrition of Boer goat .....328

## **S. Benlahrech, G. Kaci, M. Teffahi and S.M. Ounane**

Influence of inoculation and phosphorus regimes on symbiotic nitrogen fixation and phosphorus use efficiency of Algerian cowpea (*Vigna unguiculata* L. (Walp.)) landraces .....337

## **V. Bulgakov, V. Bandura, M. Arak and J. Olt**

Intensification of rapeseed drying process through the use of infrared emitters .....349

## **D. Čepauskienė, N. Pedišius and D. Milčius**

Chemical composition of agromass ash and its influence on ash melting characteristics .....357

## **V. Chiteculo, A. Brunerová, P. Surový and M. Brožek**

Management of Brazilian hardwood species (Jatoba and Garapa) wood waste biomass utilization for energy production purposes .....365

## **A. Cristobal-Sarramian and D. Atzmüller**

Yeast as a production platform in biorefineries: conversion of agricultural residues into value-added products .....377

## **L. Degola and D. Jonkus**

The influence of dietary inclusion of peas, faba bean and lupin as a replacement for soybean meal on pig performance and carcass traits .....389

<b>A. Gruduls, K. Balina, K. Ivanovs and F. Romagnoli</b>	
Low temperature BMP tests using fish waste from invasive Round goby of the Baltic Sea .....	398
<b>V. Hartová and J. Hart</b>	
Improvement of monitoring of cattle in outdoor enclosure using IQRF technology .....	410
<b>T. Jehlička and J. Sander</b>	
Differential thermal regulation of the growth of the bee colonies in the early spring period.....	416
<b>J. Jobbágy, K. Krištof, A. Schmidt, M. Križan and O. Urbanovičová</b>	
Evaluation of the mechanized harvest of grapes with regards to harvest losses and economical aspects .....	426
<b>M.A. Kamberi, S. Muji, A. Kryeziu, R. Kastrati and N. Mestani</b>	
The excretion of Ca, Mg, Zn and Cu via excreta of laying hens fed low phosphorus diets and phytase.....	443
<b>A. Karlsons, A. Osvalde, G. Čekstere and J. Pormale</b>	
Research on the mineral composition of cultivated and wild blueberries and cranberries .....	454
<b>V. Komasilovs, A. Zacepins, A. Kvišis, A. Nasirahmadi and B. Sturm</b>	
Solution for remote real-time visual expertise of agricultural objects .....	464
<b>M. Križan, K. Krištof, M. Angelovič, J. Jobbágy and O. Urbanovičová</b>	
Energy potential of densified biomass from maize straw in form of pellets and briquettes .....	474
<b>A.J. Kryeziu, N. Mestani, Sh. Berisha and M.A. Kamberi</b>	
The European performance indicators of broiler chickens as influenced by stocking density and sex.....	483
<b>J. Lev and J. Blahovec</b>	
Effect of I <sub>2</sub> /KI water solution to wheat seeds imbibition assessed by image analysis.....	492
<b>L. Litke, Z. Gaile and A. Ruža</b>	
Effect of nitrogen fertilization on winter wheat yield and yield quality .....	500

<b>K. Loit, L. Soonvald, M. Kukk, A. Astover, E. Runno-Paurson, T. Kaart and M. Öpik</b>	
The indigenous arbuscular mycorrhizal fungal colonisation potential in potato roots is affected by agricultural treatments .....	510
<b>M. Macák, V. Rataj, M. Barát and J. Galambošová</b>	
Comparison of two sowing systems for CTF using commercially available machinery .....	523
<b>J. Malat'ák, J. Velebil and J. Bradna</b>	
Specialty types of waste paper as an energetic commodity .....	534
<b>M. Mimra, M. Kavka and P. Markytán</b>	
Evaluation of break-even point and gross margin economic risks in producing winter oilseed rape .....	543
<b>K.P. Oliveira, C.F.S. Ferreira, I.F.F. Tinoco, R.R. Andrade, M. Barbari, V.M.F. Cruz, F.J.F. Baptista, M.F.A. Vieira, L. Conti and G. Rossi</b>	
Productive performance of broilers at the final stage of breeding submitted to different levels of metabolizable energy in different thermal environments .....	556
<b>G. Rossi, L. Conti, K. Al-Fartosi and M. Barbari</b>	
Implementation of practical solutions to improve buffalo breeding development in rural areas of South Iraq.....	564
<b>G. Rossi, L. Conti, G. Bambi, M. Monti and M. Barbari</b>	
Poultry farming solutions for a sustainable development of marshlands areas of South Iraq.....	574
<b>F.C. Sousa, I.F.F. Tinôco, M. Barbari, F. Baptista, C.F. Souza, A.O. Saraz, D.J.R. Coelho and A.L. Silva</b>	
Diagnosis of air quality in broilers production facilities in hot climates .....	582
<b>I. Vitázek, J. Tulík and J. Klůčik</b>	
Combustible in selected biofuels.....	593
<b>T. Yezekyan, F. Marinello, G. Armentano and L. Sartori</b>	
Analysis of cost and performances of agricultural machinery: reference model for sprayers.....	604



## Red clover drying coefficient dependences on air velocity at constant drying temperature

A. Aboltins<sup>1,\*</sup>, J. Tomsu<sup>2</sup> and P. Kic<sup>2</sup>

<sup>1</sup>Latvia University of Life Sciences and Technologies, Institute of Agricultural Machinery, Cakstes blvd.5, LV–3001 Jelgava, Latvia

<sup>2</sup>Czech University of Life Sciences Prague, Faculty of Engineering, Kamycka 129, CZ16500 Prague 6, Czech Republic

\*Correspondence: aivars.aboltins@inbox.lv

**Abstract.** The clover is widely used as feed of animals and preservation of fodder is an important problem. This paper informs about the experimental and theoretical investigations of red clover (*Trifolium pratense*) drying by forced convection. This research is focused on verification of influence of different air velocities on the drying process of red clover in thin layer in mild temperatures (22 °C) and rather high relative humidity of air (70%). The initial moisture content of clover was determined by gravimetric method using the hot air drying in the electric oven. Special device for convection drying with air flow passing through material from the bottom through supporting trays with a sieve (dimensions of mesh 3 x 4 mm) by constant temperature was used for drying when the air velocity 0.7 m s<sup>-1</sup>, 1.0 m s<sup>-1</sup>, 1.2 m s<sup>-1</sup> and 2.0 m s<sup>-1</sup>. These results was compared with drying by free convection. Changes of clover samples were determined from the measured values of weight using the gravimetric method. The function of drying coefficient  $K(t)$  is determined (using thin layer theory) and theoretical results are compared with experimental results. Using experimental results were determined relationships between air velocity and parameters included in function of drying coefficient. This allows theoretically to predict the drying process depending on the air velocity.

**Key words:** clover, conservation, drying theory, fodder, forced drying, natural drying.

### INTRODUCTION

Red clover (*Trifolium pratense*) is important forage plant grown mostly on temperate climate throughout the world. This kind of plant is easily adaptable to many natural factors as soil type, climate conditions and others. Last but not the least reason is that is excellent nitrogen fixer and also is free from many disease and insect pests, has versatile uses, and is suitable for use in crop rotations. Mostly is used for silage, hay, haylage and is especially valuable in adding nitrogen and organic matter to the soil (Riday, 2010).

Nowadays red clover improves agro-ecosystem. The main idea is that red clover is used as under-seeding plant to wheat or corn in the system strip-tillage. This results in improvements as reduction of erosion, runoff and leaching; soil temperature and moisture regulation; weed suppression; interruption of pest and disease cycles and others (Wyngaarden et al., 2015).

One of the best way to process red clover is haylage or silage system. The next popular way how to process this plant is into round bales. During drying matter of hay, it could be problem with moisture and rain. These problems can be associated with large losses and other losses can arise if we store insufficiently dried hay. For decrease drying time it should be used a mower-conditioner for cut (Rayburn, 2002). Hay bales losses are functions of hay moisture, temperature and how long the hay is exposed to these conditions (Holmes, 2004).

To develop recommendations for cutting fodder crops and drying, based on forecasts weather conditions, it can be useful a mathematical model. Important problems for future conservation of crops are related to the energy saving in drying process (Jokiniemi, et al., 2012).

A drying model can estimate the moisture content of fodder crops as a function of time for given weather conditions. Predictive model and uses the air temperature, wind speed, global radiation is shown (Atzema, 1992). The drying process of harvested grass was evaluated using numerical approaches in (Bartzanas et al., 2010).

The drying of lemon grass plant (*Cymbopogon citratus Stapf*) at different air temperatures and drying speed is described in (Coradi et al., 2014), thin-layer total drying time of some herbal leaves at different drying temperatures and velocities are determined by (Kaya & Aydin, 2009). The results of low temperature drying of hops are shown in (Hermanek et al., 2017 and Rybka et al., 2017).

The aim of this study is to determine the function of drying coefficient  $K(t)$  and to compare the theoretical results with the results of experimental measurement. The measurements were made for five air velocities, and for each situation a suitable mathematical representation of the course of the equation was found.

## MATERIALS AND METHODS

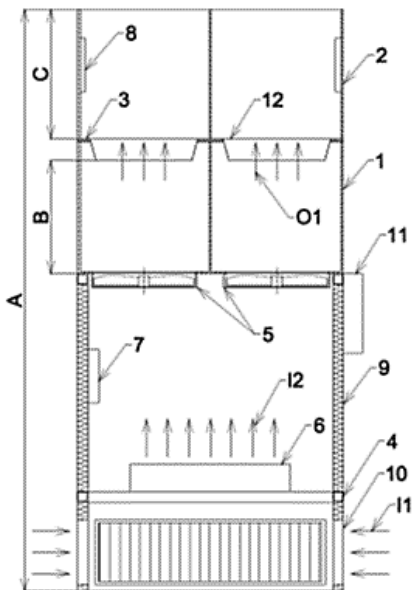
In July and August 2016, red clover drying was carried out at the Faculty of Engineering of the Czech University of Life Sciences Prague. Fig. 1 shown that for the laboratory measurements were used own design of convection system. The system is consisted from four vertical drying chambers in each of which air flows at different velocity. The velocities for each chambers were  $0.7 \text{ m s}^{-1}$ ,  $1 \text{ m s}^{-1}$ ,  $1.2 \text{ m s}^{-1}$  and  $2.0 \text{ m s}^{-1}$ . The results were compared with natural convection drying by the same temperature, but with the  $0.0 \text{ m s}^{-1}$  air velocity.

Each chamber is independent and allows for a different flow rates of air velocities. Samples for each measurement were inserted into chambers on sieve tray with mesh  $3 \times 4 \text{ mm}$  of total area approximately  $204 \text{ cm}^2$ . The airflow for drying of sample is delivered by the fan of diameter  $120 \text{ mm}$  and controlled by fan revolutions.

Red clover was cut up into pieces of  $20\text{--}50 \text{ mm}$  length. Sample of an approximate initial weight of  $45 \text{ g}$  was placed on every tray. On the sieves was placed only layer just about  $50 \text{ mm}$  which is important for our research because it is assumed that there is no moisture sharing between the layers of material.

For measured air velocity was used equipment: Anemometer CFM 8901 Master (Hygrotec Messentechnik GmbH, Germany) with resolution  $0.01 \text{ m s}^{-1}$  and accuracy  $\pm 2\%$  of final value. The sensor FHA646-E1C was used for measure humidity and air temperature. This sensor sent data to the data logger ALMEMO 2690-8 (Ahlaborn GmbH,

Germany). The measured average temperature of drying air was  $22 \pm 0.2$  °C and relative humidity  $70 \pm 0.4\%$ .



**Figure 1.** Apparatus used for drying: 1 – lower drying chamber; 2 – upper drying chamber; 3 – underlay; 4 – structure; 5 – fans; 6 – air heating; 7 – sensors; 8 – sensors; 9 – thermal insulation; 10 – inlet air; 11 – control panel; 12 – perforated tray with measured material; I1 – inlet of fresh air; O1 – air passing through perforated tray with measured material; A – overall height; B – height of lower chamber; C – height of upper chamber.

The total drying time was 164.5 h. After this time was achieved the lowest moisture content for the convective drying. During the first 0.5 hour of drying were samples measured every 10 min, the next until the 1.5 hours every 15 min, the next 2 hours every 30 min and then every 60 min. For measuring weight in regular intervals was used the laboratory weight KERN-440-35N (KERN and SOHN GmbH, Germany) with maximum load weight 400 g and with resolution 0.01 g.

The dry matter (DM) content in red clover was detected by gravimetric measurement using an MEMERT UNB-200 (MEMMERT GmbH + Co. KG, Germany) air oven under temperature 105 °C. During regular time intervals samples were weighed on a Kern 440-35N laboratory balance. The total drying time was adjusted to the need for a determination of the equilibrium moisture.

## RESULTS AND DISCUSSION

The experiment at red clover drying was made in the laboratory. Based on the results of the measurements there are calculated water loses and dry weight of original sample volume for each air velocities. The partial goal is to determine the drying coefficient  $K(t)$  that obtained by fitting the values of each measurement into the Eq. (1).

$$K(t) = \frac{-\ln \left| \frac{m - m_d}{m_s - m_d} \right|}{t}, \quad (1)$$

where  $K(t)$  – drying coefficient;  $m$  – weight of samples during drying, g;  $m_d$  – weight of dry matter, g;  $m_s$  – samples weight at the beginning of drying, g;  $t$  – time, h.

According (Aboltins, 2013) drying coefficient  $K(t)$  values are determined and approximated. For the best approximation of this data is chosen function (2).

$$K(t) = at^b, \tag{2}$$

where  $a, b$  – constants that characterize experimental conditions.

For each drying air velocity are determined  $a$  and  $b$  values from (2). These results are shown in the Table 1.

**Table 1.** The coefficients  $a, b$  values dependence from air velocity

Air velocity, $m\ s^{-1}$	$a$	$b$	$R^2$	
$v_1$	0.70	0.1446	-0.2522	0.9700
$v_2$	1.00	0.1536	-0.2757	0.9859
$v_3$	1.20	0.1690	-0.3093	0.9930
$v_4$	2.00	0.1719	-0.3037	0.9912
$v_0$	0.00	0.0338	-0.0018	0.0008

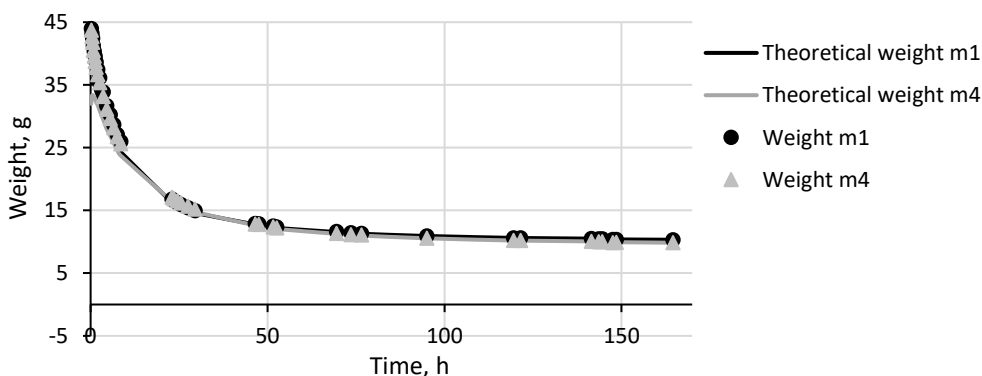
In the Table 1 is shown that the coefficient of determination for air velocities that are not equal to  $v_0$  is high. Unfortunately, Eq. (2) is not suitable for speed  $v_0$ , it is for free convection, without forced drying. The best approximation for velocity  $v_0$  is polynomial equation of second order but coefficient of determination is still less than 0.35. From this equation were obtained the coefficients  $a, b$ , for different velocities, which can also be seen in the Table 1.

Using (1) was calculated theoretically red clover during process using equation (3):

$$m_t = (m - m_d) \cdot \exp\left[-\left(\frac{a}{b+1}\right)t^b\right] + m_d, \tag{3}$$

where  $m_t$  – theoretical weight of material at the time  $t$ , g.

The comparison of theoretical and experimental data is shown in the Fig. 2. The most preferable form of red clover drying coefficient with forced air was power expression:  $K(t) = at^b$ . As it can be seen in the Fig. 2, the theoretical model of the drying process well describes the values obtained in the experiment. The greatest difference was observed at the beginning of the process and at the highest air velocity (Table 2), which could be explained by the uneven distribution of moisture in the layer.

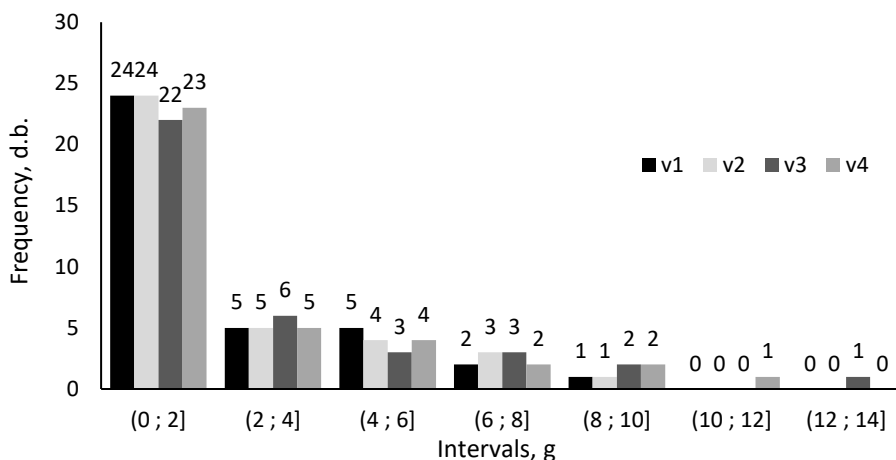


**Figure 2.** Comparison of the experimental and theoretical data:  $m_1$  ( $v_1 = 0.70\ m\ s^{-1}$ ) and  $m_4$  ( $v_4 = 2.00\ m\ s^{-1}$ ).

**Table 2.** The absolute average differences between the experimental and the theoretically calculated weight at different drying air velocity

Air velocity, m s <sup>-1</sup>	Average, g	Standard deviation, g
v <sub>1</sub>	0.70	1.856
v <sub>2</sub>	1.00	1.923
v <sub>3</sub>	1.20	2.348
v <sub>4</sub>	2.00	2.288
v <sub>0</sub>	0.00	0.471

Differences between the experimentally measured weight and the theoretically calculated weight values are plotted in the Fig. 3, where the intervals are divided into groups of 2 g. In the Fig. 3 we can also see the frequency of differences for each air velocities. The air velocity v<sub>0</sub> is not included in the graph, for which, as already mentioned, this mathematical equation is not appropriate.

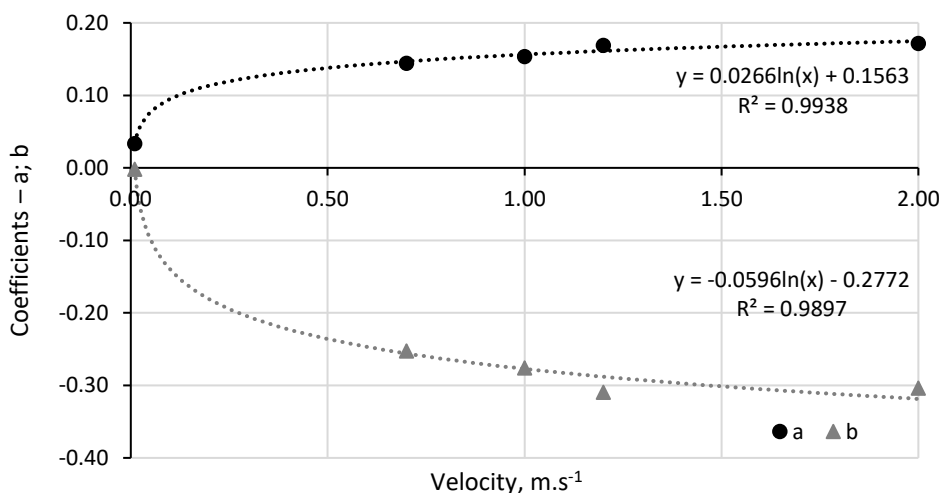


**Figure 3.** Frequency of differences between the experimental and theoretical results divided into intervals with step 2 grams.

In the Fig. 3 we can see that the most of theoretical and experimental data are the same in the first interval. The second, third and fourth intervals have a much lower frequency. The fifth, sixth, and seventh intervals, which contain only individual data and include differences greater than 8 g, can be considered as a measurement error.

As seen in Fig. 2 the highest differences are achieved by drying during the first drying hours. It can be explained with moisture on the material surface.

Assuming that the Eq. (2) which was chosen is the most appropriate mathematical expression for the type of red clover drying. A graph of coefficients *a*, *b* from the Table 1 depending on the air velocities was created.



**Figure 4.** Coefficients  $a$ ,  $b$  dependence on the drying air velocity.

It was again found the mathematical equation of the dependencies of these two parameters in the form (4, 5). High coefficients of determination are obvious in the Fig. 4. Changes of coefficients  $a$ ,  $b$  can be counted from equations:

$$a = 0.0266 \cdot \ln(v) + 0.1563, \quad (4)$$

$$b = -0.0596 \cdot \ln(v) - 0.2772, \quad (5)$$

where  $v$  – velocity of drying air,  $\text{m s}^{-1}$ .

If the velocity of drying air is known, it is possible to expect  $a$ ,  $b$  and after that calculate drying coefficient  $K(t)$ .

## CONCLUSIONS

1. The most preferable form of red clover drying coefficient with forced air was power expression. Using the experimental data and the mathematical model of the drying process, it is possible to determine the unknown parameters at each speed of drying agent.

2. The max average differences of experimental and theoretical weight values were lower than 5%.

3. The obtained relationships which connect the parameters of the drying coefficient depending on the drying agent speed allow to predict the process of drying at various speeds of unheated air. Drying time is one of main parameters which could be determined as a practical result.

4. The theoretical background solved in this paper can be used for practical information about the suitable drying process of red clover for different conservation applications with economic benefits. The principles of drying coefficient determination and optimization of drying process can be applied on another type of fodder similar to the red clover.

## REFERENCES

- Aboltins, A. 2013. Theoretical study of material drying coefficient; *Engineering for Rural Development – International Scientific Conference*. pp. 153–158.
- Atzema, A.J. 1992. A model for the drying of grass with real time weather data; *Journal of Agricultural Engineering Research* **53**, 231–247.
- Bartzanas, T., Bochtis, D., Sorensen, C., Sapounas, A. & Green, O. 2010. A numerical modelling approach for biomass field drying. *Biosystems Engineering* **106**, 458–469.
- Coradi, P.C., Melo, E.C. & Rocha, R.P. 2014. Mathematical modelling of the drying kinetics of the leaves of lemon grass (*Cymbopogon citratus Stapf*) and its effects on quality. *IDESIA* **32**(4), 43–56.
- Hermanek, P., Rybka, A. & Honzik, I. 2017. Experimental chamber dryer for drying hops at low temperatures. *Agronomy Research* **15**(3), 713–719.
- Holmes, B. 2004. Dry round hay bale storage costs. *Focus on Forage* **6**(5), 1–3.
- Jokiniemi, T., Mikkola, H., Rossner, H., Talgre, L. Lauringson, E., Hovi, M. & Ahokas, J. 2012. Energy savings in plant production. *Agronomy Research* **10**, 85–96.
- Kaya, A., Aydin, O. 2009. An experimental study on drying kinetics of some herbal leaves. *Energy Conversion and Management* **50**, 118–124.
- Rayburn, E. 2002. Proper handling and curing of hay. *Forage Manager*. pp. 5811–5814.
- Riday, H. 2010. Progress Made in Improving Red Clover (*Trifolium pratense* L.) Through Breeding. *International Journal of Plant Breeding. Global Science Books*. pp. 22–29.
- Rybka, A., Hermanek, P. & Honzik, I. 2017. Theoretical analysis of the technological process of hop drying. *Agronomy Research* **15**, 859–865.
- Wyngaarden, S.L., Gaudin, A., Deen, W. & Martin, R.C. 2015. Expanding Red Clover (*Trifolium pratense*) Usage in the Corn–Soy–Wheat Rotation. *Sustainability* **7**(11), 15487–15509.

## **Effect of thermal environment on body temperature of early-stage laying hens**

R.R. Andrade<sup>1</sup>, I.F.F. Tinôco<sup>1</sup>, C.F. Souza<sup>1</sup>, K.P. Oliveira<sup>1</sup>, M. Barbari<sup>2,\*</sup>,  
V.M.F. Cruz<sup>3</sup>, F.J.F. Baptista<sup>3</sup>, M.O. Vilela<sup>1</sup>, L. Conti<sup>2</sup> and G. Rossi<sup>2</sup>

<sup>1</sup>University of Viçosa, Department of Agricultural Engineering of Federal, Av. Peter Henry Rolfs, s/n Campus University of Viçosa, CEP: BR36570-900, Viçosa, Brazil

<sup>2</sup>University of Firenze, Department of Agricultural, Food and Forestry Systems – Via San Bonaventura, 13, IT50145 Firenze, Italy

<sup>3</sup>University of Évora, Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Apartado 94, PT7006-554 Évora, Portugal

\*Correspondence: [matteo.barbari@unifi.it](mailto:matteo.barbari@unifi.it); [rafaella.andrade@ufv.br](mailto:rafaella.andrade@ufv.br)

**Abstract.** The thermal comfort condition of early-stage laying hens can be verified by means of physiological variations. The mean surface temperature and cloacal temperature are important parameters to demonstrate the effect of the thermal environment on the birds. The objective of the present study was to correlate homeostasis and stress with physiological responses (mean surface temperature and cloacal temperature) of lightweight laying hens of the Lohmann LSL Lite line aged from one to forty-two days when submitted to different thermal environments. A total of 864 birds with the same age, origin and uniform body weight were randomly distributed in four climatic chambers. The characterization of the different environments was as follows: thermal comfort temperature (33.0–19.0 °C), two cold stress levels (28.0–17.0 °C and 25.0–17.0 °C) and one level of heat stress (38.0 °C – 22.0 °C). The experiment was performed in a completely randomized design in the subdivided plots scheme, with four treatments in the plots and the evaluations (days) in the subplots. The means were compared using the Tukey test, adopting the 5% probability level. The birds maintained the physiological responses, based on cloacal and surface temperatures, within the normal range for all evaluated treatments during the period between 01 and 42 days of life. The surface temperature of the birds varied as a function of the air temperature of each breeding environment, with lower surface temperatures for mild cold and moderate cold treatments.

**Key words:** environmental conditions, poultry, surface temperature, layer chicks.

### **INTRODUCTION**

In poultry farming, maximum productivity is achieved when birds are introduced in an environment that provides minimal energy exchanges and meets the welfare needs of the animal. Birds are classified as homeothermic because they retain (to a certain extent of adversity) body temperature constant or within a tolerable range, even if considerable variations occur in the external environment.



Animals of chicken species, like all homeothermic species, maintain a constant body temperature (41.1 °C) and are quite sensitive to climatic changes, and may suffer from adaptive issues under conditions of variation in the breeding environment (Albino et al., 2014). The amount of thermal energy stored per unit of body mass is determined by the body temperature of the bird, energy that can be increased or decreased by the processes of thermogenesis and thermolysis (Castilho et al., 2015).

Animal welfare is the full state of physical and mental health, in which the animal is in harmony with the environment where it lives, and a perceptual change to the state of comfort of the animal can be verified through its surface temperature (Camerini et al., 2016).

Nascimento et al. (2014), in a study conducted to evaluate the thermal comfort of broiler chickens in two aviaries with different air conditioning systems, found that in conditions of thermal comfort the superficial temperatures of the birds are strongly associated with the surface temperatures of the facility.

The temperature of the cloaca indicates the temperature of the body centre and can be considered as an important parameter to assess changes in the environmental conditions where the animals live (Brown-Brandtl et al., 2003). The variation in cloacal temperature indicates that the superficial heat exchanges of the skin are not enough to maintain the homeothermia (Nascimento, 2010).

To obtain better performance in poultry breeding, it is important to verify the interaction between animal and environment in order to maximize the energy available for production. For this reason researches on interaction between animals and environment have to be incentivized.

In this sense, this research was conducted with the objective to correlate homeostasis and stress with physiological responses (mean surface temperature and cloacal temperature) of laying hens of the Lohmann LSL Lite line, from one to forty – two days of age, when submitted to different thermal environments.

## **MATERIALS AND METHODS**

### **Characterization of the facilities used during the initial phase**

The experiment was developed in four climatic chambers with the following dimensions: 3.2 m long x 2.44 m wide x 2.38 m high, located in the experimental area of the Centre for Research in Ambience and Engineering of Agroindustrial Systems (AMBIAGRO) of the Department of Agricultural Engineering of the Federal University of Viçosa, in Viçosa, Minas Gerais, Brazil. Each climate chamber is equipped with an electric resistance air heater with 2,000 W of power, a hot-cold split air conditioner of 3,500 W and an air humidifier with a capacity of 4.5 L and a mist rate (average value) of 300 ml.h<sup>-1</sup>. The heater and humidifier are operated by means of an MT-531R Plus temperature and humidity electronic controller, which has the following specifications: control temperature ranging from -10 °C to 70 °C with a resolution of 0.1 °C, control humidity ranging from 20% to 85%.

The installation has also two AMB axial fans (model FD08025 S1M; DC 12 V, 0.15 A), used for the renewal of the air inside the climatic chambers during the whole experimental period.

In each of the four climatic chambers used in this work four different thermal environments were established, constituting four treatments: one of the temperature ranges was considered as the comfort range recommended in the literature (TCL = Comfort Temperature by Literature) (Management Guide Lohmann LSL LITE, n.d.), the others represented two levels of cold stress, mild cold (MiC) and moderate cold (MoC) and one level of heat stress, moderate heat (MoH). Considering that the thermal requirement of the birds varies with the age, it was tried to represent this requirement in each week of life of the layer hens during the experimental period. The variation of the weekly temperature occurred until the fifth week, and the sixth week remained with a temperature similar to that of the fifth week.

Table 1 shows the different temperatures used during the experimental phase.

**Table 1.** Air temperature in the internal environment of the climatic chambers, in °C, depending on the treatment and age of the layer chicks

Thermal environment	Temperature (°C)				
	1–7 days	8–14 days	15–21 days	22–28 days	29–42 days
Moderate heat (MoH)	38	31	29	26	22
Literature approach (TCL)	33	28	26	23	19
Mild Cold (MiC)	28	25	23	20	17
Moderate Cold (MoC)	25	22	20	17	17

Management Guide Lohmann LSL LITE (s.d); Albino et al. (2014); Ferreira (2016).

The air relative humidity was monitored and values maintained throughout the experimental period and in all treatments around 60%, in a range between 55 and 65%, since it is considered an adequate value for poultry production, regardless of the age of the birds (Tinôco, 2001; Ferreira, 2016).

### Instruments and measurements used in the characterization of environments

Since the birds were subjected to continuous stress, from the first day of experiment the values of air temperature ( $T_{air}$ ), relative humidity (RH) and black globe temperature (BGT) were recorded every 5 minutes, 24 hours per day, throughout the experimental period.

To measure air temperature and RH, HOBO® T / RH dataloggers, model U14-001 (-20 °C to + 70 °C), with an accuracy of 0.7 °C were installed. In order to obtain the BGT, in each climatic chamber a black globe was installed including inside a Testo temperature sensor, model 174, with a resolution of 0.1 °C, measuring range from -30 to 70 °C and accuracy of ± 0.5 °C. The sensors were installed at the height of the birds, in the centre of each climatic chamber. Based on the records, the Black Globe Humidity and Temperature Index (BGHI) was calculated by means of the Eq. 1 (Buffington et al., 1981).

$$BGHI = BGT + DPT (0.36) + 41.5 \quad (1)$$

where BGHI = Black Globe Humidity and Temperature Index; BGT = black globe temperature, in °C; DPT = dew point temperature, in °C.

### **Management of laying birds during the initial phase**

The experiment was carried out with laying birds from one to forty two days old, considered the initial stage for laying hens. During the experimental phase, 864 lightweight laying hens of the Lohmann LSL Lite line were housed in cages, distributed homogeneously in four climatic chambers (four treatments), totalling 216 birds per treatment. The cages are 0.50 m<sup>2</sup> in surface (0.50 m wide x 1.0 m long) and 0.5 m high, being six units per chamber.

From the first day to the end of the fourth week, each cage housed 36 chickens in order to guarantee a density of 140 cm<sup>2</sup> bird<sup>-1</sup>. From the beginning of the fifth week until the end of the sixth week, the density was of 285 cm<sup>2</sup> bird<sup>-1</sup>, which corresponds to 18 birds in each cage (Patterson & Siegel, 1998; Management Guide Lohmann LSL Lite, n.d., 2016). This procedure was adopted to guarantee the density used by the poultry industry under field conditions, for each of the different ages.

For the period from one to forty-two days the water and feed supply was *ad libitum*, and this management occurred twice a day (7 and 17 h), in order to keep the drinkers and the feeders always supplied.

The experiment was conducted in a completely randomized experimental design, with four treatments (moderate heat, recommended comfort, mild cold and moderate cold), and in subplots with six replications. The data were evaluated through analysis of variance and the means compared using the Tukey test, adopting the level of 5% of probability. The results were interpreted statistically using the System Program for Statistical Analysis and Genetics – SAEG (2007).

### **Collection of data on physiological variables of birds**

The average surface temperature and cloacal temperature of the birds were measured weekly. For this purpose, ten birds from each experimental unit were chosen at random, totalling 60 birds per treatment.

For the measurement of the mean surface temperature (MST), the wing, head, cinnamon and back of the birds were monitored with the aid of a digital infrared thermometer, with a laser sight (Instrutherm® Instruments of Measurement Ltda, São Paulo, BR, model TI-860), amplitude from -30 °C to 270 °C, accuracy ± 2.5% of reading, resolution 1 °C and emissivity fixed to 0.95. The average surface temperature (MST) was calculated on the basis of equation 2 (Richards, 1971).

$$\text{MST} = (0.12 T_w) + (0.03 T_h) + (0.15 T_p) + (0.70 T_b) \quad (2)$$

where  $T_w$  = wing surface temperature (°C);  $T_h$  = head surface temperature (°C);  $T_p$  = cinnamon surface temperature (°C);  $T_b$  = back surface temperature (°C).

## **RESULTS AND DISCUSSION**

The values of temperature and relative humidity of the ambient air, and respective values of BGHI, related to chickens from one to forty two days of life, for each treatment are presented in Table 2.

**Table 2.** Average and standard deviations of the values of air temperature ( $T_{\text{air}}$ ), air relative humidity (RH) and black globe temperature and humidity index (BGHI) for each climatic condition evaluated in the period from 1 to 42 days

Thermal environment	$T_{\text{air}}$ ( $^{\circ}\text{C}$ )	RH (%)	BGHI
		(1–7 days)	
Moderate heat (MoH)	37.9 ± 0.2	55.5 ± 2.4	89.0 ± 1.5
Literature approach (TCL)	33.0 ± 0.4	56.5 ± 1.2	82.7 ± 1.3
Mild Cold (MiC)	28.0 ± 0.3	62.4 ± 2.5	76.3 ± 0.5
Moderate Cold (MoC)	25.0 ± 0.2	61.6 ± 1.5	72.3 ± 0.5
		(8–14 days)	
Moderate heat (MoH)	31.1 ± 0.5	60.1 ± 2.0	80.7 ± 0.6
Literature approach (TCL)	28.2 ± 0.5	62.6 ± 2.4	76.9 ± 0.8
Mild Cold (MiC)	25.1 ± 0.6	63.1 ± 1.5	71.8 ± 1.4
Moderate Cold (MoC)	22.1 ± 0.6	62.9 ± 2.0	69.1 ± 1.2
		(15–21 days)	
Moderate heat (MoH)	29.1 ± 0.4	61.1 ± 2.2	76.9 ± 1.1
Literature approach (TCL)	26.0 ± 0.5	60.4 ± 2.4	72.4 ± 0.9
Mild Cold (MiC)	23.1 ± 0.5	60.9 ± 0.2	70.9 ± 1.4
Moderate Cold (MoC)	20.1 ± 0.2	64.1 ± 0.8	66.3 ± 1.1
		(22–28 days)	
Moderate heat (MoH)	26.0 ± 0.6	62.3 ± 0.5	73.8 ± 1.0
Literature approach (TCL)	23.1 ± 0.7	62.8 ± 0.7	70.7 ± 1.2
Mild Cold (MiC)	20.1 ± 0.5	60.3 ± 0.2	66.3 ± 0.8
Moderate Cold (MoC)	17.0 ± 0.5	64.6 ± 0.5	63.6 ± 0.6
		(29–42 days)	
Moderate heat (MoH)	22.1 ± 0.4	64.6 ± 0.2	70.2 ± 2.4
Literature approach (TCL)	19.2 ± 0.5	62.7 ± 1.2	66.4 ± 1.3
Mild Cold (MiC)	17.0 ± 0.2	64.5 ± 0.5	63.8 ± 0.2
Moderate Cold (MoC)	17.0 ± 0.2	64.2 ± 1.3	63.6 ± 0.2

It is observed that the mean values of air temperature and relative humidity remained close to the values proposed for each thermal environment, showing an adequate control of the environment inside the climatic chambers.

### Physiological parameters of hens in different environmental conditions

#### Cloacal temperature

Table 3 shows the mean values of cloacal temperature, in  $^{\circ}\text{C}$ , for laying birds of the Lohmann LSL Lite line, from 1 to 42 days of age, for the respective combinations of days and thermal environments (MoH, TCL, MiC, MoC).

It was observed that the thermal environment factor, given by the different temperatures in each of the four climatic chambers, significantly influenced ( $p < 0.05$ ) the cloacal temperature of the birds only when they were at 8 and 22 days of age, with higher values for the treatment moderate heat, compared to the other treatments.

In the first days of life, chickens do not have their thermoregulatory system fully developed yet, so they require that the environmental temperature remains within the comfort range in order to maintain homeothermia. Outside of these thermal ranges, the uncontrolled process begins, with elevation or reduction of the internal temperature of the body, depending on whether the environment is very hot or very cold, respectively.

Based on this premise, it can be inferred that the temperatures used during the trials did not negatively affect the laying birds from the physiological point of view giving a situation of lack of homeothermic control.

Furlan & Macari (2002) cite the 41.1 °C rectal temperature of birds as the lower limit of the thermal stress condition. When this limit is not reached, physiological mechanisms are triggered to maintain body temperature, which characterizes the cold stress condition.

It can be observed that the birds in the mild cold and moderate cold treatments, with the ages of 08 and 22 days, presented stress condition, indicating that the sensible heat exchange mechanism was not totally sufficient for maintain body temperature within acceptable limits. In this situation, birds need to use other mechanisms, such as increased food intake, to maintain body temperature.

This behaviour observed in laying hens during the three weeks of life is in line with the studies of Cassuce (2011). The author evaluated the cloacal temperature of broilers during the initial phase in different thermal environments (39–33, 36–30, 33–27, 30–24 and 27–21 °C) and observed a statistical difference between the treatments in the third week of life of the birds, with lower rectal temperature for birds submitted to temperature of 21 °C.

Thus, birds submitted to the treatments MoH and TCL were able to maintain body temperature within normal values during the whole experimental phase, demonstrating the ability of birds to adapt to higher temperatures, avoiding the occurrence of hyperthermia.

It is also observed that birds with up to 29 days of recommended comfort treatment presented absolute values of cloacal temperature lower than the moderate heat treatment, although for both treatments, the values were within acceptable limits.

It was verified that, in the birds with age of 15, 29, 36 and 42 days of age, the cloacal temperature remained stable in all studied treatments, not differing among them ( $P > 0.05$ ). It can be concluded, therefore, that none of the temperatures used in the trials, for the aforementioned ages, causes a situation of stress due to cold or heat, showing the adaptability of the birds.

### Surface temperature

Table 4 presents the results of the average surface temperature (MST) of the birds according to the different thermal environments evaluated (MoH, TCL, MiC, MoC).

It was verified that there was a significant difference ( $p < 0.05$ ) in the results of the surface temperature of the birds in relation to the thermal environment and days of evaluation, being these always higher in the treatment MoH in relation to all the others,

**Table 3.** Average cloacal temperature values, in °C, for laying birds of the Lohmann LSL Lite line, aged from 01 to 42 days of age, in relation to age and thermal conditions (Moderate heat: MoH; comfort recommended by the literature: TCL; mild cold: MiC; moderate cold: MoC)

Days of age of birds	Cloacal temperature			
	MoH	TCL	MiC	MoC
8	41.57 <sup>a</sup>	41.19 <sup>b</sup>	40.89 <sup>c</sup>	40.70 <sup>c</sup>
15	41.32 <sup>a</sup>	41.11 <sup>a</sup>	41.18 <sup>a</sup>	41.09 <sup>a</sup>
22	41.32 <sup>a</sup>	41.17 <sup>ab</sup>	41.08 <sup>ab</sup>	40.92 <sup>b</sup>
29	41.34 <sup>a</sup>	41.20 <sup>a</sup>	41.26 <sup>a</sup>	41.11 <sup>a</sup>
36	41.37 <sup>a</sup>	41.46 <sup>a</sup>	41.47 <sup>a</sup>	41.33 <sup>a</sup>
42	41.37 <sup>a</sup>	41.51 <sup>a</sup>	41.54 <sup>a</sup>	41.49 <sup>a</sup>

The average values followed by at least one letter in the row do not differ, at the 5% probability level by the Tukey test.

for the birds with age of 08, 15, 22 and 29 days. In birds with age of 36 and 42 days higher surface temperatures were found when submitted to the treatments MoH and TCL in comparison to the others, however without significant differences between the first two mentioned.

These results confirm the direct relationship between the body temperature and the environmental temperature, highlighting the strong effect of the thermal environment on the birds, which can modify the physiological response in relation to the situation.

Andrade et al. (2017), when evaluating the surface temperature of laying birds in the initial stage of creation by means of thermographic images, observed the effect that the different temperatures exert on the birds, being able to modify the physiological response according to the thermal environment.

During the first and third week of life of the birds, in the cold stress treatments, it was observed that the birds presented in addition to the decrease of the surface temperature, decrease of the cloacal temperature with values lower than the values of the other treatments, which indicates that the birds could not maintain body temperature constant, presenting cloacal temperature below the recommended level.

According to Ruzal et al. (2011), part of the physiological responses that explain the direct relationship between ambient temperature and poultry surface temperature may be related to redistribution of blood flow in the body, because exposure to heat causes peripheral vasodilation.

## CONCLUSIONS

The birds submitted to the treatments moderate heat, recommended comfort, mild cold and moderate cold kept the physiological responses, based on cloacal and surface temperatures, within the normal range during the period between 01 and 42 days of life.

Under the specific conditions of the present experiment, the surface temperature of the birds varied according to the air temperature of each breeding environment, with lower surface temperatures for mild cold and moderate cold treatments.

ACKNOWLEDGEMENTS. To CAPES, CNPq, FAPEMIG and the LOHMANN DO BRASIL.

**Table 4.** Mean values of mean surface temperature (MST), in °C, for laying birds of the Lohmann LSL Lite line, aged from 01 to 42 days of age, in relation to age and thermal conditions (Moderate heat: MoH; comfort recommended by the literature: TCL; mild cold: MiC; moderate cold: MoC)

Days of age of birds	MST			
	MoH	TCL	MiC	MoC
8	48.47 <sup>a</sup>	45.05 <sup>b</sup>	40.28 <sup>c</sup>	37.36 <sup>d</sup>
15	44.19 <sup>a</sup>	42.09 <sup>b</sup>	39.00 <sup>c</sup>	35.73 <sup>d</sup>
22	41.51 <sup>a</sup>	39.72 <sup>b</sup>	36.75 <sup>c</sup>	33.35 <sup>d</sup>
29	39.29 <sup>a</sup>	37.41 <sup>b</sup>	34.09 <sup>c</sup>	32.89 <sup>c</sup>
36	34.48 <sup>a</sup>	34.18 <sup>a</sup>	32.11 <sup>b</sup>	31.28 <sup>b</sup>
42	37.37 <sup>a</sup>	37.27 <sup>a</sup>	33.83 <sup>b</sup>	32.48 <sup>b</sup>

The average values followed by at least one letter in the row do not differ, at the 5% probability level by the Tukey test.

## REFERENCES

- Albino, L.F.T., Carvalho, B.R., Maia, R.C. & Barros, V.R.S.M. 2014. *Creation and Feeding Hens*. Learn Easy, Viçosa, 376 pp. (in Portuguese).
- Andrade, R.R., Tinôco, I.F.F., Baêta, F.C., Barbari, M., Conti, L., Cecon, P.R., Cândido, M.G.L., Martins, I.T.A. & Teles Junior, C.G.S. 2017. Evaluation of the surface temperature of laying hens in different thermal environments during the initial stage of age based on thermographic images. *Agronomy Research* **15**, 629–638.
- Brown-Brandl, T.M., Yanagi Júnior, T., Xin, H., Gates, R.S. & Bucklin, R.A. 2003. A new telemetry system for measuring core body temperature in livestock and poultry. *Applied Engineering in Agriculture* **19**, 583–589.
- Buffington, D.E., Colazo-Arocho, A. & Canton, G.H. 1981. Black globe humidity index (BGHI) as comfort equation for dairy cows. *Transactions of ASAE* **24**, 711–714.
- Camerini, N.L., Silva, R.C., Nascimento, J.W.B., Oliveira, D.L. & Souza, B.B. 2016. Surface temperature variation of laying hens created in two creation systems using Thermography. *Scientific Agriculture in the Semi-Arid* **12**, 145–152 (in Portuguese).
- Castilho, V.A.R., Garcia, R.G., Lima, N.D.S., Nunes, K.C., Caldara, F.R., Nääs, I.A., Barreto, B. & Jacob, F.G. 2015. Welfare of laying hens in different densities of housing. *Brazilian Journal of Biosystems Engineering* **9**, 122–131 (in Portuguese).
- Cassuce, D.C. 103f. The thermal comfort bands determination in poultry in different ages created in Brazil. Thesis (PhD in Agricultural Engineering), Federal University of Viçosa, 2011 (in Portuguese).
- Ferreira, R.A. 2016. *Greater production with better environment for poultry, pigs and cattle*. Learn Easy, Viçosa, 296 pp. (in Portuguese).
- Furlan, R.L. & Macari, M. 2002. Thermoregulation. In: MACARI, M.; FURLAN, R.L. *Fisiologia aviária aplicada a frango de corte*. 2ed. Jaboticabal: FUNEP, 209–230. (in Portuguese).
- Management guide Lohmann LSL-Lite (n.d.). [www.hylinena.com/UserDocs/products/Lohmann\\_LSL-Lite.pdf](http://www.hylinena.com/UserDocs/products/Lohmann_LSL-Lite.pdf). Accessed 11.8.2016.
- Nascimento, G.R., Nääs, I.A., Baracho, M.S., Pereira, D.F. & Neves, D.P. 2014. Infrared thermography in the estimation of thermal comfort of broilers. *Brazilian Journal of Environmental Agricultural Engineering* **18**, 658–663 (in Portuguese).
- Nascimento, S.T. 2010. Determination of heat balance in broiler chickens through body temperatures. 120f. Doctoral thesis. Dissertation (Master's Degree in Agricultural Environmental Physics) -Unique Superior School of Agriculture 'Luiz de Queiroz', University of São Paulo, Piracicaba, SP, 2010.
- Patterson, P.H. & Siegel, H.S. 1998. Impact of cage density on pullet performance and blood parameters of stress. *Poultry Sci.* **77**, 32–40.
- Richards, S.A. 1971. The significance of changes in the temperature of the skin and body core of the chicken in the regulation of heat loss. *J. Physiol.* **216**, 1–10.
- Ruzal, M., Shinder, D., Malka, I. & Yahav, S. 2011. Ventilation plays an important role in hens' egg production at high ambient temperature. *Poultry Sci.* **90**, 856–862.
- SAEG System for Statistical Analysis. 2007. Version 9.1: Fundação Arthur Bernardes - UFV – Viçosa.
- Tinôco, I.F.F. 2001. Industrial aviculture: new concepts of materials, conceptions and constructive techniques available for Brazilian poultry houses. *Brazilian J. Poultry Sci.* **3**, 1–26.

## The role of field beans in nutrition of Boer goat

E. Aplocina

Latvia University of Life Sciences and Technologies, Institute of Agrobiotechnology,  
Liela street 2, LV3001 Jelgava, Latvia

Correspondence: [elita.aplocina@llu.lv](mailto:elita.aplocina@llu.lv)

**Abstract.** A study on the effective using of field beans to Boer mother goats feeding was carried out on a farm whose main activity is the production of goat meat. Since the Boer goat breed is still very rare in Latvia, there is a lack of experience in the feeding of meat goat. Goat productivity was analyzed according to the birth weight of goat kids and kids live weight at 50 days, as well as the average live weight gain of kids per day up to 50 days old. For control group goats a relatively high energy shortage (14% of the daily requirement) and protein deficiency (29% of the daily requirement) were observed in feed ratio. Adding fodder beans and maize into the feed, it is possible to optimize feed ratio for energy and protein supply. Experimental activities had shown that by the inclusion of field beans and maize in feed ratio of goat's mothers, the average increase in live weight of kids up to 50 days old was by 58.2% higher ( $P < 0.05$ ) than that of kids in which goats' mothers received only oats as concentrated feed. By the optimization of feed ratio, it is possible to achieve a higher milk productivity of goat mothers and hence larger live weight gain for kids, which reduces the feed costs by up to 9% for 1kg of live weight. By optimizing the breeding and feeding of mother goats and kids, it is possible to achieve greater animal fast-growing, hence, more efficient and cost-effective management.

**Key words:** Boer goat, live weight, gain.

### INTRODUCTION

Goat is thought to have been the earliest animal domesticated besides sheep and dogs. At the present time, goats provide the principle source of animal protein in North African and Middle Eastern nations. Goat is also important in the Caribbean, in Southeast Asia, and developing tropical countries. Three quarters of all the goats in the world are located in the developing regions of the world. Sheep and goats are the major source of livelihood for millions livestock farmers (Hasnain, 1985; Correa, 2016).

Meat is the primary reason to raise goats, which is why meat goats constitute the majority of the world's goat production systems. Goat meat comprises 63 percent of all red meat that is consumed worldwide (Correa, 2016).

The sub-optimal productivity of the existing flocks of goats is mainly attributed to low genetic potential, nutritional and managerial inadequacies. However, some breeds of goats offer a high potential for meat and milk production. One of the traits of economic importance in goats is birth weight. The diversity in performance traits of goats may be attributed to several genetic and non-genetic factors. Although any programme of breed improvement is based on the maximum exploitation of genetic



variation, yet these traits also vary due to certain environmental factors, e.g. climate and seasonal differences, sex of the kid, type of birth and age of the dam. It is, therefore, imperative to estimate the magnitude of all such factors, so that the genetic variation among animals can be used to devise effective breeding plans for their improvement (Afzal et al., 2004).

In the Baltic States goat farming focuses mainly on milk production, however, recently there has been increased interest and demand for goat meat. For milk production most common breeds in Latvia are Latvian local and Saanen, also German White noble and the Alps breed, but the majority of the meat-type goats in Latvia are of the Boer breed and crossbreeds (Selegovska & Spruzs, 2010). The Boer breed was developed in South Africa for the purpose of meat production. This breed is known for its large frame size, muscularity, and characteristic white body and brown or red-colored head (Van Nieker & Casser, 1988).

Boer goats were imported into Latvia in 2005, and used in cross breeding for the improvement of goat meat quality and quantity. If you breed meat goats, then you will not get milk because goats give milk only for kids. Meanwhile, there will be a lot of meat production and goat meat is very valuable. The favorite is 6–8-week old goat kid meat, which is very similar to lamb. Kids are often slaughtered at the age of 3 to 5 months and weight from 10 to 20 kg. Kids do not store much body fat. Many goats are older and heavier when marketed, but most, except aged cull goats, are slaughtered at less than a year of age. The meat of older goats is darker and less tender, but more juicy and with more flavour than that of kid. The meat from males is lighter in color and lower in fat. The meat from females is more desirable for steaks and chops, and is more tender (Piliena & Spruzs, 2007).

Feed can account for up to 50–60% of total production costs, and the goal of providing livestock with high quality feeds must be met in a manner that allows the animals' needs to be met without jeopardizing sustainability while also being economically feasible for the farmer (Vaarst et al., 2004). Usually, nutrient requirements are defined for a certain level of production, using factorial approaches (i.e. calculating requirements) as a function of body weight, physiological status and level of performance, using established factors for use of nutrients and energy (Nutrient requirement..., 2007). The probability of nutrition-related problems increases with increasing level of production, decreasing forage quality and lack of home-grown feedstuffs with a high nutrient concentration. Problems may occur also, in connection with an unbalanced supply of different nutrients. The magnitude of imbalance between protein and energy intake will depend, among other things, on the soil type, the proportion of legumes in the diet, and the availability of feedstuffs with relatively high energy content (Baars, 1998). Typically, goat farmers will focus on forage and pasture systems and use less concentrates and mineral supplements than intensive foreign farmers. Under these circumstances, nutrition will probably limit milk and meat production and eventually affect the milk's and meat's nutrient content (Krutzinna et al., 1996). Because extensive farms rely on home-grown feedstuffs, much more than intensive farms, low feedstuff quality, which may temporarily occur because of unfavorable conditions, will be important for the nutrition of ruminants (Olesen, 1999). Temporary nutrient deficits can be covered by using supplementary feedstuffs such as concentrates or different mineral sources.

The nutritional requirements of goats managed primarily for milk production and those managed primarily for meat production are quite similar with perhaps two notable differences. First, dairy goats are expected to milk at relatively high and persistent levels throughout a 9–10 month lactation; meat goats need only achieve a 4–7 month lactation with high initial milk flow, persistency beyond 4 months being of lesser concern. Secondly, dairy goats are typically fed considerable concentrates (grain mixtures) to encourage maximum and persistent milk flow. In contrast, lactating meat goats are not usually fed concentrates in addition to their forage diet because the extra kid growth achieved from the extra milk may well not repay the added costs. As always, special circumstances may occasionally alter normal cost-benefit calculations. In those situations in which the plants are too low in protein (or in which forage quantity is much reduced), additional protein must be offered to maintain acceptable goat performance. Protein supplementation may take many forms and cost per unit of protein may vary widely. High protein supplemental feedstuffs, used only occasionally by meat goat owners, are soybean meal, peas and field beans meal, urea and others. Choosing between alternative high protein feedstuffs is largely an economic decision (Pinkerton & Pinkerton, 2015).

Boer's goat's mothers have good maternal properties and calm character. Boer sires may also be used for crossbreeding with other related dairy goat breeds, taking into account that the live weight of kids at birth will be 10–18% higher than that of dairy goats. Therefore, for the crossing, select large goats with a well-developed part of the hip, which are usually observed for mild births. Boer goat productivity indicators are: fertility 180–200%; live weight gain of kid 180–230 g per day; goat live weight 65–75 kg; height 65–55 cm; the live weight of the sire is 90–100 kg, the height 75–90 cm. The following main tasks have been identified in Latvia to improve the breeding value of goats: live weight gain of kids till age of 50 days should be 200–230 g per day; fertility 180%–200%; kids at weaning 170%; live weight at birth 3.2–3.8 kg (Ciltsdarba programma..., 2013).

The effect of breed and diet on goat breeding properties and carcass characteristics has been investigated in only a limited number of studies (Oman & Waldron, 1999), and we will try to find the answer to the question of how to improve Boer goat productivity.

The objective of our study was to assess the influence of field beans on Boer goat productivity and make recommendations for feeding of the mother goat.

## **MATERIALS AND METHODS**

Data on pedigree breeding and performance records of Boer goats maintained at a farm whose main activity is the production of goat meat during the period of two years were used. The farm has 23 goat mothers at 1<sup>st</sup> control period and 13 at 2<sup>nd</sup> research period and 1 purebred Boer sire. Since Boer goats are still very rare in Latvia, there are no pure-breed goat mothers in the farm, but cross-breeds of different grades. Generally, the does were bred once a year in autumn (September – November) and kids were born during subsequent spring (February – April). The Boer goat does not have seasonal breeding as is the case for dairy goats. The male and female kids are processed into meat at the age of 4 months, but some female kids are kept for breeding. In the summer, animal basic feed is a pasture grass and oats, in winter – fodder beetroot, hay, corn and oats. As the farm does not pay much attention to optimizing of feed ratio, the goat first breeding takes off only at the age of 2 years when optimal live weight is achieved.

The study started in early spring when goat mothers (Research group) after kidding were fed with hay (1.5 kg per day for goat) and as concentrated feed they received farm-produced oats (0.2 kg per day for goats) and purchased field beans (0.3 kg per day for goat) and maize (0.2 kg per day for goat). The study was conducted in a period from the time when the first kids were born to the time when the last spring kids reached the age of 50 days. Goat productivity was evaluated according to the kids live weight at birth and live weight of kids at age of 50 days, as well as the average live weight gain of kids per day up to age of 50 days.

In each study year goat kids were weighed at birth and at the age of 50 days with an electronic scale (accuracy of 0.01 kg). Absolute live weight gain per day for analyzed kids was calculated by formula (1):

$$a = (W_t - W_0)/t \tag{1}$$

where a – live weight gain per day, g;  $W_t$  – live weight at the end of period (50 days), g;  $W_0$  – live weight at the beginning of period (at the birth), g; t – period (50), days.

The obtained data was compared with the same goat productivity indicators of the previous year (Control group), when the does after kidding received only the hay (1.5 kg per day for goat) and oats (0.6 kg per day for goat). In addition, phosphorus-containing mineral feed was fed to ensure an optimum ratio of Ca and P in the feed ratio to a range of 1.6–2 : 1.

The analysis of obtained data was conducted according to research scheme (Table 1).

**Table 1.** The research scheme

Groups	Number of does	Feedstuffs
1st control group	23	Hay + oats
2nd research group	13	Hay + oats + field beans + maize

The following feed nutrient biochemical parameters were established before the start of the trial according with generally accepted methods of analysis: dry matter (DM) according ISO 6496:1999 method; neutral detergent fiber (NDF) according LVS EN ISO 16472:2006; acid detergent fiber (ADF) according LVS EN ISO 13906:2008; crude protein (CP) according LVS EN ISO 5983-2:2009; calcium (Ca) according LVS EN ISO 6869:2002; phosphorus (P) according ISO 6491:1998; ash according ISO 5984:2002/ Cor 1:2005, but undegraded intake protein content (UIP), net energy for lactation (NEL) and digestibility were calculated based on the results of the analysis performed. The quality indicators for nutrients were determined by the accredited laboratory of Agronomic analysis of the Latvia University of Agriculture.

Collected data was analyzed with mathematical data processing methods.

## RESULTS AND DISCUSSION

The nutritional requirements for animals were determined by the live weight of goat's (average 60 kg) and milk yield (1.4 kg on average), based on the Latvia and United States standard rules for Boer goat feeding (Nutrient requirement..., 2007). Goats of control group were fed according of the farm's usual scheme (Table 2).

**Table 2.** Nutrients of feed ration

Feed nutrient	Requirement	1st control group		2nd research group	
		Total	Difference, %	Total	Difference, %
DM, kg	1.8	1.9	+5	1.9	+5
NDF, kg	0.7	0.8	+14	0.8	+14
NEL, MJ	12.7	10.9	-14	11.8	-7
CP, g	236.0	167.0	-29	222.0	-6
UIP, g	76.0	58.0	-24	73.0	-4
Ca, g	9.0	10.0	+11	9.5	+5
P, g	5.0	5.4	+8	5.6	+12

After the analysis of feed ration chemical composition, we found that the animals are not provided with sufficient energy and protein levels, which has an effect on the productivity indices of Boer goat. The base feed rate of control group shows a relatively high level of energy shortage (14% of the requirement), which could be explained by the low quality of grass feed and the lack of protein (29% of the requirement). By the addition of fodder beans into the feed ration of Research group, it is possible to optimize feed ration for energy and protein supply. If the farm has produced low-quality grass fodder - hay, which contains only 8.15% of protein in the dry matter and 60.91% NDF, then it is not possible to balance the feed ration with all the nutrients required. The maximum of NDF level in feed ration is exceeded, which limits the intake and use of valuable nutrients. In countries where goat breeding is developed, the feeding of the dairy goats is dominated by the feeding of concentrated feed and the use of grass fodder is only for the provision of fiber. But it is also considered as cost-effective to grass fodder with small concentrated feed additives for meat goats. In Latvia, the feeding of ruminant animals is dominated by the feeding of grass fodder, as it is the cheapest feedstuff that a farmer can grow and prepare on his own farm (Piliena & Spruzs, 2007).

The experimental farm deals with the production of meat goats, but, as this is a fairly new livestock sector in Latvia, the farm has mother goats with different crossing level. When evaluating reproduction indices in the farm, the results of the study show (Table 3) that better reproduction rates were reported by Boer goat mothers of 1st crossing level where Boer goats are crossed with Latvian Native goats, while lower fertility was observed in goats with higher Boer breed crossing level. In Latvia, 15% of all registered animals are pure-bred Boer animals (Ciltstarba programma..., 2013), while there is only 1 pure-bred sire in this research farm.

**Table 3.** Fertility rate of mother goats

Boer breed crossing level	Number of mother goats		Fertility, %	
	1st control	2nd research	1st control	2nd research
0%	1	1	200.0 ± 0.00	200.0 ± 0.00
1st – 50%	11	9	209.1 ± 16.26*	177.7 ± 22.22
2nd – 75%	11	3	163.6 ± 20.33*	133.3 ± 67.42
Average in Latvia	110		120.0	

\* – for the fertility, traits signed with asterisks shows significant differences ( $P < 0.05$ ) between traits.

According to the Boer goat breeding program the fertility should be 180 to 200%, and the live weight gain of the kids to age of 50 days should be 180–230 g per day (Ciltstarba programma..., 2013).

Analyzing the performance indices of the kids (Table 4), we conclude that Boer goat mothers with 1st and 2nd crossing level had heavier new-born kids in 1st control group (no significant differences), while at the age of 50 days kids were heavier in 2nd research group ( $P < 0.05$ ) from 2nd crossing level mothers (average 14.15 kg). In 2nd research group these kids also showed the highest live weight gain ( $P < 0.05$ ) per day (0.218 kg per day), which is a very good indicator. When comparing the fertility rates of the female goat population with the Latvia average indices, we conclude that in the farm, in 2nd research group, these parameters are optimal and are in line with the Boer goat breeding program.

**Table 4.** Goat productivity indices in a farm

Boer breed crossing level	Number of kids		Live weight at birth, kg		Live weight at age of 50 days, kg		Live weight gain per day, g	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0%	2	2	3.95 ± 0.05 <sup>A</sup>	3.30 ± 0.20 <sup>B</sup>	10.70 ± 0.20	10.50 ± 0.50*	140 ± 3.2	144 ± 6.0*
1st – 50%	23	16	4.14 ± 0.14 <sup>A</sup>	3.42 ± 0.16 <sup>B</sup>	9.89 ± 0.35 <sup>A</sup>	11.79 ± 0.35 <sup>*B</sup>	115 ± 8.3 <sup>A</sup>	170 ± 7.2 <sup>*B</sup>
2nd – 75%	18	4	4.18 ± 0.13 <sup>A</sup>	3.25 ± 0.32 <sup>B</sup>	8.87 ± 0.48 <sup>A</sup>	14.15 ± 0.35 <sup>**B</sup>	94 ± 8.4 <sup>A</sup>	218 ± 7.7 <sup>**B</sup>
Average in Latvia	66		3.60		11.67		168	

\* – for the live weight at age of 50 days and live weight gain per day in 2<sup>nd</sup> research group, different number of asterisks indicates significant differences ( $P < 0.05$ ); A, B – traits with different superscriptions shows significant differences between trial goat groups within separate goat productivity indices ( $P < 0.05$ ).

Comparing goat trial groups and analyzing the effects of different feed rations on goat productivity, the data of newborn kids live weight and kids live weight at the age of 50 days depending on the kid's sex was analyzed (Table 5). Analyzing the data, we can see that the 1st control group kids had higher birth weight ( $P < 0.05$ ) than that of the 2nd research group kids, but there no significant differences between female and male kids within goat group. After supplementary feeding of 2<sup>nd</sup> research group goat mothers with field beans, which affected the milk yield, the growth rate of kids increased. The average increase in live weight of kids per day in 1st group reached only 110 g per day for a 50-day period.

**Table 5.** Productivity of goats according of kids sex

Group	Sex	Number of kids	Live weight, kg		Live weight gain per day till 50 days, kg
			New born	At 50 days	
1st control	Female	23	4.05 ± 0.11	9.19 ± 0.36	0.10 ± 0.008
	Male	20	4.26 ± 0.15	9.85 ± 0.44	0.11 ± 0.008
	Average		4.15 ± 0.09*	9.50 ± 0.28*	0.11 ± 0.006*
2nd research	Female	10	3.43 ± 0.13	12.03 ± 0.60	0.17 ± 0.011
	Male	12	3.33 ± 0.21	12.16 ± 0.16	0.18 ± 0.009
	Average		3.38 ± 0.13**	12.10 ± 0.34**	0.17 ± 0.007**

\* – for the live weight and live weight gain per day, different number of asterisks within each productivity indices indicates significant differences ( $P < 0.05$ ).

Following the Boer goat breeding program, it is necessary to achieve at least 180 g of live weight gain per day, and in 2nd group with optimized goat feeding we achieved more efficient and cost-effective farming, reaching 170 g live weight gain for female kids and 180 g for male kids. Feeding of field beans as additional concentrated feed to goat mothers, the average live weight gain for kids was by 58.2% higher than in 1<sup>st</sup> group, where goat's received only oats as a concentrated feed, and this difference is significant ( $P < 0.05$ ).

In addition to the different feed rations, the effect of litter size on goat's milk yield, kids fast-growing and live weight gain was also analyzed (Table 6).

**Table 6.** Live weight of kids according the litter size

Group	Litter size	Live weight, kg		Live weight gain per day till 50 days, kg	Number of kids
		New born	At 50 days		
1st control.	1	3.93 ± 0.33	10.08 ± 0.98*	0.12 ± 0.016*	6
	2	4.27 ± 0.09	10.06 ± 0.28*	0.12 ± 0.006*	28
	3	3.92 ± 0.25	7.36 ± 0.30**	0.07 ± 0.007**	9
2nd research	1	3.50 ± 0.16*	12.64 ± 0.83*	0.18 ± 0.014	5
	2	3.41 ± 0.19*	12.36 ± 0.35*	0.18 ± 0.009	14
	3	3.00 ± 0.00**	10.00 ± 0.58**	0.14 ± 0.012	3

\* – within each group and each productivity indicator, different number of asterisks indicates significant differences ( $P < 0.05$ ).

Single born kids were heavier than the multiple born kids (Table 6), as they had better opportunities in the uterus of their dams as compared to multiple kids. When analyzing the kids fast-growing and live weight indicators, it was found that there are significant differences ( $P < 0.05$ ) between single or twin with triplet born kids live weight at 50 days (in both goat groups) and live weight gain (in 1<sup>st</sup> group), and the average live weight of triplet born kids at 50 days is even 20 to 31% lower than that of single or twin kids. Also the male kids were heavier than female twin born or triplet born kids. It may be due to the fact that the gestation period of does carrying male kids is usually slightly longer (1–2 days) than those carrying female kids (Afzal et al., 2004).

Comparing the live weight and live weight gain of different crossing level kids, it was found that better fast-growing indices showed kids with higher level (90%) of Boer breed. The average live weight gain in a day to the age of 50 days in kids from 2nd research group with a higher crossing level was 210 to 230 grams per day, which is a very good indicator and is relevant to the characteristics of the fast growing of Boer goat (Ciltsdarba programma..., 2013). Such fast growing indicators can only be achieved with optimized feed rations, where the goat mothers, in addition to the low quality basic diet also receive protein feed. Assessing feed costs for the feeding of the mother's and analyzing the kids live weight, the calculations of feed costs for goats were made. In 1st control group when goats received only hay and oats, the daily ration cost per animal was EUR 0.24 per day, while in 2nd research group where field beans were included in the ration, the price of daily ration was EUR 0.29 (Table 7).

**Table 7.** Economical effect of different feed rations

Group	Feed cost to 1 goat per day, EUR	Kids live weight gain per day per 1 goat, kg	Feed cost for 1 kg of live weight gain, EUR
1st control	0.24	0.22	1.09
2nd research	0.29	0.29	1.00

Including of fodder beans and maize in feed ration at a price of 300 EUR t<sup>-1</sup> increased feed costs per 1 goat per day. However, with optimizing of feed ration it is possible to achieve a higher milk yield of goats and hence also a higher live weight gain for kids, which reduces feed costs by up to 9% for live weight growth.

## CONCLUSIONS

Results of this study provide new knowledge about the influence of field beans on Boer goat productivity. Adding fodder beans and maize into the feed, it is possible to optimize feed ratio for energy and protein supply. Experimental activities had shown that by the inclusion of 0.3 kg of field beans and 0.2 kg maize in feed ratio of goat's mothers, the average increase in live weight gain of kids up to 50 days old was higher than that of kids in which goats' mothers received only oats as concentrated feed. By the optimization of feed ratio, it is possible to achieve a higher milk productivity of goat mothers and hence higher live weight gain for kids, which reduces the feed costs for 1kg of live weight. By optimizing the breeding and feeding of mother goats and kids, it is possible to achieve greater animal fast-growing, hence, more efficient and cost-effective management.

**ACKNOWLEDGEMENTS.** Financial support for the research project was provided by Latvia Ministry of Agriculture.

## REFERENCES

- Afzal, M., Javed, K. & Shafiq, M. 2004. Environmental effects on birth weight in Beetal goat kids. *Pakistan Vet. J.* **24**(2), 104–106.
- Baars, T. 1998. Future systems in organic farming: grassland and fodder production. The future of organic farming systems. *Proceedings of the Fourth ENOF Workshop / The European Network for Scientific Research coordination in organic farming*: Edinburg, UK, pp. 39–48.
- Correa, J.E. 2016. Nutritive value of goat meat. *Alabama Cooperative Extension System UNP-0061*, 1–4.
- Ciltsdarba programma Būru šķirnes kazām 2013.–2018. gadam: <http://www ldc.gov.lv> (in Latvian).
- Hasnain, H.U. 1985. Sheep and Goat in Pakistan. *FAO: Animal production health paper 56*, FAO. Quetta, Pakistan.
- ISO 6491:1998. Animal feeding stuffs. Determination of phosphorus content. ISO/TC34/SC10, ICS: 65.120, ed.2, 7 pp.
- ISO 5984: 2002/Cor.1:2005. Animal feeding stuffs. Determination of crude ash. ISO/TC34, Food products SC 10 ed.3, 10 pp.
- ISO 6496:1999. Animal feeding stuffs. Determination of moisture and other volatile matter content. ISO/TC34/SC10, ICS: 65.120, ed.2, 7 pp.

- Krutzinna, C., Boehncke, E. & Herrmann, H.J. 1996. Organic milk production in Germany. *Biological agriculture and horticulture* **13**, 351–358.
- LVS EN ISO 13906:2008. Animal feeding stuffs. Determination of acid detergent fibre (ADF) and acid detergent lignin (ADL) contents. ISO 13906:2008, ISO/TC34/SC10, 17 pp.
- LVS EN ISO 16472:2006. Animal feeding stuffs. Determination of amylase-treated neutral detergent fibre content. ISO 16472:2006, ISO/TC34/SC10, 15 pp.
- LVS EN ISO 5983-2:2009. Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content. ISO 5983-2:2009, ISO/TC34/SC10, ICS: 65.120, ed. 2. part 2, 15 pp.
- LVS EN ISO 6869:2002. Animal feeding stuffs. Determination of calcium, magnesium and sodium. ISO 6869:2000, ISO/TC34/SC10, ed.1, 15 pp.
- Nutrient Requirements of small ruminants, 2007. National Research Council, National Academy Press, 362 pp.
- Olesen, I., Lindhard, E. & Ebbesvik, M. 1999. Effects of calving season and sire's breeding value in a dairy herd during conversion to ecological milk production. *Livestock Production Science* **61**, 201–211.
- Oman, J.S. & Waldron, D.F. 1999. Effect of breed-type and feeding regimen on goat carcass traits. *Animal Science* **77**, 3215–3218.
- Piliena, K. & Spruzs, J. 2007. *Kazkopiba*. Talsi, 99 pp. (in Latvian).
- Pinkerton, F. & Pinkerton, B. 2015. Feeding programs for meat goats. <http://www.goatworld.com>.
- Selegovska, E. & Spruzs, J. 2010. Interaction between goat nutrition and meat composition *Animal Husbandry* **56**, 64–74.
- Vaarst, M., Roderick, S., Lund, V. & Lockeretz, W. 2004. *Animal health and welfare in organic Agriculture*. CAB International, 426 pp.
- Van Niekerk, W.A. & Casey, N.H. 1988. The boer goat II. Growth, nutrition requirements, carcass and meat quality. *Small Ruminant Research* **1**, 355–368.



## **Influence of inoculation and phosphorus regimes on symbiotic nitrogen fixation and phosphorus use efficiency of Algerian cowpea (*Vigna unguiculata* L. (Walp.)) landraces**

S. Benlahrech\*, G. Kaci, M. Teffahi and S.M. Ounane

Integrative Breeding of Vegetable Production Laboratory, National High School of Agronomy, Avenue Hassen Badi, El Harrach, DZ16200 Algeria

\*Correspondence: [benlahrechsamia@gmail.com](mailto:benlahrechsamia@gmail.com)

**Abstract.** To study the genotypic variation of cowpeas on plant growth and phosphorus (P) uptake which is a function of different P regimes in the soil rhizosphere, 6 landraces of cowpea commonly found in northern Algeria (NE4, NE10, NE11, NKT5, NKT7, NKB7) and 4 landraces from Sahara in southern Algeria (NAG4, NAG5, NAT2 and ND3) were studied during 2013–2014 in greenhouse. They were inoculated with *Mesorhizobium* sp. (S1), *Bradyrhizobium* sp. (S2) and co-inoculation (S1–S2) under three P treatments: no P supply (P0), soluble P (PP) and insoluble P (TCP). Only varieties commonly found in northern Algeria nodulated with soluble P (PP) and inoculation containing *Mesorhizobium* sp. (S1). As a result of the symbiosis, the use of S1 has significantly increased shoot dry weight by 22%, total P content by 20% and P use efficiency for symbiotic nitrogen fixation by 18% compared to no inoculation (T). The landraces from the northern of Algeria expressed a higher growth than those from Sahara in the south of Algeria, especially three of them (NE4, NE10 and NE11) who showed a high performance under all P regimes. We suggest that these three landraces may be useful for improving symbiotic nitrogen fixation in cowpeas when growth is limited by low-P soils and that they could contribute to sustainable farming systems through reduction of farmer's dependence on fertilizers.

**Key words:** Cowpea, Landraces, Nitrogen fixation, Phosphorus, Rhizobia, Symbiosis.

### **INTRODUCTION**

Legumes as a source of symbiotic fixation of atmospheric nitrogen play a major environmental role in cropping systems, which may save nitrogen fertilizer (Graham, 2008). Cowpea (*Vigna unguiculata* L. (Walp.)) is an important leguminous crop that is used for live stocks as feed, as green vegetables, as well as dry beans for human consumption (Murillo–Amador et al., 2006; Goenaga et al., 2008). In Algeria cowpea is mainly consumed for home consumption and subsistence and considered to have therapeutic properties.

Symbiotic nitrogen fixation (SNF) by legume symbionts, or the ability to convert di-nitrogen to ammonia, occurs only within nodules, which are formed specifically in response to the bacterial symbionts (Beattie, 2007). In addition, SNF is highly sensitive to environmental stresses, such as phosphorus deficiencies (Drevon & Hartwig, 1997). The P requirement of legumes is increased when the legume is dependent on N<sub>2</sub> fixation (Leidi & Rodriguez–Navarro, 2000; Gentili & Huss–Danell, 2003). Nodule growth is

more sensitive to P deficiency than plant growth (Drevon & Hartwig, 1997). Thus, P deficiency leads to nodulation delay (Kouas et al., 2005). Indeed, SNF is an energetically expensive process which requires more inorganic P than mineral nitrogen assimilation (Vadez & Drevon, 2001).

Phosphorus (P) is one of the most important plant growth-limiting nutrient in soils besides nitrogen (Deubel & Merbach, 2005; Richardson et al., 2009). Low phosphorus fertility of the soils may be due to low total P content, particularly in soils with low organic matter content. The low availability of P is often due to its insoluble association with such cations as Ca, Al or Fe and its adsorption on surfaces of mineral phases (Alkama et al., 2012).

Ghalmi et al. (2010) identified the various landraces of cowpea cultivated in Algeria, mainly from two regions: Kabylia in northern Algeria (Tizi-Ouzou) and Saharian oases (El Gole'a, Adrar) in southern Algeria. They identified two different cultivar groups of cowpea: Melanophthalmus in Kabylia and Biflora in the Sahara.

Our investigation is the first study to determinate if the geographical origin of accession affects the phosphorus use efficiency (PUE) in Algerian cowpeas. The purpose of the present work was firstly to characterize different cowpea landraces for their ability to establish a symbiosis with rhizobia by checking the effectiveness and infectivity of the strains used, and secondly to investigate their efficiency in nitrogen fixation, and their tolerance to P deficiency in order to reduce production costs and dependence of farmers on soil fertilizers.

## MATERIALS AND METHODS

The present study was conducted during 2013–2014 in pots in a greenhouse at the Experimental Station of ENSA (Ecole Nationale Supérieure Agronomique), Algiers, (36° 30. 50'N). It included ten landraces of cowpeas (*Vigna unguiculata* (L.) (Walp.)) obtained from collection of ENSA (Ghalmi et al., 2010). Seeds characteristics are given in Table 1.

**Table 1.** Characters of Algerian cowpea accessions

NKB7	Bejaia, North	Holstein	Absent	Kidney	Smooth	0.9	23.46
NKT7	Tizi Ouzou, North	Cream	Black	Kidney	Smooth	1.2	27.34
NKT5	Tizi Ouzou, North	Black	Absent	Ovoid	Smooth	0.5	7.83
NAG5	Adrar, Oasis	Black	Black	Globose	Rough	0.5	14.38
NAG4	Adrar, Oasis	Black	Absent	Ovoid	Smooth	0.4	9.49
NAT2	Adrar, Oasis	Brown	Absent	Ovoid	Smooth	0.4	10.82
NE4	El Kala, North	Cream	Black	Globose	Smooth	0.5	14.87
NE10	El Kala, North	Brown	Absent	Ovoid	Smooth	0.5	13.43
NE11	El Kala, North	Black	Absent	Ovoid	Smooth	0.6	15.33
ND3	Djanet, Oasis	Cream	Absent	Rhomboid	Smooth	0.4	10.1

SC – Seed Color; EC – Eye Color; SSH – Seed Shape; ST – Seed texture; SL – Seed Length (cm); WHS – Weight of Hundred Seeds (g).

## Soil

The soil (obtained from the Institut Technique des Cultures Maraîchères Industrielles' experimental station, Algiers (36° 45. 24.3'N)) was selected for its low phosphorus content. It was sterilized and divided into 480 sterile pots with a capacity of 10 kg.

The soil has undergone physical–chemical analysis: (granulometry, pH, and electrical conductivity (CE), CaCO<sub>3</sub> content, Total–N, Total–P and Olsen–P), granulometrical and chemical soil properties of experimental soil are given in Table 2.

**Table 2.** Granulometrical and chemical soil properties

Clay %	Loam %	Sandy %	pH	CE Ms cm <sup>-1</sup>	CaCO <sub>3</sub> %	Total–N g kg <sup>-1</sup>	Total–P mg kg <sup>-1</sup>	Olsen–P mg kg <sup>-1</sup>
20.3	21.5	58.2	7.1	3.725	13.54	0.7	235	20.3

Thus, Olsen–P value reveals that the initial soil (P0 regime) without P input has a low available P rate; 10 times lower than the total P content. This is related to the fact that the soil is calcareous and that much of the total P can be precipitated. The mechanisms of precipitation of P in calcareous soil have been studied previously by Tunesi et al. (1999). In this case P0 is considered as deficient P.

## Bacteria

For this study, two isolates of bacteria (a fast–growing strain as *Mesorhizobium sp.* (S1), and slow–growing strain as *Bradyrhizobium sp.* (S2)) were isolated from northern Algeria' cowpea nodules and obtained from collection of ENSA, Algiers, Algeria. Isolates were cultivated on Yeast Extract Agar medium (YEAM), inoculums were prepared in YEAM without addition of Agar.

## Culture conditions and experimental device

The ten landraces were grown under three different P regimes (deficient P (P0), soluble inorganic P as KH<sub>2</sub>PO<sub>4</sub> (PP) 0.2 g per 10 kg of soil and insoluble Tricalcium phosphate as Ca<sub>3</sub> (PO<sub>4</sub>)<sub>2</sub>, 6H<sub>2</sub>O (TCP) 0.7 g per 10 kg of soil). These P regimes were combined with four inoculation types (control without inoculant (T), inoculated with *Mesorhizobium sp.* (S1), *Bradyrhizobium sp.* (S2) or doubly inoculated (S1–S2)). The experiment was organized into a split–plot design with four replications for each combination of landraces, P regime and inoculation type, providing the 480 pots (10 x 3 x 4 x 4). The seeds of cowpeas were surface–sterilized using absolute ethanol for 10 seconds followed by the sodium hypochlorite at 12 ° for 5 minutes; followed by 10 washes in sterile distilled water, and then sown. The seedlings were inoculated by covering with 1ml of bacterial culture containing 10<sup>8</sup>bacteria mL<sup>-1</sup> and 10<sup>9</sup>bacteria mL<sup>-1</sup> respectively for *Mesorhizobium sp.* and *Bradyrhizobium sp.*

## Data collection

At full flowering stage, which is spread out according to the precocity of the landraces, shoots were separated from roots at the cotyledon node, dried for 48 h at 75 °C and weights recorded. However, for landraces which has nodulated, nodules were detached from the roots, counted, dried and dry weight determined.

### Total P concentration

Plant P uptake corresponded to the total P taken up by plants during plant growth. Total P concentration in plant (shoot, root and nodules) was determined by green malachite method after digestion by nitric and perchloric acids according to Valizadeh et al. (2003). It was calculated as follows: P uptake (total P content) = [P concentration in shoot ( $\text{mg g}^{-1}$ )  $\times$  shoot dry weight (g)] + [P concentration in root ( $\text{mg g}^{-1}$ )  $\times$  root dry weight (g)] + [P concentration in nodule ( $\text{mg g}^{-1}$ )  $\times$  nodule dry weight (g)] / 1,000.

### Phosphorus use efficiency (PUE)

PUE represents the efficiency of use of phosphorus by the plant. There are several ways to design and calculate PUE, from physiological point of view to agronomic point of view (Baligar et al., 2011; MacDonald et al., 2011). In this study, we chose to calculate a physiological PUE which is the ratio between the dry weight of the whole plant and the concentration of phosphorus in the plant (Vadez & Drevon, 2001).

### Statistical analyzes

Effects of inoculation and P nutrition on plant growth, nodulation, P concentration in plant and plant P uptake were tested using multi-way analyses of variance (ANOVA) with R software, version 3.0.2 (R Core Team, 2013), using Rcmdr package (Fox, 2005) and RcmdrPlugin.EZR (Kanda, 2013), considering landraces, inoculation treatments and soil P regimes as factors. The means were compared by Tukey's multiple comparison test at 0.05 probability. Graphics were performed using RcmdrPlugin.FactoMineR (Husson et al., 2014) and RcmdrPlugin.KMggplot2 (Triad & Kengo, 2013).

## RESULTS AND DISCUSSION

### Plant Growth expression under different P regimes and inoculation types

In order to assess the effects of P deficiency on growth and SNF, the dry weight of the different landraces was evaluated considering shoot measurement under different P regimes and different inoculation types (Fig. 1). The ANOVA results of shoot dry weight are reported in Table 3.

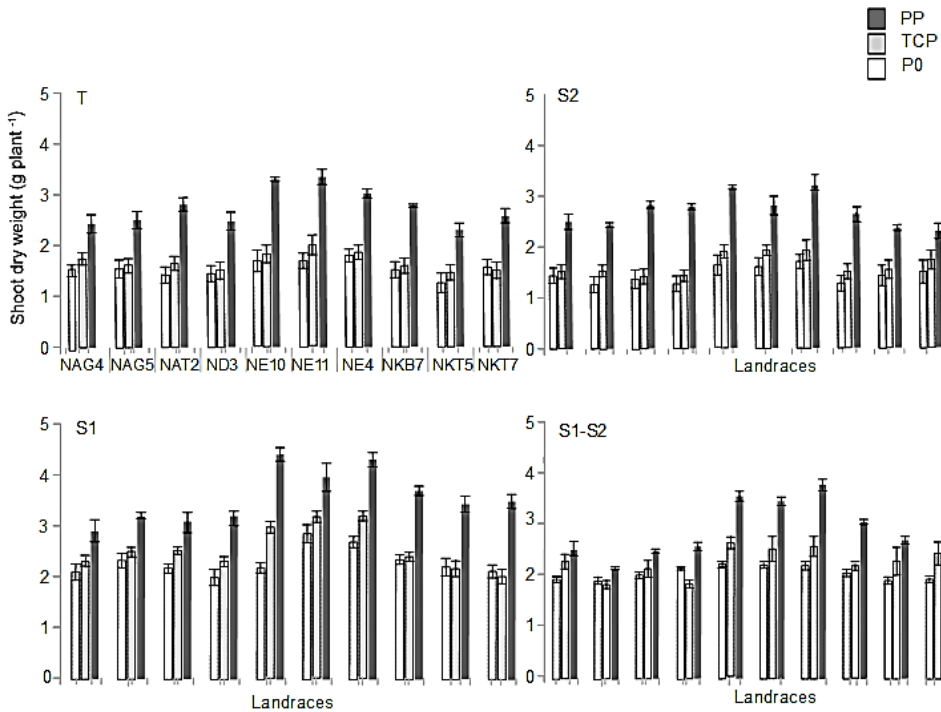
**Table 3.** ANOVA results of sDW

	Sum Sq	Df	F value	Pr(> F)
Factor1.P regime	319.6	2	293.8886	< 2.2e-16 ***
Factor2.Landraces	10.6	9	2.1617	0.024105 *
Factor3.Inoculation	68.8	3	42.2055	< 2.2e-16 ***
Factor1 X Factor2	28.1	18	2.8712	9.461e-05 ***
Factor1 X Factor3	12.0	6	3.6657	0.001512 **
Factor2 X Factor3	16.4	27	1.1156	0.317789
Factor1XFactor2XFactor3	19.9	54	0.6771	0.959624
Residuals	195.7	360		

\*\*, \*\*\* – Significant difference at  $p < 0.01$  and  $p < 0.001$ , respectively.

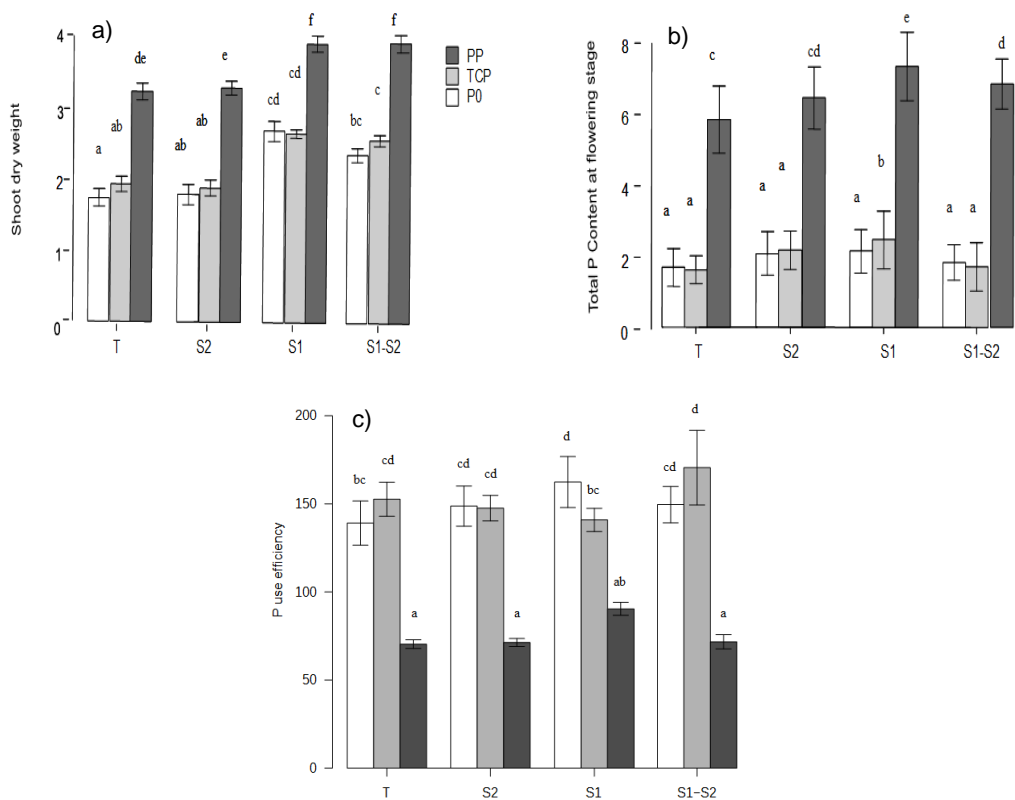
The results showed that the P regime, landraces and the inoculation type significantly affected the shoot dry weight (sDW). There were also interaction effects between P regime and landraces and P regime and inoculation type.

sDW varied between 1.4 and 3.2 g sDW pl<sup>-1</sup> for NKT5 and NE11 without inoculation (Fig. 1, T), between 1.2 and 3.1 g sDW pl<sup>-1</sup> for NAG5 and NE11 for inoculation S2 (Fig. 1, S2), between 2 and 4.5 g sDW pl<sup>-1</sup> for NAG4 and NE10 for inoculation S1 (Fig. 1, S1) and between 1.8 and 3.3 g sDW pl<sup>-1</sup> for NAG5 and NE4 for co-inoculation S1–S2 (Fig. 1, S1–S2). Landraces NE10 and NE4 expressed the highest production potential with more than 4 g sDW pl<sup>-1</sup> under soluble P regime (PP). Growth under TCP varied between 1.3 pl<sup>-1</sup> for landraces from Saharian region and 3 g sDW pl<sup>-1</sup> for landraces from the north of Algeria. For all P regimes, the highest sDW were found for the landraces NE4, NE11, and NE10, from the north of Algeria.



**Figure 1.** Effect of P regimes on shoot dry weight for landraces under control without inoculation (T), inoculation with (S2), inoculation with (S1) and co-inoculation (S1–S2). Data are means and SD of 4 harvested plants.

As indicated by the ANOVA in Table 3 and by Fig. 2, a, P regimes and inoculation significantly affected the shoot biomass. For a given inoculation type, the soluble P regime (PP) resulted in a significant increase in the shoot biomass compared to insoluble P (TCP) and deficient P (P0) regimes. Also, for a given P regime, the shoot dry weight significantly increased with inoculation with the fast growing strain *Mesorhizobium sp.* (S1) or S1–S2, compared to no inoculation (T) or inoculation with the slow growing strain *Bradyrhizobium sp.* (S2). Then, inoculation S1 increased shoot dry weight not only for PP regime but also for TCP and P0 regimes by 22% and 30% respectively (Fig. 2, a)



**Figure 2.** Effect of P regimes (P0, TCP and PP) and inoculation types (T, S2, S1 and S1–S2) on shoot dry weight (g plant<sup>-1</sup>) (a), on total P content (mg P g plant<sup>-1</sup> DW) (b) and on P use efficiency (P<sup>-2</sup> dry weight mg<sup>-1</sup> P) (c). Values are the means of 40 replicates. Bars indicate standard errors. Mean values labelled with the same letter were not significantly different at  $p < 0.05$ .

### Variability in phosphorus use efficiency (PUE) response for SNF

In order to evaluate the PUE of landraces, a first evaluation of the impact of P deficiency was performed by measuring the total P content of the plants at flowering stage.

Table 4 shows the ANOVA results for total P content in the plants. This P content was significantly influenced by the P regime, the inoculation type, and the landraces, with also an interaction between P regime and inoculation. Under soluble P regime (PP), total P content increased, by more than 20% and 16% respectively for inoculation with S1 and co-inoculation with S1–S2 compared to no inoculation (T) (Fig. 2, b). Under insoluble P regime (TCP), inoculation with S1 significantly increased P content by 20% compared to the other inoculation types (Fig. 2, b).

Furthermore, in response to P deficiency, plants can adjust their internal P requirement by optimizing the metabolic phosphorus use efficiency (PUE) by producing overall more biomass per unit of P consumed, which in fixing more N<sub>2</sub> per unit of P for a SNF-dependent legume (Vadez & Drevon, 2001). In Table 5 are reported the ANOVA results of physiological PUE.

From this ANOVA, P regimes and inoculation type significantly affected the P use efficiency. There was also an interaction between P regimes and inoculation type.

The inoculation with *Mesorhizobium sp.* (S1) significantly increased the PUE by about 8% and 11% respectively for soluble P (PP) and (P0) compared to other inoculation type (Fig. 2, c). Whereas, the co-inoculation S1–S2 significantly increased the PUE by more than 10% under insoluble P. (Fig. 2, c) shows a strong decrease of PUE for PP regime compared to P0 regime and TCP regime.

**Table 4.** ANOVA results of total P content

	Sum Sq	Df	F value	Pr(>F)
Factor1.P regime	2294.8	2	2175.1601	< 2.2e-16 ***
Factor2.Landraces	10.2	9	2.1409	0.02561 *
Factor3.Inoculation	35.1	3	22.1865	3.327e-13 ***
Factor1 X Factor2	3.8	18	0.4038	0.98678
Factor1 X Factor3	37.1	6	11.7201	5.245e-12 ***
Factor2 X Factor3	8.7	27	0.6078	0.94052
Factor1XFactor2 XFactor3	16.6	54	0.5835	0.99151
Residuals	189.9	360		

\*, \*\*\* – Significant difference at  $p < 0.05$  and  $p < 0.001$ , respectively.

**Table 5.** ANOVA results of PUE

	Sum Sq	Df	F value	Pr(>F)
Factor1.P regime	554021	2	60.6961	< 2e-16 ***
Factor2.Landraces	78297	9	1.9062	0.04996 *
Factor3.Inoculation	9845	3	0.7191	0.54111
Factor1 X Factor2	39945	18	0.4862	0.96337
Factor1 X Factor3	64143	6	2.3424	0.03120 *
Factor2 X Factor3	77921	27	0.6324	0.92467
Factor1XFactor2 XFactor3	96135	54	0.3901	0.99997
Residuals	1642999	360		

\*, \*\*, \*\*\* – Significant difference at  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ , respectively.

### Plant nodulation

Dry matter and number of nodules were evaluated in the different landraces (Fig. 3).

No nodules were found without supply of P (P0) and with supply of insoluble P (TCP P regime), and nodulation was only observed under soluble P regime (PP). This shows that nodulation does not occur in P deficiency conditions.

The landraces and the inoculation type affected significantly the nodule dry weight (nDW) as indicated by the ANOVA results of nodule dry weight for the 6 landraces having nodules under PP regime (Table 6).

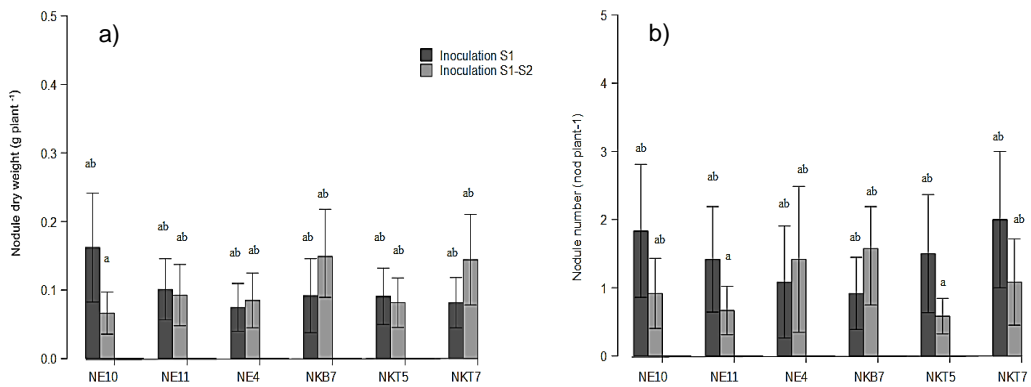
**Table 6.** ANOVA results of nodule dry weight

	Sum Sq	Df	F value	Pr(>F)
Factor1.Landraces	0.5913	9	2.5197	0.008013 **
Factor2.inoculation	0.7487	3	9.5710	3.912e-06 ***
Factor1 X Factor2	0.8432	27	1.1976	0.229169
Residuals	11.4732	440		

\*\*, \*\*\* – Significant difference at  $p < 0.01$  and  $p < 0.001$ , respectively.

The nodulation only occurred for landraces from the north of Algeria, namely; NE10, NE11, NE4, NKT7, NKB7, and NKT5, and for the inoculations including *Mesorhizobium sp.* (S1 or S1–S2). For these northern landraces nodule dry weight nDW was found to vary between 50 and 180 mg nDW  $\text{pl}^{-1}$  for double inoculation with S1–S2 and between 80 and 230 mg nDW  $\text{pl}^{-1}$  for inoculation with S1 (Fig. 3, a).

Within the landraces that provided nodules, the nodule number was also influenced by the inoculation type and the cowpea landraces as indicated by Fig. 3, b, for inoculation with S1, the 6 landraces showed high nDW for S1. On the other hand, for double inoculation S1–S2, NE10, NE4 NKB7, and NKT7 significantly showed a higher nodule number than NE11 and NKT5.

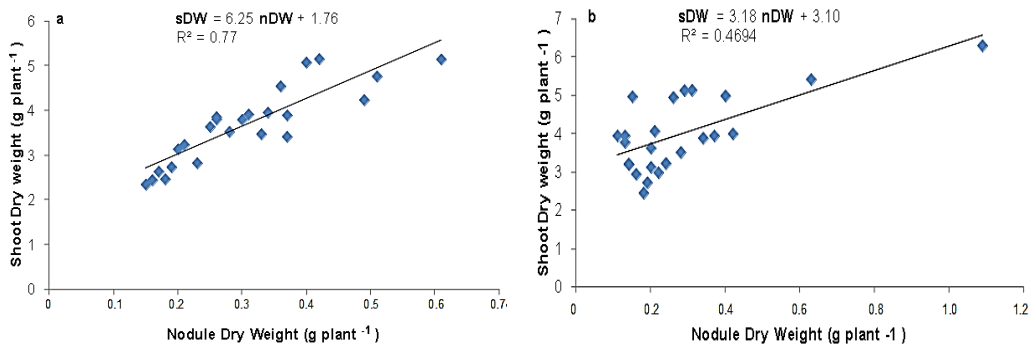


**Figure 3.** Effect of landraces and inoculation on nodule dry weight ( $\text{g plant}^{-1}$ ) (a) and nodule number (b) for PP regime. Values are the means of 4 replicates. Bars indicate standard errors. Mean values labeled with the same letter were not significantly different at  $p < 0.05$ .

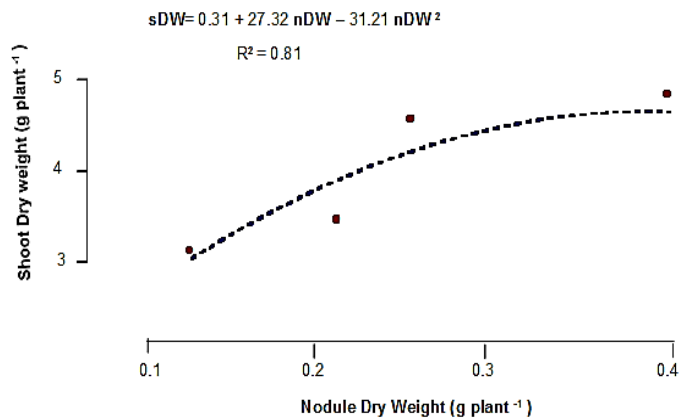
### Efficiency in use of the rhizobial symbiosis for plant growth

In order to assess the efficiency in use of the rhizobial symbiosis (EURS), the values of the shoot biomass were plotted against their corresponding nodule biomass. The slopes of the regressions were considered as an estimate of the EURS. This was achieved under PP regime (the only one having provided nodules) with S1 inoculation type (Fig. 4, a) and co-inoculation S1–S2 (Fig. 4, b). We first proceeded by grouping together all the landraces having provided nodules (i.e the northern landraces). Then we proceeded for each landrace. Fig. 4, a shows, for all the northern landraces together, that shoot and nodule biomass of northern landraces were positively correlated (up to  $R^2 = 0.77$ ) under inoculation type S1. Whereas Fig. 4, b shows a positive correlation between shoot and nodule biomass up to  $R^2 = 0.46$ . However, by considering landraces individually, only landrace NE10 showed a positive correlation between sDW and nDW under inoculation S1 with  $R^2 = 0.81$  (Fig. 5).





**Figure 4.** Global relationship between nodule dry weight and shoot dry weight for northern landraces under PP regime for inoculation S1(a) and co-inoculation S1–S2 (b). Data are means standard errors of 24 replicates.



**Figure 5.** Correlation between nodule dry weight and shoot dry weight for NE10 under inoculation S1 and PP regime. Data are means standard errors of 4 replicates.

## GENERAL DISCUSSION

The aim of this research was to investigate the ability of ten different cowpea landraces to establish a symbiosis with rhizobia and their efficiency in nitrogen fixation. Nodulation did not occur for all landraces, the process was observed only for northern landraces under PP regime and inoculations including S1 (Fig. 3). Thus, *Mesorhizobium sp.* (S1) was better at infecting than *Bradyrhizobium sp.* (S2). The lack of infectivity of S2 can be explained by the fact that legume root nodules may contain a range of bacteria that are unable to nodulate their hosts (Sprent, 2009).

Depret & Laguerre (2008), reported that different rhizobial genotypes may be better at infecting legume roots and producing effective nodules at different stages of host development. However, the nodulation for SNF with co-inoculation S1–S2 for PP showed a slight decrease compared to S1, which may have resulted in competition established between both of rhizobia strains. Wielbo et al. (2007) demonstrated that

competitiveness is affected by edaphic factors and soil nutrients, in this case by P availability.

No nodulation of *Mesorhizobium sp.* (S1) under TCP and P0 may be explained by a low tolerance to low levels of P for the fast-growing strains (Raman et al., 2006).

Landraces from oasis in Saharian region showed no nodulation with the two rhizobial strains that we tested so low SNF capacity. This might be attributed to their original domestication promoting a high level of specificity for the symbiosis rhizobia-cowpea genotype. In fact S1 and S2 are strains isolated from northern landraces, where they are adapted.

The nodulation and as a result, the SNF in the cowpea landraces, was found to be influenced by P deficiency. Thus, P deficiency particularly affects the rhizobial symbiosis (Attar, 2014). In fact, P appears to be essential for both nodulation and nitrogen fixation (Rai, 2006).

The increased plant growth under PP shown in Fig. 1 is likely attributed to enhanced P nutrition (Fig. 1). In addition to nitrogen-fixation, enhancement of legume symbioses is often manifested by an increase in the phosphorus content of the plant (Vessey, 2003).

The increase of shoot dry weight with S1 under P0 and TCP could result from a phosphatase release by S1, which can contribute to increase P availability to the plant. This could explain the increase of P content observed with S1 under P0 and TCP (cf. Fig. 2,b).

The greater sDW for NE4, NE11, and NE10 landraces under all P regimes compared to others landraces may be related to enhanced phosphorus use efficiency. In fact, one of the strategies employed by plants in response to P deficiency consists in optimizing the metabolic phosphorus use efficiency (PUE). This is achieved by producing higher overall biomass per unit of P consumed, through fixing more N<sub>2</sub> per unit of P for an SNF-dependent legume (Vadez & Drevon, 2001).

Our findings presented in Fig. 4 also revealed a positive correlation for inoculation S1 and co-inoculation (S1-S2) under PP regime between the biomass of symbiotic nodules nDW and the shoot dry weight sDW. This correlation may indicate that the symbiotic nitrogen fixation is effective under P sufficient conditions. The increase in EURS suggests high regulation between EURS and the plant P requirement, probably in relation to the high energy requirement of the SNF process (Lazali et al., 2013). This finding is in agreement with previously published data (Attar, 2014).

## CONCLUSIONS

The presented research was focused on the influence of inoculation and phosphorus regimes on symbiotic nitrogen fixation and phosphorus use efficiency of Algerian cowpea landraces. It was determined that:

- Soluble P enhances the growth of plants, their total P content and nodulation.
- The fast-growing *Mesorhizobium sp.* (S1) is better at infecting and effectiveness than the slow-growing *Bradyrhizobium sp.* (S2).
- S1 increased shoot dry weight under P0 and insoluble P (TCP). This could be due to releasing of phosphatase by S1, which can contribute to enhance P availability.

- Only northern landraces showed nodulation with soluble P regime and inoculations with fast growing strains *Mesorhizobium* sp. (S1). As the soil was sterilised before inoculation and as the tested strains came also from the northern region, this suggests that there are probably regional adaptations between the rhizobia strains and the cowpea landraces.
- The strain S1 significantly increased the phosphorus use efficiency by 8% under P soluble conditions.
- The landraces NE4, NE11 and NE10 exhibited a higher performance under all P regimes with S1 inoculation. Therefore, these landraces may be used as a source of genetic diversity for cowpea production to improve PUE for SNF potential, and their adaptation to the climatic conditions of the South could be tested.

However, further studies on field experiments are needed to identify whether similar results may be observed with a more extensive array of cowpea landraces and rhizobia potentially adapted to southern landraces whether their potential differences in PUE are associated with genotype–rhizobia interaction in adaptation to low–P soils.

ACKNOWLEDGEMENTS. This work was supported by the laboratory of Laboratoire d'Amélioration Intégrative des Productions Végétales (LAIPV) from the 'Ecole Nationale Supérieure Agronomique' (ENSA) of Algiers. We are grateful to Dr Ghalmi N. and his team for putting at our disposal the cowpea collection.

## REFERENCES

- Alkama, N., Ounane, G. & Drevon, J.J. 2012. Is genotypic variation of H<sup>+</sup> efflux under P deficiency linked with nodulated–root respiration of N<sub>2</sub>–Fixing common–bean (*Phaseolus vulgaris* L.)?. *Journal of plant physiology* **169**, 1084–1089.
- Attar, H. 2014. Phosphorus Availability and Proton Efflux of Nodulated–root Varies among Common–bean Genotypes (*Phaseolus vulgaris*) in Rhizobox. *International Journal of Agriculture, Environment and Biotechnology* **7**, 391–401.
- Baligar, V.C., Fageria, N.K. & He, Z.L. 2001. Nutrient use efficiency in plants. *Communications in Soil Science and Plant Analysis* **32**(7–8), 921–950.
- Beattie, G.A. 2007. Plant–associated bacteria: survey, molecular phylogeny, genomics and recent advances. In *Plant–associated bacteria*. Springer, Netherlands, pp.1–56.
- Depret, G. & Laguette, G. 2008. Plant phenology and genetic variability in root and nodule development strongly influence genetic structuring of *Rhizobium leguminosarum biovar viciae* populations nodulating pea. *New Phytologist* **179**, 224–235.
- Deubel, A. & Merbach, W. 2005. Influence of microorganisms on phosphorus bioavailability in soils. In *Microorganisms in soils: roles in genesis and functions*. Springer, Berlin, Heidelberg, pp.177–191.
- Drevon, J.J. & Hartwig, U.A. 1997. Phosphorus deficiency increases the argon–induced decline of nodule nitrogenase activity in soybean and alfalfa. *Planta* **201**, 463–469.
- Fox, J. 2005. The R Commander: A Basic Statistics Graphical User Interface to R. *Journal of Statistical Software* **14**(9), 1–42.
- Gentili, F. & Huss–Danell, K. 2003. Local and systemic effects of phosphorus and nitrogen on nodulation and nodule function in *Alnus incana*. *J. Exp. Bot.* **54**, 2757–2767.
- Ghalmi, N., Malice, M., Jacquemin, J.M., Ounane, S.M., Mekliche, L. & Baudoin, J.P. 2010. Morphological and molecular diversity within Algerian cowpea (*Vigna unguiculata* (L.) Walp.) landraces. *Genetic resources and crop evolution* **57**, 371–386.

- Goenaga, R., Gillaspie, V. & Quiles, A. 2008. Assessing yield potential of cowpea genotypes grown under virus pressure. *Hort Sci.* **43**, 673–676.
- Graham, P.H. 2008. Ecology of the root–nodule bacteria of legumes. In: Dilworth, M.J., James, E.K., Sprent, J.I. and Newton, W.E. (eds) *Leguminous nitrogen–fixing symbiosis*. Springer, Netherlands, pp.23–58.
- Husson, F., Josse, J. & Le, S. 2014. RcmdrPlugin.FactoMineR: Graphical User Interface for FactoMineR. R package version 1.5-0. <http://CRAN.R-project.org/package=RcmdrPlugin.FactoMineR>
- Lazali, M., Zaman-Allah, M., Amenc, L., Ounane, G., Abadie, J. & Drevon, J.J. 2013. A phytase gene is overexpressed in root nodules cortex of *Phaseolus vulgaris*–rhizobia symbiosis under phosphorus deficiency. *Planta* **238**(2), 317–324.
- Leidi, E.O. & Rodriguez–Navarro, D.N. 2000. Nitrogen and phosphorus availability limit N<sub>2</sub> fixation in bean. *New Phytol.* **147**, 337–346.
- Kanda, Y. 2013. Investigation of the freely available easy-to-use software {EZR} for medical statistics. *Bone Marrow Transplantation* 2013 **48**, 452–458, advance online publication, December 3, 2012.
- Kouas, S., Labidi, N., Debez, A. & Abdelly, C. 2005. Effect of P on nodule formation and N fixation in bean. *Agron. Sustain.* **25**, 389–393.
- MacDonald, G.K., Bennett, E.M., Potter, P.A. & Ramankutty, N. 2011. Agronomic phosphorus imbalances across the world's croplands. *Proceedings of the National Academy of Sciences* **108**(7), 3086–3091.
- Murillo–Amador, B., Troyo–Diéguez, E., García–Hernández, J.L., López–Aguilar, R., Ávila–Serrano, A.G., Zamora–Salgado, S., Rueda–Puente, E.O. & Kaya, C. 2006. Effect of NaCl salinity in the genotypic variation of cowpea (*Vigna unguiculata*) during early vegetative growth. *Hort Sci.* **108**, 423–431.
- Rai, M. 2006. *Handbook of microbial biofertilizers*. CRC, New York, USA, 385p.
- Raman, N., Selvaraj, T. & Rai, M. 2006. Tripartite relationship of Rhizobium, AMF, and host in growth promotion. In: M. Rai (Eds). *Handbook of Microbial Biofertilizers*, New York, USA, pp.51–88.
- R Core team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Richardson, A.E., Hocking, P.J., Simpson, R.J. & George, T.S. 2009. Plant mechanisms to optimize access to soil phosphorus. *Crop Pasture Sci.* **60**, 124–143
- Sprent, J. 2009. *Legume nodulation*. John Wiley & Sons, United Kingdom, pp.36–45.
- Triad S. & Kengo N. 2013. RcmdrPlugin.KMggplot2: An Rcmdr Plug-In for Kaplan–Meier Plots and Other Plots by Using the ggplot2 Package. R package version 0.2-0. <http://CRAN.R-project.org/package=RcmdrPlugin.KMggplot2>.
- Tunesi, S., Poggi, V. & Gessa, C. 1999. Phosphate adsorption and precipitation in calcareous soils: the role of calcium ions in solution and carbonate minerals. *Nutrient Cycling in Agroecosystems* **53**(3), 219–227.
- Vadez, V. & Drevon, J.J. 2001. Genotypic variability in P use efficiency for symbiotic N<sub>2</sub> fixation in common–bean (*Phaseolus vulgaris* L.). *Agronomie* **21**, 691–699.
- Valizadeh, G.R., Rengel, Z. & Rate, A.W. 2003. Response of wheat genotypes efficient in P utilization and genotypes responsive to P fertilisation to different P banding depths and watering regimes. *Australian Journal of Agricultural Research* **54**, 59–65.
- Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and soil* **255**, 571–586.
- Wielbo, J., Marek–Kozaczuk, M., Kubik–Komar, A. & Skorupska, A. 2007. Increased metabolic potential of *Rhizobium spp.* is associated with bacterial competitiveness. *Canadian journal of microbiology* **53**, 957–967.

## **Intensification of rapeseed drying process through the use of infrared emitters**

V. Bulgakov<sup>1</sup>, V. Bandura<sup>2</sup>, M. Arak<sup>3</sup> and J. Olt<sup>3,\*</sup>

<sup>1</sup>National University of Life and Environmental Sciences of Ukraine, 15, Heroyiv Oborony street, UA03041 Kyiv, Ukraine

<sup>2</sup>Vinnitsia National Agrarian University of Ukraine, Soniachna street 3, UA21008 Vinnitsia, Ukraine

<sup>3</sup>Estonian University of Life Sciences, Kreutzwaldi 56, EE51014 Tartu, Estonia;

\*Correspondence: [jyri.olt@emu.ee](mailto:jyri.olt@emu.ee)

**Abstract.** The theoretical basis of the agricultural material drying techniques has been formed in the world for two and a half centuries. Nevertheless, despite the multitude of studies well presented in the literature, the industry still lacks universally recognized methods of design calculation for the majority of drying problems. Presumably, this is due to the fact that the dehydration of foodstuffs is one of the most energy-intensive and complicated work processes. In the process of thermal treatment during the drying, the physical state of the water in the treated item changes, the properties of the item itself are altered.

Recently, the drying of foodstuffs by means of infrared (IR) radiation has been gaining ever wider acceptance. At the same time, the technologies for dehydrating foodstuffs and materials are now far ahead of the theory of drying. Not only the classical literature on the drying problems, but even special studies on the subject cannot provide any specific recommendations for the design of installations with the electro-magnetic principle of energy input. Whereas that is exactly the type of installations that are now actively making headway, while offering serious challenges in their modelling. For that reason, experimenting has until now been the only reliable way of their studying. Despite the available extensive scientific literature on the drying, including works on IR units, the practical issues of engineering IR driers have not been tackled. All the existing studies consider solely particular cases. This study discusses the process of drying the fixed bed of rapeseeds with the use of the electromagnetic infrared emitter. The energy intensity of the process and the optimum thickness of the product bed are determined. The aim of the paper is to examine the effect that the operating condition parameters (energy input intensity, temperature, grain bed thickness) have on the drying kinetics and the energy characteristics of the process. The methods of research are based on the thermophysical analysis of the material's structure. The experimental studies were carried out with the use of monitoring and measuring equipment, up-to-date methods and instruments, including solutions developed by the authors. The analytical treatment was performed with the use of the software packages: MathCAD, Excel. The sample testing and examination took place in the laboratory and the methods were in compliance with the applicable standards. A comprehensive experimental investigation of the effect that the operating parameters (specific load and power) have on the rape seed IR-drying kinetics had been accomplished

**Key words:** agricultural engineering, rape, infrared emitters, drying, load.

## INTRODUCTION

The thermal treatment (heating) of grain is applied, as is known, in order to disinfect the grain prior to putting it to storage, predry it in order to improve the efficiency of the separation, micronisation of cereals, fodder grain crops, intensify the process of drying and oil extraction from oil crops. In this process, different equipment is employed in each case in terms of the method of heat input. In the equipment that is used most frequently for the preheating and drying of grain (cascade heaters, tower driers with air ducts), direct contact between the grain and the heat carrier gas or heat carrier-heated surface (gas duct, shelf etc.) is utilised. The main way of intensifying the heat and mass transfer between the surface and the heat carrier is increasing the latter's flow rate. But, the potential of the said process intensification method is limited by the removal of the material from the treatment zone and the inefficient excessive consumption of energy, which is lost with the exhaust heat carrier. It is possible to reduce the total amount of heat carrier and, accordingly, the heat consumption, if a no-contact method of thermal energy input is used. One of the efficient methods of transferring heat to grain without contacting it is the infrared irradiation of a layer of dispersed material.

Today's interpretation of the process of dehydration during drying is based on the scientific fundamentals of the heat and mass transfer during the change of state (Orsat et al., 2007; Wang et al., 2007; Wang et al., 2011) and the theory by Rebinder & Shchukin (1973) about the forms of bonding between moisture on the one hand and colloid and capillary-porous materials on the other hand. The theory of drying, the foundations of which were laid by A.V. Lykov (1968), had evolved in the works (Ginsburg, 1985). There are schools of: convection drying (Nikitenko et al., 2008; Aboltins & Upitis, 2012; Doymaz, 2014), filtration drying (Barna et al., 2013), dielectric drying (Jones & Rowley, 1996), dispersed material drying (Burdo, & Bezbah, 2008; Burdo et al., 2017), vibratory fluidized bed drying (Kats & Mazor, 2010).

Infrared radiation is the electromagnetic radiation within a wavelength range of 0.75 to 1,000  $\mu\text{m}$ . This range is divided into the three bands: near infrared (0.75–1.4  $\mu\text{m}$ ), intermediate infrared (1.4–3  $\mu\text{m}$ ) and far infrared radiation (3–1,000  $\mu\text{m}$ ).

The absorption of infrared radiation in the absorption spectra of foodstuffs of plant and animal origin results from the combined absorption by all components constituting the cells. Accordingly, superposition of individual lines is responsible for the appearance of the continuous spectrum with wavelengths of  $\lambda > 3.0 \mu\text{m}$  (Sandu, 1986).

It has been established (Wang et al., 2007) that the interaction between light and foodstuff manifests in the continuous reflection of heat and dispersion of light. The heat reflection is determined by the colour of the material. In case of near infrared radiation, around 50% of all rays reflect back, while far infrared radiation reflects at a rate of less than 10%. Experimental observations (Krishnamurthy et al., 2008) have shown that, as the thickness of the layer becomes greater, the radiation admittance declines and the reflection from the surface increases.

Overall, it has been noted (Sandu, 1986; Krishnamurthy et al., 2008; Das et al., 2009; Riadh et al., 2015) that solid materials absorb infrared radiation only in the thin surface layer. Another finding has been that with the temperature of the IR emitter getting lower the main radiation wavelength shifts towards the area of lesser absorption of infrared rays by the product. The development of continuous-operation dryers equipped with far and near infrared spectrum units can allow reducing the material costs,

duration of drying and process temperatures. Anyway, the thickness of the product layer may not exceed 5 mm.

In order to ensure the reduced metal intensity of the equipment, the uniform distribution of the heat flows over the receiving surfaces and the improved quality of the semi-processed plant raw materials, reflectorless infrared film driers are used most frequently. Our experimental investigations on this issue have shown that infrared film driers have low metal consumption rates. Moreover, the emitter is easy to install, has low inertia, the low temperature of the working surface (45–85 °C) and the IR-wave length (3–15 μm) that is acceptable for drying plant raw materials, in particular, rape seeds (Kiptela et al., 2017)

The synergistic effect due to the combination of IR heating and convection drying has shown in the reduction of the process duration by 43% and the energy consumption by 63% as compared to the conventional convection drying. Also, it has been found that infrared radiation improves the effectiveness of inactivation of enzymes, pathogenic germs, thus contributing to the improvement of the product’s quality and its shelf life.

When determining the optimum operating mode in the drying of rape seeds with a purpose of bringing them to the standard condition, several factors have to be taken into account, depending on the following use of the seeds – either for industrial processing or as seed grain (Table 1).

It appears that the mentioned observations could be useful in the development of improved grain drying facilities.

The aim of the study is to examine the effect that the operating parameters (energy input intensity, temperature, grain bed thickness) have on the drying kinetics and the energy characteristics of the process.

**Table 1.** Rapeseed shaft drying modes in relation to end use of seeds (Beregovaya & Stankevits, 2001)

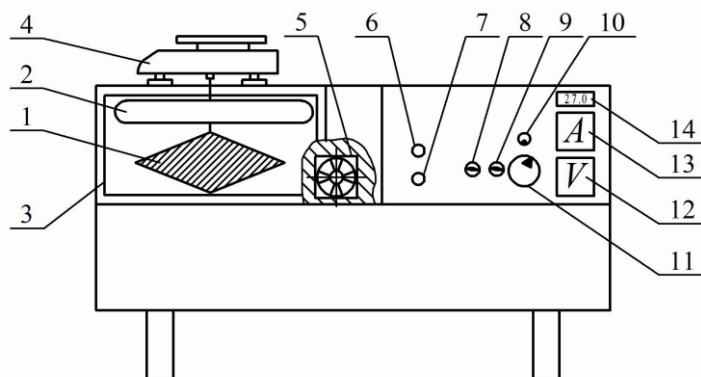
Initial moisture content, $\omega$ (%)	Maximum temperature (°C)	
	drying medium	seeds
<b>1. Seeds for foodstuffs and combined fodders</b>		
$\omega \leq 10$	85	62
$10 < \omega \leq 15$	83	56
$15 < \omega \leq 20$	80	52
$20 < \omega \leq 25$	78	50
$\omega > 25$	75	48
<b>2. Seeds for sowing</b>		
$\omega \leq 17$	65	40
$17 < \omega \leq 19$	60	37
$\omega > 19$	55	35

## MATERIALS AND METHODS

The investigation with the use of a fixed bed of rape seeds was carried out on the test bench (Figs 1 and 2) comprising IR-chamber, electronic scales, two IR-generators, trays with grain, system of measurement for registering the temperature of grain and air in the chamber as well as the energy consumption.

The movement of air in the chamber was created by a fan. The square tray measuring 200 mm x 200 mm with a 10-mm high flange was made of metal wire mesh. The tray hung from the scales on a system of wire cables with adjustable lengths, which enabled setting the required distance between the product and the emitters within a range of 7–15 cm.

The role of IR-radiation generators was played by quartz short-wave luminous elements with a wattage of 550 W, which ensured the attainment of the required temperature conditions virtually in 30 seconds. The supply voltage was controlled by the system comprising a laboratory transformer and a 2.5 accuracy class ammeter and a 1.5 accuracy class voltmeter. The readings of the ammeter and voltmeter were used to find the consumed power.



**Figure 1.** Schematic representation of experimental unit for drying with the use of infrared emitter: 1 – tray; 2 – infrared emitter; 3 – drying chamber; 4 – electronic scales; 5 – fan for drawing air out of chamber; 6 – unit start switch; 7 – unit stop switch; 8 – fan start switch; 9 – infrared emitter start switch; 10 – changeover switch for indication of temperature of product or air in chamber; 11 – infrared emitter power control; 12 – voltmeter; 13 – ammeter; 14 – indicator of temperature of product and air in chamber.

The velocity of the air leaving the chamber was measured with electronic anemometer-thermometer-hygrometer Testo 410-2. The air velocity was changed within a range of 0.5–2.5 m s<sup>-1</sup> by adjusting the rpm of the fan motor with the use of the laboratory autotransformer. The system of measurement of the temperature of the product and the air in the chamber comprised two Chromel-Copel thermocouples shielded from the exposure to infrared radiation by asbestos rope and aluminium foil and a digital microvoltmeter PT-0193 with ambient temperature compensation.



**Figure 2.** General appearance of the laboratory experimental unit.



## RESULTS AND DISCUSSION

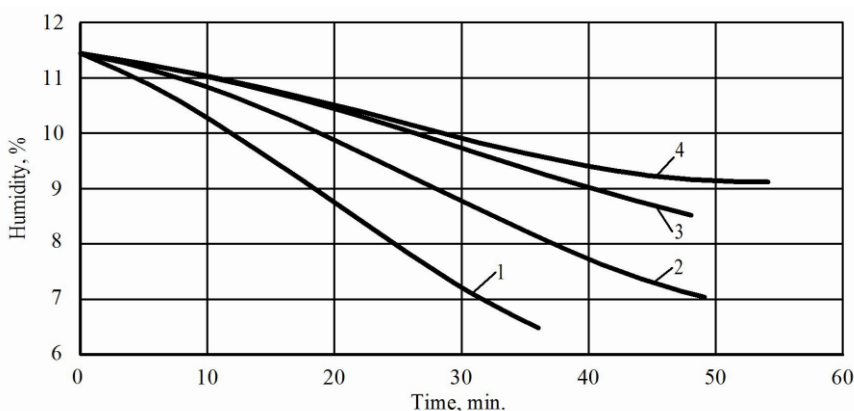
The experimental part of the investigation included series of experiments with different loads (grain weights) and different inputs of electromagnetic radiation power into the chamber. The soft- and hardware system of the test unit registered the resulting changes of the bed's weight and temperature.

The electronic scales registered the product mass variations. The reduction of mass provided the basis for determining the amount of extracted moisture. In advance, the initial moisture content in grain, which was equal to 11.39 % in our experiment, and the mass of dried grain had been found using the conventional methods of drying to a constant weight.

The rapeseeds were placed on the hanger of the scales in the centre of the chamber. The computer continuously processed the information sent from the scales and thermocouples and output to the monitor display the trend lines for the mass of product, product dehydration amount and rate, product temperature, chamber air temperature, dry and wet bulb thermometer measurements of the air leaving the chamber.

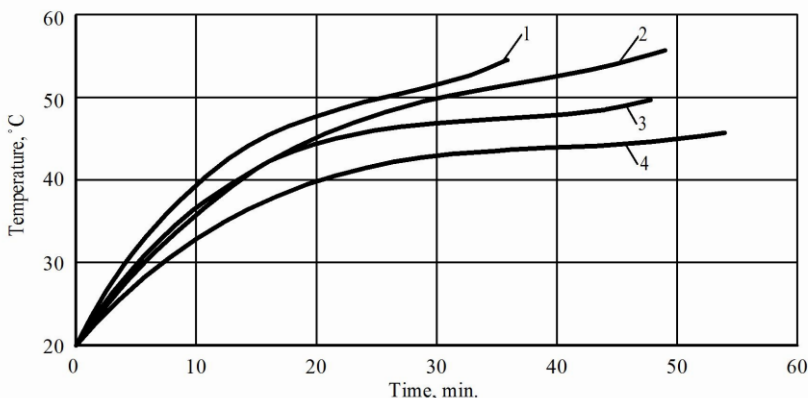
Samples of rapeseeds with masses of 100; 200; 300; 400 g were placed on the tray, which created specific loads of 2.5; 5; 7.5; 10 kg m<sup>-2</sup> respectively. After that, the tray was placed into the test unit with an IR emitter and immediately exposed to IR radiation with a specifically set power of 100, 200, 300, 400, 500 W, which had continued until the product temperature became equal to 70 °C. At the same time, the readings of product mass, product temperature and air temperature were continuously recorded.

The effect of the rapeseed load variation on the drying process is shown in Fig. 3. The results of the investigations represented by the following curves correspond to a distance of 10 cm between the emitter and the surface of the seed bed on the tray. That was the distance (maintained the same for different bed thicknesses), at which the most optimal results had been obtained.



**Figure 3.** Dependence between reduction of humidity content and rape seed load at constant power of  $N_e = 120$  W: 1 – mass of 100 g, layer thickness of 4 mm; 2 – 200 g, 8 mm; 3 – 300 g, 12 mm; 4 – 400 g, 16 mm.

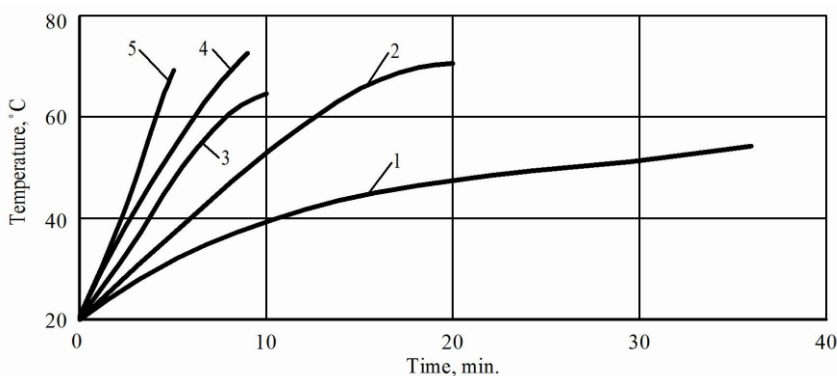
The comparison of the curves (Figs 3 and 4) reveals that the product temperature variation for all loads does not exceed 10 °C (Fig. 4), while an increase in the bed thickness results in the less intensive dehydration (Fig. 3).



**Figure 4.** Rape seed thermograms at constant emitter power of  $N_e = 120$  W, different loads and layer thicknesses: 1 – 100 g, layer thickness of 4 mm; 2 – 200 g, layer thickness of 8 mm; 3 – 300 g, layer thickness of 12 mm; 4 – 400 g, layer thickness of 16 mm.

The results of the described investigation prove that drying with the use of an IR emitter with a power rating of 300 W provides the fullest moisture evaporation, but consumes more time. The other option with an increased power rating of 400...500 W allows increasing the dehydration rate, but in that case the heated product will reach faster the critical thermal point, which can significantly impair its properties.

It has been established that the drying of rapeseeds at a drying medium temperature of 100 °C has no effect on the digestibility of their proteins, while the uniform thermal dehydration of rapeseeds at a temperature of 70 °C improves the nutritive properties of their proteins by increasing their digestibility from 61% to 64%. For that reason, the latter temperature was chosen for our experiments.



**Figure 5.** Rapeseed thermograms at varying emitter power: 1 – 100 W; 2 – 200 W; 3 – 300 W; 4 – 400 W; 5 – 500 W.

It can be seen from the above thermograms (Fig. 5) that a power rating of 100 W provides for the gradual heating of the product, but the duration of the process is rather long. When a power rating of 200 W is applied, adequate heating is facilitated, but with considerably reduced time consumption. Power ratings of 300 W and higher cause the product heating too fast.

## CONCLUSIONS

1. A comprehensive experimental investigation of the effect that the operating parameters (specific load and power) have on the rape seed IR-drying kinetics has been carried out with the use of the laboratory experimental unit that was developed specially for the investigation.

2. The results of the experimental investigation prove that the reduction of the moisture content in the product improves substantially due to the use of IR emitters. Also, it has been established that raising the specific load of rapeseeds to  $7.5 \text{ kg m}^{-2}$  results in the increase of the moisture removal rate, while further increasing the specific load is inadvisable, as the moisture removal will not improve any more, the latter presumably explained by the insufficient penetration of the IR rays into all parts of the loaded product.

3. When the specific load of rapeseeds exceeds  $7.5 \text{ kg m}^{-2}$ , IR-heating works only in the surface layers of the grain bed, the deeper layers are heated only because of the thermal conductivity of the grain, which significantly reduces the efficiency of the drying process.

## REFERENCES

- Aboltins, A. & Upitis, A. 2012. Experimental and theoretical investigation of agricultural material drying process. *Engineering for Rural Development. 11th International Scientific Conference on Engineering for Rural Development*, Jelgava, Latvia; 24 May 2012 through 25, pp. 93–98.
- Barna, I., Gumnytskyi, Y.M. & Atamanyuk, V. 2013. Intradiffusion mass transfer during drying of slag gravel raw granule. *Chemistry and Chemical Technology* **7**(4), 461–465.
- Beregovaya, O.M. & Stankevits, G.N. 2001. Research into rape seed drying process. *Current trends in technologies and mechanization of processes for food processing and production*, Bulletin of Harkow State University, **5**, pp. 282–288 (in Ukrainian).
- Burdo, O.G. & Bezbah, I.V. 2008. Rotating heat pipes in devices for heat treatment of the food-stuffs. *Applied Thermal Engineering* **28**(4), 341–343.  
DOI: 10.1016/j.applthermaleng.2006.02.021
- Burdo, O., Bandura, V., Zykov, A., Zozulyak, I., Levtrinskaya, J. & Marenchenko, E. 2017. Development of wave technologies to intensify heat and mass transfer processes. *Eastern European Journal of Enterprise Technologies* **4**(11–88), 34–42, DOI: 10.15587/1729-4061.2017.108843.
- Das, I., Das, S.K. & Bal, S. 2009. Drying kinetics of high moisture paddy undergoing vibration-assisted infrared (IR) drying. *Journal of Food Engineering* **95**(1), 166–171.  
DOI: 10.1016/j.jfoodeng.2009.04.028
- Doymaz, I. 2004. Convective air drying characteristics of thin layer carrots. *Journal of Food Engineering* **61**(3), 359–364. DOI: 10.1016/S0260-8774(03)00142-0.
- Ginsburg, A.S. 1985. *Design and engineering of drying installations for the food industry*. Moscow: Food Industry, 336 pp. (in Russian).

- Jones, P.L. & Rowley, A.T. 1996. Dielectric Drying. *Drying Technology*, **14**(5), 1063–1098, DOI: 10.1080/07373939608917140.
- Kats, V.Y. & Mazor, G. 2010. Drying of granules in vibrating suspended bed: Engineering simulation. *Russian Journal of Applied Chemistry* **83**(9), 1707–1716. DOI: 10.1134/S1070427210090399.
- Kiptela, L.V., Zagorulko, A.M., Zagorulko O.E. & Ljashenko, B.V. 2017. Enhancement of IR emitter for application in reflectorless plant raw material dryer, 1–12. DOI: 10.15587/2312-8372.2017.98068 (in Ukrainian).
- Krishnamurthy, K., Khurana, H.K., Soojin, J., Irudayaraj, J., Demirci, A. 2008. Infrared heating in food processing: An overview. *Comprehensive Reviews in Food Science and Food Safety* **7**(1), 2–13, DOI: 10.1111/j.1541-4337.2007.00024.x.
- Lykov, A.V. 1968. *Theory of drying*. Moscow: Energy, 472 pp.
- Nikitenko, N.I., Snezhkin, Y.F. & Sorokovaya, N.N. 2008. Development of a theory and methods for calculating the heat and mass transfer in drying a porous body with multicomponent vapor and liquid phases. *Journal of Engineering Physics and Thermophysics* **81**(6), 1153–1167. DOI: 10.1007/s10891-009-0132-x.
- Orsat, V., Yang, W., Changrue, V. & Raghavan, G.S.V. 2007. Microwave-assisted drying of biomaterials. *Food and Bioproducts Processing* **85**(3 C), 255–263. DOI: 10.1205/fbp07019.
- Rebinder, P.A. & Shchukin, E.D. 1973. Surface phenomena in solids during the course of their deformation and failure. *Soviet Physics – Uspekhi* **15**(5), 533–554, DOI: 10.1070/PU1973v015n05ABEH005002.
- Riadh, M.H., Ahmad, S.A.B., Marhaban, M.H. & Soh, A.C. 2015. Infrared Heating in Food Drying: An Overview. *Drying Technology* **33**(3), 322–335. DOI: 10.1080/07373937.2014.951124.
- Sandu, C. Infrared Radiative Drying in Food Engineering: A Process Analysis. 1986. *Biotechnology Progress* **2**(3), 109–119, DOI: 10.1002/btpr.5420020305.
- Wang, Y., Li, Y., Wang, S., Zhang, L., Gao, M. & Tang, J. 2011. Review of dielectric drying of foods and agricultural products. *International Journal of Agricultural and Biological Engineering* **4**(1). DOI: 10.3965/j.issn.1934-6344.2011.01.0-0.
- Wang, X., Cao, R. & Sun, C. 2007. Application of infrared radiation technology on processing agriculture biological materials. *Nongye Jixie Xuebao/Transactions of the Chinese Society of Agricultural Machinery* **38**(7), 177–182.

## **Chemical composition of agromass ash and its influence on ash melting characteristics**

D. Čepauskienė<sup>1,\*</sup>, N. Pedišius<sup>1</sup> and D. Milčius<sup>2</sup>

<sup>1</sup>Lithuanian Energy Institute, Laboratory of Heat–Equipment Research and Testing, Breslaujos str. 3, LT–44403 Kaunas, Lithuania

<sup>2</sup>Lithuanian Energy Institute, Center for Hydrogen Energy Technologies, Breslaujos str. 3, LT–44403 Kaunas, Lithuania

\*Correspondence: Deimante.Cepauskiene@lei.lt

**Abstract.** The increasing demand for biofuels leads to a growing need for agromass, such as herbaceous plants and agricultural waste. However, agromass contains high quantities of alkali metals, mainly potassium and sodium, which limits agromass usage in thermoconversion processes. Alkali metals react with other ash forming elements which leads to ash related problems such as agglomeration, fouling and slagging during agromass burning. In this study, chemical composition and melting behaviour of ashes formed at 550 °C are investigated. Three herbaceous plants (reed canary grass, hemp, orchard grass), four types of agricultural waste (straws of rye, wheat, buckwheat and canola) and two types of woody biomass (birch, spruce) are selected. Ash melting behaviour, composition and bulk structure are determined using a high temperature furnace with a video camera, inductively coupled plasma atomic emission–spectrometry and X–ray diffraction technique, respectively. Ash melting behaviour of selected agromass types shows that the hemp ash has the highest shrinkage starting temperature which reaches 1,079 °C. This is due to the high content of calcium and low content of potassium and sodium in hemp ash. Three main components calcium carbonate, potassium sulfate and potassium chloride have been identified in ashes after agromass and woody biomass are heat–treated at temperature of 550 °C.

**Key words:** agromass, agrofuels, ash, ash melting, alkali metals.

### **INTRODUCTION**

A growing demand for energy, decreasing resources of fossil fuel and increasing environmental pollution induce search for alternatives of fossil fuel. One of them is biofuel, which until now, was mainly produced from woody biomass. The consumption of such biomass has risen during the past two decades, growing at an annual rate of over 2% (Patel & Gami, 2012). Yet not only woody biomass can be used for biofuel production, but also herbaceous plants of short rotation and agricultural waste such as straw (hereinafter – agro biomass or in abbreviated form – agromass). The fuel produced from agromass is usually called agrofuel. However, agrofuel has several negative properties related to high amounts of alkali and alkaline earth metals as well as chlorine (Cl) and sulphur (S). Therefore, despite the well–known advantages of agromass usage such as carbon dioxide neutrality and high energy generation potentiality, efficient and

low-emission burning of such fuels is still limited due to technical problems associated with corrosion of equipment, ash agglomeration, fouling and slagging of partially fused deposits on furnace walls and convection heat surfaces.

The presence of alkali metals, such as potassium (K) and sodium (Na), has the greatest impact on ash melting during combustion. High contents of alkali metals and silicon (Si) results in the formation of sticky molten K silicates that melt under low temperature of approximately 750 °C. Such viscous ash sticks to the unburned fuel particles, which leads to agglomeration (Thy et al., 2006; Magzdiarz et al., 2016). Due to high content of K in the ash, K silicates as well as other compounds of K (chlorides, sulphates, carbonates) are formed. Melting point of these K compounds is low too, i.e. merely 770 °C (Wang et al., 2012). Na, which transformation mechanism in ash is similar to K, also reduces ash melting temperature. However, the content of Na in ash is very low, so for the following reason Na content is not distinguished separately and is attributed to K (Niu et al., 2016).

Woody biomass composition is quite stable, and contains large quantities of calcium (Ca) and Si, yet less phosphorus (P) and K. On the contrary, agromass composition varies greatly. Both grasses and straws contain large quantities of Si and K. Smaller amounts of Ca and magnesium (Mg) are found in grasses, the same goes for aluminium (Al) and Na in straw. Aquatic biomass contains large quantities of K and Mg though less Al and Si (Vassilev et al., 2013). Moreover, the composition of agromass depends on the soil type, harvesting season (Niu et al., 2014), climate, type of fertilizer, its concentration and frequency of fertilization (Schiemenz & Eichler-Lobermann, 2010) and impurities that fall in during preparation and transportation of agromass (Wang et al., 2012).

Ash melting temperature depends not only on elemental composition of ash as a mineral matter, but also on the form of mineral compounds. It was determined, that potassium chloride and sulfate (KCl, K<sub>2</sub>SO<sub>4</sub>) are the dominant alkali-containing compounds that influence ash related issues. K<sub>2</sub>SO<sub>4</sub> mainly accumulates on high temperature heating surfaces, while KCl accumulates on low temperature heating surfaces (Niu et al., 2016). However, mineral composition and chemical transformation mechanisms of agromass ash compounds are not fully investigated and further studies are required.

This study mostly focuses on determination of chemical composition and fusion properties of ash of selected types of agromass and woody biomass. The aim is to extend and improve the knowledge on agromass types which are less investigated and analyzed. Ash melting stages related to the shrinkage starting temperature (SST), deformation temperature (DT), hemisphere temperature (HT) and flow temperature (FT) and their dependence on the ash elemental (Ca, K, Si, P, Mg, Al, Na) and mineral composition of ash are determined. These results can be beneficial with the goal to provide general understanding to industrial users about the agromass diversity and which types of agromass are suitable to use in combustion technologies.

## MATERIALS AND METHODS

Nine different samples divided into three main groups, i.e. agricultural waste, herbaceous plants and woody biomass were selected for the analysis. For the group of agricultural waste, rye, wheat, buckwheat and canola straws were selected, because in

Lithuania the annual amount of this harvest waste reaches 3 million tons and only a small part of it is used according to its original purposes, as animal litter or feed. Pellets of orchard grass, reed canary grass and hemp were selected for the group of herbaceous plants. Two wood species spruce and birch (with bark) were selected for comparison.

All samples were dried in low temperature laboratory furnace under the temperature of 105 °C and later grinded with a mill. The ash content of all samples was determined using a muffle furnace (Nabertherm LVT/9/11/P330) at 550 °C according to ISO 18122:2016. Ashing experiment was carried out three times for each sample. Ash melting behaviour was conducted twice in muffle furnaces CARBOLITE with the monochromatic video camera CAF DIGITAL 380–415V with reference to CEN/TS 15370–1. Ashes were grinded with a pestle to obtain particles with a size of less than 0.075 mm. Using a mould with a defined pressure, a moistened (with ethanol) ash powder was used to form cylindrical ash samples with a size of 5 x 5 mm. Samples were heated in ash fusion furnace under reducing atmosphere. A furnace temperature was raised up to 550 °C. Then temperature was gradually raised 5 °C min<sup>-1</sup> and photos were automatically made every interval of 2 °C till temperature has reached 1,600 °C. The temperature at which the phase of the sample changed was recorded.

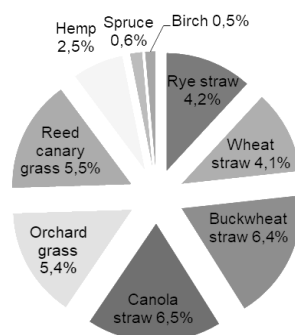
Wet-digestion of the ashes and raw materials was applied before elemental composition analysis using a microwave oven Multiwave 3000 according to ISO 16967:2015. Samples were mineralised with solvent of H<sub>2</sub>O<sub>2</sub> (30%), HNO<sub>3</sub> (65%) and HF (40%). In order to prevent Si contamination from glass, plastic vessels were used. The concentration of elements K, Ca, Mg, Na, Al, Si, P were measured using inductively coupled plasma atomic emission-spectrometry (ICP-OES) OPTIMA 8000 CROSS FLOW.

Volumetric structure of ash was determined using XRD Bruker AXS D8 diffractometer. Each sample was scanned for 45 minutes using  $\Theta$ -2 $\Theta$  modification in the interval from 20° to 70°. A source of X-ray CuK $\alpha$ 1. Compounds identification were performed through comparison with standards of EVA Search-Match program from PDF-2 database.

## RESULTS AND DISCUSSION

Accomplished analysis revealed that buckwheat (6.4%) and canola (6.5%) straws have the highest ash content (Fig. 1).

Ash content of herbaceous plants, excluding hemp, is also similar to ash content of straw. Ash content of hemp is more than twice lower (2.5%) than the other herbaceous plants. The difference in ash content between various types of agromass can be explained by few factors. Firstly, ash content and its amount depend on the genetic plant properties and its parts used for production of biofuel (Monti et al., 2008). This part of inorganic components source is the agromass itself. Secondly, quite large part of inorganic components, such as potassium, nitrogen, phosphorus and others which are necessary for plant growth, come with fertilizers.



**Figure 1.** Ash content (% db.) of agromass and woody biomass.

Herbaceous plants, which are specifically cultivated for energy purposes, are fertilized in order to increase the quantity of agromass. The effects of climate, soil composition, harvesting and fuel preparation also can not be underestimated.

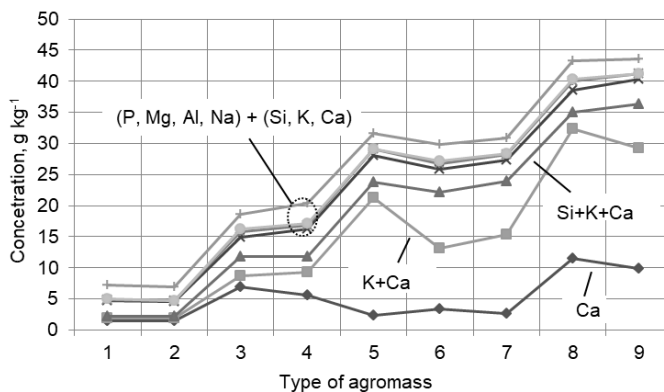
The results of ash melting behaviour are presented in Table 1. The obtained data shows that straw ashes have the lowest shrinkage starting temperature (SST), whereas the SST temperatures of herbaceous plants are even higher than the ones of woody biomass. SST temperature is the most important since it is the initial phase of ash melting. Melting formation at low temperatures makes ashes particularly troublesome because it induces stickiness of ashes which causes formation of slag.

**Table 1.** Ash melting temperatures of investigated types of biomass (°C)

	SST	DT	HT	FT
<b>Herbaceous plants</b>				
Reed canary grass	946	979	1,147	1,164
Hemp	1,079	1,418	1,490	1,496
Orchard grass	1,061	1,167	1,263	1,287
<b>Agricultural waste</b>				
Rye straw	739	875	1,163	1,185
Wheat straw	783	869	1,099	1,175
Buckwheat straw	698	751	1,544	1,549
Canola straw	722	1,061	1,497	1,502
<b>Wood</b>				
Birch	888	1,477	1,514	1,526
Spruce	849	1,497	1,523	1,531

Hemp is considered to be one of the most potent herbaceous plant for production of agrofuel because its ash melts at higher temperatures. Ash melting behaviour and slag formation depends on a component of the lowest melting temperature, chemical composition, combustion duration and conditions, because the longer fuel/ash is kept in high temperature, the more favourable are conditions for slagging (Fang & Jia, 2012).

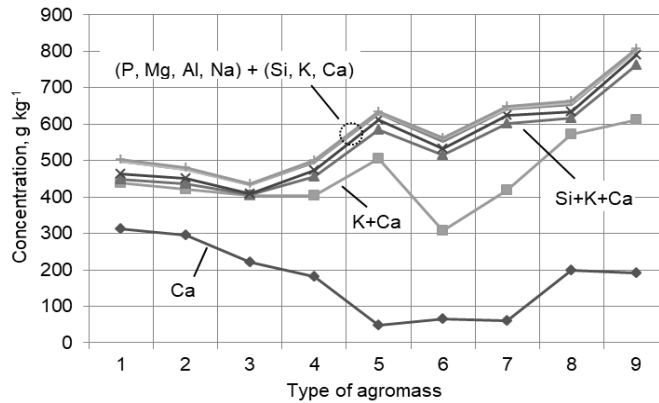
The results of elemental composition of raw agromass and woody biomass as well as its ash are shown in Fig. 2 and Fig. 3.



**Figure 2.** Elemental composition of agromass: 1 – birch; 2 – spruce; 3 – hemp; 4 – orchard grass; 5 – reed canary grass; 6 – rye straw; 7 – wheat straw; 8 – canola straw; 9 – buckwheat straw.



As can be seen from these Figures, the elemental composition of agromass, woody biomass and its ash is well correlated. Three elements Ca, K and Si make up the main part or the total content of all elements which increases consistently from the smallest total concentration value for the woody biomass to the largest value for the straw. For the investigated types of agrobiomass, the ratio of these values approaches 10 (Fig. 2). This trend is mainly due to an increase of K in grasses and especially in straw. The other elements (P, Na, Mg, Al) in the agrobiomass are also clearly visible, but their impact to the total element concentration is almost identical for all agrobiomass and woody biomass types. According to the elemental composition, the herbaceous plants such as hemp and orchard grass are closest to the woody biomass.



**Figure 3.** Elemental composition of agromass ashes: 1 – birch; 2 – spruce; 3 – hemp; 4 – orchard grass; 5 – reed canary grass; 6 – rye straw; 7 – wheat straw; 8 – canola straw; 9 – buckwheat straw.

The composition of ash is characterized by a significantly weaker dependence on the type of agromass. The main reason of such trend is the decreasing amount of Ca and K in the grass and straw ashes. Composition of hemp ash is clearly distinguishable. The total concentration of all elements in its ash is lower than a single one for wood ash. Compared to other herbaceous plants and agricultural wastes, hemp has a larger woody part of the stem, which affects the elemental composition of hemp ashes.

As mentioned before, the usage of fertilizers has also a substantial influence on the properties of agrobiomass, its ash, and elemental composition. Higher concentrations of Si in agricultural waste and herbaceous plants ashes may be due to agromass contamination with soil. However, the complex transformation of compounds takes place during combustion which results in reduction of some elements, such as K, in ash.

The results in Table 2 show that only a few cases exhibit a sufficient level of correlation which allows to draw conclusions about ash melting behaviour dependence on the content of a single element.

Si has the greatest influence on the temperatures of DT, HT and FT. High amount of Si cause a decrease of these temperatures. The effect of Ca is more clear in the final ash melting stages. On the contrary, the effect of Na and K is more evident at the initial phases of ash melting, where the higher quantities of these elements reduces the SST values. Conclusions about the effects of P, Mg and Al can not be made since the determination coefficients are less than 0.25. It can be concluded that a single element does not directly affect the ash melting behaviour. Summarizing the analysis data it can

be assumed that higher amounts of Si, Na and K reduces ash melting temperature while Ca raises it. Similar results were obtained by other authors (Niu et al., 2010, Paulrud et al., 2001).

**Table 2.** Correlation of the ash melting behaviour and individual elements

	SST	DT	HT	FT
Ca	-0.24 ( $R^2 = 0.06$ )	0.36 ( $R^2 = 0.13$ )	0.65 ( $R^2 = 0.43$ )	0.66 ( $R^2 = 0.44$ )
K	-0.63 ( $R^2 = 0.39$ )	-0.67 ( $R^2 = 0.45$ )	-0.44 ( $R^2 = 0.19$ )	-0.44 ( $R^2 = 0.20$ )
Si	-0.41 ( $R^2 = 0.17$ )	-0.86 ( $R^2 = 0.74$ )	-0.79 ( $R^2 = 0.62$ )	-0.78 ( $R^2 = 0.60$ )
P	-0.37 ( $R^2 = 0.14$ )	-0.02 ( $R^2 = 0.00$ )	-0.25 ( $R^2 = 0.06$ )	-0.24 ( $R^2 = 0.06$ )
Mg	-0.45 ( $R^2 = 0.20$ )	0.21 ( $R^2 = 0.04$ )	-0.06 ( $R^2 = 0.00$ )	-0.05 ( $R^2 = 0.00$ )
Al	-0.50 ( $R^2 = 0.25$ )	-0.42 ( $R^2 = 0.18$ )	-0.35 ( $R^2 = 0.12$ )	-0.36 ( $R^2 = 0.13$ )
Na	-0.68 ( $R^2 = 0.46$ )	-0.06 ( $R^2 = 0.00$ )	-0.01 ( $R^2 = 0.00$ )	-0.01 ( $R^2 = 0.00$ )

Several chemical changes of ash mineral content take place during combustion in high temperatures. Si melts partially or completely and reacts with other ash forming elements, and mainly silicates of alkali metals (K and Na) are formed. Dissociation of carbonates, chlorides and other salts takes place. Alkali metals and heavy metals become volatile and evaporate. Due to this it is very important to determine the mineral composition of ash.

Analysis of such results, indicating the qualitative presence of crystalline minerals in the ash and melted ash samples are presented in Table 3 and Table 4. It has been found out that ash does not have a clear crystal structure and consists of oxides, silicates, carbonates, sulphates and phosphates, which are the main compounds that are formed during combustion. Ash forming compounds are largely very different depending on the agromass type. However, three main components calcium carbonate ( $\text{CaCO}_3$ ), potassium sulfate ( $\text{K}_2\text{SO}_4$ ) and potassium chloride (KCl) are the most commonly found in the ashes of agromass and woody biomass that are heat – treated at temperature of 550 °C.

**Table 3.** Mineral composition of agromass and woody biomass ashes (550 °C)

<b>Herbaceous plants</b>	
Reed canary grass	KCl, $\text{K}_2\text{SO}_4$ , $\text{Ca}_4\text{O}(\text{PO}_4)_2$ , $\text{SiO}_2$ , $\text{KCaPO}_4$
Hemp	$\text{CaCO}_3$ , $\text{Ca}_4\text{O}(\text{PO}_4)_2$ , $\text{SiO}_2$
Orchard grass	KCl, $\text{Ca}_4\text{O}(\text{PO}_4)_2$ , $\text{SiO}_2$ , $\text{KCaPO}_4$ , $\text{Na}_2\text{Ca}_3\text{Si}_2\text{O}_8$ , $\text{KMg}_2\text{Al}_{15}\text{O}_{25}$
<b>Agricultural waste</b>	
Rye straw	$\text{CaCO}_3$ , $\text{K}_2\text{S}_4\text{O}_6$ , $\text{SiO}_2$ , $\text{MgSiO}_3$
Wheat straw	KCl, $\text{K}_2\text{SO}_4$ , $\text{KCaPO}_4$ , $\text{SiO}_2$
Buckwheat straw	KCl, $\text{CaCO}_3$ , $\text{K}_2\text{SO}_4$ , $\text{K}_2\text{S}_2\text{O}_6$ , $\text{K}_2\text{Ca}(\text{CO}_3)_2$
Canola straw	KCl, $\text{CaCO}_3$ , $\text{K}_2\text{SO}_4$ , $\text{K}_2\text{Ca}(\text{CO}_3)_2$ , $\text{Ca}_2\text{SiO}_4$
<b>Wood</b>	
Birch	$\text{CaCO}_3$ , $\text{K}_2\text{SO}_4$ , $\text{K}_2\text{Mg}(\text{PO}_3)_4$ , KMnP, $\text{MnSO}_4$
Spruce	$\text{CaCO}_3$ , $\text{K}_2\text{SO}_4$ , $\text{K}_2\text{Mg}(\text{PO}_3)_4$ , KMnP, $\text{MnSO}_4$

There is also some difference between mineral composition of ash of the same group. Exclusively in the case of woody biomass ash, the identified compounds were the same:  $\text{CaCO}_3$ ,  $\text{K}_2\text{SO}_4$ , potassium magnesium phosphate ( $\text{K}_2\text{Mg}(\text{PO}_3)_4$ ), potassium manganese phosphide (KMnP) and manganese sulphate ( $\text{MnSO}_4$ ). The dominant components of agricultural waste are KCl,  $\text{CaCO}_3$  and  $\text{K}_2\text{SO}_4$ , whereas a vast majority

of other K compounds are dominated. Hemp ash is the most distinctive among herbaceous plants. The following three main compounds  $\text{CaCO}_3$ , calcium oxophosphate ( $\text{Ca}_4\text{O}(\text{PO}_4)_2$ ) and silica ( $\text{SiO}_2$ ) have been identified. Ash of other herbaceous plants also contained KCl, but no  $\text{CaCO}_3$  has been found in them. All other identified compounds are largely the same and K, Ca, P are predominated in composition. It means that alkali metals play a key role in formation of ash. For plants K is one of the most important nutrient that is absorbed from the soil in the form of dissolved salts, especially for one – year rotation fast-growing plants. Therefore, compounds with K, mainly KCl,  $\text{K}_2\text{SO}_4$ ,  $\text{CaCO}_3$ , dominate in agromass ash.

Analysis of molten ash, which is obtained at FT (Table 4), reveal that silicates, aluminates and oxides are the main components. Results show that aluminium oxide ( $\text{Al}_2\text{O}_3$ ) and spinel ( $\text{MgAl}_2\text{O}_4$ ) are compounds that have been identified in melted ash of selected woody biomass and agromass samples. In this case, it is also necessary to consider that ash was melted on porcelain plates. Therefore, peaks of aluminium oxide ( $\text{Al}_2\text{O}_3$ ) and silica ( $\text{SiO}_2$ ) may be a result of a tray.

**Table 4.** Mineral composition of molten agromass and woody biomass ashes (at FT)

<b>Herbaceous plants</b>	
Reed canary grass	$\text{Al}_2\text{O}_3$ , $\text{MgAl}_2\text{O}_4$ , Si
Hemp	$\text{MgAl}_2\text{O}_4$ , $\text{Ca}_2\text{Al}_2\text{SiO}_7$ , $\text{CaMg}_2\text{Al}_6\text{O}_{12}$ , $\text{CaNa}_3\text{Al}(\text{P}_2\text{O}_7)_2$ , $\text{CaAl}_4\text{O}_7$
Orchard grass	$\text{Al}_2\text{O}_3$ , $\text{KCaPO}_4$ , $\text{SiO}_2$ , $\text{K}_2\text{Si}_4\text{O}_9$ , $\text{K}_{0.85}\text{Al}_{0.85}\text{Si}_{10.15}\text{O}_2$ , $\text{KP}_6\text{O}_{18}$
<b>Agricultural waste</b>	
Rye straw	$\text{Al}_2\text{O}_3$ , Si
Wheat straw	$\text{Al}_2\text{O}_3$ , $\text{MgAl}_2\text{O}_4$ , $\text{KAlSi}_2\text{O}_6$ , Si
Buckwheat straw	$\text{Al}_2\text{O}_3$ , $\text{Ca}_2\text{Al}_2\text{SiO}_7$ , $\text{CaAl}_{12}\text{O}_{19}$ , $\text{KAl}_3\text{Si}_3\text{O}_{11}$ , $\text{MgAl}_2\text{O}_4$ , $\text{KAlP}_2\text{O}_7$
Canola straw	$\text{MgAl}_2\text{O}_4$ , $\text{Ca}_2\text{Al}_2\text{SiO}_7$ , $\text{Ca}_3(\text{PO}_4)_2$
<b>Wood</b>	
Birch	$\text{Al}_2\text{O}_3$ , Al, $\text{Ca}_3\text{Si}_2\text{O}_7$ , $\text{Al}(\text{PO}_3)_3$
Spruce	$\text{Al}_2\text{O}_3$ , $\text{MgAl}_2\text{O}_4$ , $\text{Ca}_5(\text{PO}_4)_2\text{SiO}_4$ , $\text{SiO}_2$ , $\text{Ca}_3\text{Al}_2\text{P}_2\text{Si}_2\text{O}_{15}$ , $\text{Ca}_3\text{Al}_2(\text{SiO}_4)_3$

Mineral composition of molten herbaceous plant ashes are quite different: orchard grass ash forming compounds mainly contain K, reed canary grass ash – Mg, whereas hemp ash – mostly Ca.

Molten ash mineral composition of agricultural waste is even more diverse. However, two main compounds, i.e.  $\text{Al}_2\text{O}_3$  and  $\text{MgAl}_2\text{O}_4$  are typical for this entire group of agromass.  $\text{Al}_2\text{O}_3$  and many other Ca compounds have been identified in molten woody biomass ash.

It should be noted that mineral composition of molten hemp ash is very similar to mineral composition of molten woody biomass ash where Ca and Mg compounds are predominant.

In general, mineral composition of molten ash has less K compounds, so it can be assumed that through thermo-chemical reactions some ash elements become gaseous and are removed with volatile matter. Compounds with higher melting points are found in this type of ash.

The results obtained in this study are in good agreement with the data and conclusions of other authors (Thy et al., 2006, Niu et al., 2016, Fang & Jia, 2012), regarding the influence of temperature on ash forming compounds and their transformation into high melting temperature forms while temperature increasing.

## CONCLUSIONS

From all studied agromass species, hemp has been distinguished as the most suitable for agrofuel production due to the lowest ash content (2.5%), highest SST temperature (1,079 °C) and appropriate mineral composition of ash.

Furthermore, it has been determined that higher amounts of Si, K and Na reduce ash melting temperature while Ca raises it. However, the correlation between fusion temperatures and concentration of separate elements is strong only in the case of Si. It means that a single chemical element does not directly affect the temperature of ash melting phase. Summarizing the analysis data of ash mineral composition it has been concluded that ash does not have a clear crystalline structure and oxides, silicates, carbonates, sulphates, and phosphates are the main components of which the most common compounds are CaCO<sub>3</sub>, K<sub>2</sub>SO<sub>4</sub>, KCl. The main compounds found in molten ash are silicates, aluminates and oxides, most often in the form of Al<sub>2</sub>O<sub>3</sub> and MgAl<sub>2</sub>O<sub>4</sub>.

## REFERENCES

- Fang, X. & Jia, L. 2012. Experimental study on ash fusion characteristics of biomass. *Bioresource Technology* **104**, 769–774.
- Magzdiarz, A., Dalai, A.K. & Kozinski, J.A. 2016. Chemical composition, character and reactivity of renewable fuel ashes. *Fuel* **176**, 135–146.
- Monti, A., Virgilio, N. & Venturi, G. 2008. Mineral composition and ash content of six major energy crops. *Biomass and Bioenergy* **32**, 216–223.
- Niu, Y., Tan, H. & Hui, S. 2016. Ash-related issues during biomass combustion: Alkali-induced slagging, silicate melt-induced slagging (ash fusion), agglomeration, corrosion, ash utilization, and related countermeasures. *Progress in Energy and Combustion Science* **52**, 1–61.
- Niu, Y., Tan, H., Wang, X., Liu, Z., Liu, H., Liu, Y. & Xu, T. 2010. Study on fusion characteristics of biomass ash. *Bioresource Technology* **101**, 9373–9381.
- Niu, Y., Zhu, Y., Tan, H., Hui, S., Jing, Z. & Xu, W. 2014. Investigation on biomass slagging in utility boiler: criterion numbers and slagging growth mechanisms. *Fuel Processing Technology* **128**, 499–508.
- Patel, B. & Gami, B. 2012. Biomass characterization and its use as solid fuel for combustion. *Iranica Journal of Energy & Environment* **3**(2), 123–128.
- Paulrud, S. 2001. Upgraded Biofuels - Effects of quality on Processing, Handling characteristics, Combustion and Ash melting. *Doctoral thesis (Unit of Biomass Technology and Chemistry)*. Umeå: Swedish University of Agricultural Sciences. 53 pp.
- Schiemenz, K. & Eichler-Lobermann, B. 2010. Biomass ashes and their phosphorus fertilizing effect on different crops. *Nutrient Cycling in Agroecosystems* **87**(3), 471–482.
- Thy, P., Jenkins, B.M., Grundvig, S., Shiraki, R. & Leshar, C.E. 2006. High temperature elemental losses and mineralogical changes in common biomass ashes. *Fuel* **85**, 783–795.
- Vassilev, S.V., Baxter, D., Andersen, L.K. & Vassileva, C.G. 2013. An overview of the composition and application of biomass ash. Part 1. Phase-mineral and chemical composition and classification. *Fuel* **105**, 40–76.
- Wang, L., Hustad, J.E., Skreiberg, Ø., Skjevraak, G. & Grønli, M. 2012. A critical review on additives to reduce ash related operation problems in biomass combustion applications. *Energy Procedia* **20**, 20–29.

## **Management of Brazilian hardwood species (Jatoba and Garapa) wood waste biomass utilization for energy production purposes**

V. Chiteculo<sup>1,\*</sup>, A. Brunerová<sup>2,\*</sup>, P. Surový<sup>1</sup> and M. Brožek<sup>2</sup>

<sup>1</sup>Czech University of Life Sciences Prague, Faculty of Forestry and Wood Sciences, Department of Forest management, Kamýcká 129, CZ165 00 Prague, Czech Republic

<sup>2</sup>Czech University of Life Sciences Prague, Faculty of Engineering, Department of Material Science and Manufacturing Technology, Kamýcká 129, CZ165 00 Prague, Czech Republic

\*Correspondence: brunerova@tf.czu.cz; chiteculo@fld.czu.cz

**Abstract.** In the Federative Republic of Brazil, Jatoba (*Hymenaea courbaril*) and Garapa (*Apuleia leiocarpa*) trees are intensively harvested. The yield of one log is approximately 45–55%, which indicates a great amount of produced wood waste biomass. Present research monitored the suitability of wood waste biomass from Jatoba and Garapa trees for bio-briquette for solid biofuel production. The research was focused on chemical parameters, and energy potential of such biomass kinds. Jatoba wood waste biomass was used for the production of bio-briquette fuel and its final mechanical quality was investigated by determination of their mechanical quality indicators. Results of chemical analysis (in wet basis) exhibited great level of ash content in case of both species (Jatoba – 0.31%, Garapa – 3.02%), as well as high level of energy potential; net calorific value equal to 18.92 MJ kg<sup>-1</sup> for Jatoba and to 18.395 MJ kg<sup>-1</sup> for Garapa. Analysis of elementary composition proved following levels of oxygen content: Jatoba – 41.10%, Garapa – 39.97%. Mechanical analysis proved bio-briquette samples volume density  $\rho$  equal to 896.34 kg m<sup>-3</sup> which indicated quality bio-briquette fuel, while the level of rupture force RF occurred at a lower level – 47.05 N mm<sup>-1</sup>. Most important quality indicator, the mechanical durability DU, unfortunately, occurred at a lower level; DU = 77.6% compared to the minimal level of bio-briquette fuels intended for commercial sales which must be > 90%. Overall analysis proved materials suitability for energy generation purpose with certain limitations which can improve by changing production parameters of briquetting.

**Key words:** Briquetting, direct combustion, renewable energy, waste management.

### **INTRODUCTION**

Management is the process of reaching organizational goals and by hardwood species management we mean integration and coordination of series of actions towards the achievement of a specific objective. It follows value chain wood waste generated by wood processing industry.

Wood processing is a downstream activity of the forestry because it adds economic value to logs, diversifies the products and increases the incomes (Israel & Bunao, 2017).

All products and services have environmental impacts, from the extraction of raw materials to the production or manufacture, distribution, use, and disposal. However, wood is a renewable resource used in wood industry and for energy production (Jungmeier et al., 2002; Kim & Song, 2014). Wood waste is produced by a number of sectors as part of the municipal waste stream. The value chain of wood involves cutting logs, sawn into the timber and transported to manufacturers who transform and obtain outputs that generate wood waste (Kaplinsky, Memedovic, Morris, & Readman, 2003).

In the late 19<sup>th</sup> century, worldwide trade in wood furniture grew by 36%, faster than merchandise trade as a whole (26.5%), apparel and footwear (32%). Solid wood furniture represents one of the rawest materials for manufacturing high-end designed products (Kaplinsky et al., 2003). Wood waste from furniture, construction arises in different forms ranging from untreated, off-cuts, to treated wood containing preservatives and via a variety of post-consumer waste which can be used for feedstock or combustion (Owoyemi et al., 2016; Huron et al., 2017). The over-extraction of wood resources, linked with clearing for agricultural purposes and indiscriminate burning creates disorder which aggravates the wellbeing of the forests. In Brazil, many highly valued timber species occur at extremely low densities yet are intensively harvested with little regard for impacts on population structures and dynamics of forest (Schulze et al., 2008). For instance, Jatoba and Garapa wood are used for flooring, furniture, cabinetry, tool handles, boat building and other special items (Meier, 2015). The production, exploitation, and processing of wood represent one of the main pillars of Amazon economy. There are more than 71 zones of wood extraction which extracts about 14.2 million cubic meters of logs per year which generate 5.8 million cubic meters of lumber with about 59% of wood waste materials (Marchesan, 2012). The yield of one log of Jatoba, for instance, is approximately 45–55%, the remains fall into the category of by-products such as dust, sawdust, chips, barks, rags, trimmings, and tips.

The importance of wood waste management is the worldwide spread of techniques focused on the reduction and re-utilization of these waste materials as regards the policies of each country (Jungmeier et al., 2002; European Commission, 2010; Bittencourt et al., 2015). Considering the need to reduce carbon emission, wood waste resources provide an alternative energy that helps to reduce the emission from landfill (Röder & Thornley, 2017). The utilization of most common products of wood waste for recycling and energy production using biomass has been highlighted in the field of energy and sustainability mainly for environmental conservation (Altafini et al., 2003; Daian & Ozarska, 2009a; Raud et al., 2014). Studies of awareness on wood waste utility, economic benefits, and energy consumption are summarised in Table 1.

One of the factors affecting the utilization of wood waste is the dependency on fossil fuel as a source of energy production. At a time when fossil fuels were much cheaper than wood, wood wastes were destroyed by burning (Top, 2015). The amount of carbon dioxide released into the atmosphere during burning or decomposition of wood is the same as that which a tree absorbs during growth. In developing countries about 70% of energy is supplied from fossil fuels and the remaining 30% is from renewable sources. Environment impacts due to fossil fuel use include global warming, air quality deterioration, oil spills and acid rain (Ming et al., 2014; Patel, 2014). However, the use of wood pellets and briquettes from wood waste contributes less to air pollution than fossil fuel (Giuntoli et al., 2013; Kim & Song, 2014; Singh et al., 2016). Wood waste is considered a potential alternative for energy production. It does not compete with food

and feed production with no direct impact on soil. Contrarily, it contributes to generating energy security for the local population in the places where wood waste originates from (Bergeron, 2016).

Classification of wood waste biomass varies from country to country. In European Union legislation, waste management option is ranked in order of environmental preference with the first priority being the reduction or avoidance of waste and the recovering of energy (Knauf, 2015; van Dam, 2013). However, the classification obeys three categories: *Clean untreated* wood (an e.g. pallet, wooden boxes, scraps lumber and, plywood) which can sometimes contain nails, bolts or screws (Fig. 1, c, d); *slightly treated wood waste* (wood painted or coated) and *heavily treated* (impregnated wood waste).

*Clean untreated* wood waste biomass is widely used for commercial production of bio-briquette fuel due to its suitable chemical and mechanical parameters. The technology of high-pressure briquetting operates without using any external binders, thus, ensures a high level of bio-briquette final quality (Emerhi, 2011). Differences within energy potentials and level of suitability of specific wood types can be found between deciduous and coniferous trees, trees from tropical and temperate zones and even different parts of the trees (trunk, branch, bark).

In consequence, the aim of present paper is to state the potential of Jatoba and Garapa wood waste biomass for energy generation in the form of bio-briquette fuel. The aim was supported by chemical analysis of the wood waste biomass kinds (which contained of testing of basic chemical parameters and elementary composition of investigated samples) and subsequent mechanical analysis of bio-briquette samples produced from such materials (which contained of testing of mechanical quality indicators of investigated bio-briquette samples).



**Figure 1.** Different forms of wood waste biomass: a) pile of sawdust; b) wooden offcuts or scraps from lumber; c) wood chips; d) old pallets.

**Table 1.** Examples of studies on wood waste management: main features

Authors (year)	Country	Category	Methodology	Area of impact
Huron et al. (2017)	FRA	Treated wood waste	Lab analysis	Environmental awareness
Bergeron (2016)	CHE	Climate change	Review plus modeling	Wood waste utility
Tatàno et al. (2009)	ITA	Waste management	Lab analysis	Energy content
Kim & Sang (2014)	PRK	Climate change	LCA according to ISO 14040	Environmental awareness
Massote et al. (2013)	BRA	Waste management	CP methodology by UNEP	Wood waste utility
Hiramatsu et al. (2002)	JPN	Waste management	Lab analysis	Wood waste utility
Moreno & Font (2015)	ESP	Untreated waste management	Lab analysis	Wood waste utility
Joshi et al. (2015)	USA	Waste management	Questionnaire survey	Wood waste utility
Top (2015)	TUR	Waste management	Questionnaire survey	Economic awareness
Knauf (2015)	DEU	Energy policy	A review of LCA methodology	Environmental awareness
Röder et al. (2014)	UK	Waste management	LCA according to ISO 14040	Environmental awareness
Warnken (2008)	AUS	Waste management	LCA methodology	Environmental awareness
Daian & Ozarska, (2009b)	AUS	Waste management	Questionnaire survey	Economic awareness

FR – France; NGA – Nigeria; CHE – Switzerland; ITA – Italy; PRK – Republic of Korea; BRA – Brazil; JPN – Japan; ESP – Spain; USA – United States of America; TUR – Turkey; DEU – Germany; UK – United Kingdom; AUS – Australia.

## MATERIALS AND METHODS

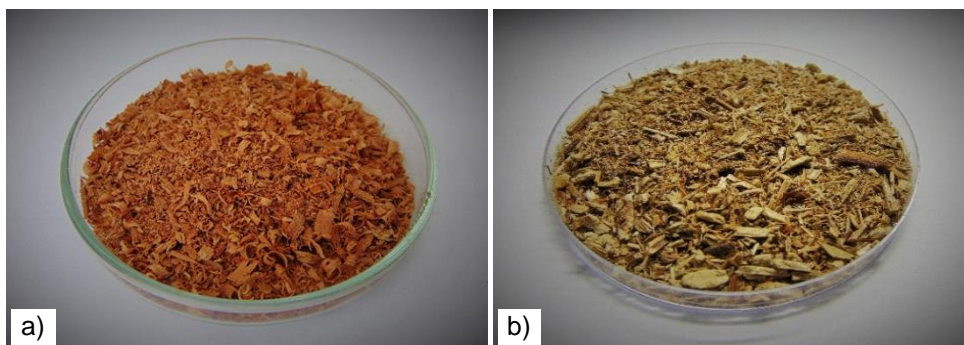
The present chapter is divided into three subchapters sorted chronologically in accordance with the experimental measurements process. Nevertheless, the whole process of biomass definition investigated materials selection; preparation and subsequent testing performed according to international technical standards requirements. Specifically, we used following technical standards requirements: EN 14918 (2010), ISO 1928 (2010), EN 15234–1 (2011), EN 643 (2014), EN ISO 16559 (2014), EN ISO 17225–1 (2015), EN ISO 17831–2 (2015), EN ISO 18122 (2015), EN ISO 18134–2 (2015), EN ISO 16948 (2016) and EN ISO 18123 (2016).

### Investigated samples

The samples used for this paper originated from Brazil in form of rough sawn lumber, kiln dried (KD) at 10–12%, fumigated and then processed in the Czech Republic into profiles for furniture industries. The samples investigated came from trees harvested in 2015 and processed in 2017. The collected waste samples are produced during the cutting of boards in the form of fine dust, during drilling and milling operations for production of outdoor furnitures.



Generated biomass was primarily processed in the effort to meet the requirements for bio-briquette production; such preparation mainly contained from drying (suitable moisture content < 10%) and milling and grinding (suitable particle size < 10 mm). Investigated samples in prepared suitable form are expressed in Fig. 2.



**Figure 2.** Investigated wood waste biomass samples prepared for testing: a) Jatoba; b) Garapa.

### **Chemical quality indicators**

Experimental measurements performed within the chemical analysis stated the safety and suitability of investigated wood waste biomass for direct combustion (energy generation). Following laboratory testing was performed. The measurements were repeated; at least three reliable results were acquired for every sample with respect to the reliability of obtained results and to the behavior of the sample during testing.

### **Moisture and ash content**

Determination of investigated samples moisture content  $M_c$  (%) was performed according to the mandatory technical standard EN 18134-2 (2015) by using of thermo-gravimetric analyser LECO, type TGA 701 (Saint Joseph, United States). Ash content  $A_c$  (%), was determinate using the same equipment in accordance to the mandatory technical standard EN ISO 18122 (2015). Primarily, the samples were dried at 107 °C and further, the samples were burned at 550 °C until their constant weight.

### **Elementary composition**

The content of Carbon C (%), Hydrogen H (%), Nitrogen N (%), Sulphur S (%) and Oxygen O (%) was determined by using of laboratory instrument LECO, type CHN628+S (Saint Joseph, United States) which used helium as carrier gas. The process of testing is completely defined by the mandatory technical standard EN ISO 16948 (2016). Investigated samples were burned in Oxygen while produced flue gases were analyzed.

### **Calorific values**

The results values of gross calorific value GCV ( $\text{MJ kg}^{-1}$ ) were obtained during experimental measurement described in mandatory technical standard EN 14918 (2010) by using of isoperibol calorimeter LECO, type AC 600 (Saint Joseph, United States). However, the results of net calorific value NCV ( $\text{MJ kg}^{-1}$ ) were calculated according to the mandatory technical standard ISO 1928 (2010).

### Mechanical quality indicators

Set of experimental practical tests was used for determination of the final mechanical quality of produced bio-briquette samples. Together, the result values of following tests describe the efficiency of such densification process, thus, the suitability of investigated materials for bio-briquette production and appropriateness of such bio-briquette samples for commercial sale.

### Volume density

The dimensions of produced bio-briquette samples were used for the statement of their volume density  $\rho$  ( $\text{kg m}^{-3}$ ). Following formula (1) was used for its calculation:

$$\rho = \frac{m}{V} \quad (1)$$

where  $\rho$  – volume density ( $\text{kg m}^{-3}$ );  $m$  – bio-briquette samples mass (kg);  $V$  – bio-briquette samples volume ( $\text{m}^3$ ).

### Mechanical durability

Mechanical durability  $DU$  (%), the most important indicator of bio-briquette samples mechanical quality, as stated by the mandatory technical standard EN ISO 17831-2 (2015). A dustproof rotating drum (Fig. 3) was used for experimental part of the testing; the bio-briquette samples was primarily weighted and then placed into the drum and then subjected to the controlled impacts due to the drum rotation.

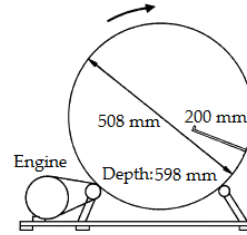
After a precisely stated time of rotation, the tested bio-briquette samples were removed from the drum and weighted. The following formula (2) was used to calculate mechanical durability  $DU$  (%) level of the samples.

$$DU = \frac{m_a}{m_e} \cdot 100 \quad (2)$$

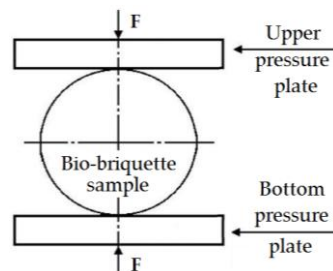
where  $DU$  – mechanical durability (%);  $m_a$  – samples weight after testing (g);  $m_e$  – samples weight before testing (g).

### Rupture force

Statement of rupture force  $RF$  ( $\text{N mm}^{-1}$ ) is not stated by any mandatory technical standards, but it is based on previous experimental testing of Brožek et al. (2012) and (Brožek, 2015). The principle of the test (as shown in Fig. 4) consists in the loading of the force to the bio-briquette sample and subsequent measurement of the maximal force before samples disintegration.



**Figure 3.** Scheme of a mechanical durability equipment testing  $DU$  (CULS, 2013).



**Figure 4.** The principle of rupture force ( $RF$ ) testing.

## RESULTS AND DISCUSSION

Present chapter reports and evaluates obtained observations and result values of specific experimental tests. In respect to the ‘Materials and Methods’ chapter distribution, there are also three subchapters related to the specific biomass or bio-briquette samples parameters.

### Investigated samples

The experimental part of present research related to the bio-briquette samples production is unfortunately represented only by the utilization of the Jatoba wood waste biomass.

The process of bio-briquette samples production was not possible in case of Garapa wood waste biomass due to its limitations. Mentioned limitations were related to the area of materials origin and its transportation. The available amount of material did not correspond to the required quantity material necessary for bio-briquette samples production and testing. Nevertheless, utilization of Jatoba wood waste biomass for bio-briquette production resulted in bio-briquette samples expressed in Fig. 5.



**Figure 5.** Produced bio-briquette sample before experimental testing.

The observation of produced bio-briquette samples dimensions (expressed in Table 2) was important for further calculations and evaluation of subsequent experimental tests.

**Table 2.** Dimensions of produced Jatoba bio-briquette samples

Bio-briquette sample	Mean height (mm)	Mean diameter (mm)	Mean weight (g)
Jatoba	$54.51 \pm 9.15$	$51.45 \pm 0.39$	$101.78 \pm 13.26$

± – standard deviation.

### Chemical quality indicators

Data obtained during chemical analysis described the suitability of investigated materials for direct combustion that ensured environmental conservation but also described energy potential and burning efficiency of such biofuels. The detailed values are noted in Table 3 and Table 4.

**Table 3.** Chemical parameters of investigated wood waste biomass samples (w.b.)

Waste biomass sample	W <sub>c</sub> (%)	A <sub>c</sub> (%)	GCV (MJ kg <sup>-1</sup> )	NCV (MJ kg <sup>-1</sup> )
Jatoba	7.46	0.31	20.16	18.92
Garapa	7.77	3.02	19.61	18.39

W<sub>c</sub> – moisture content; A<sub>c</sub> – ash content; GCV – gross calorific value; NCV – net calorific value; w. b. – wet basis. All values expressed here are average values.

**Table 4.** The chemical composition of investigated wood waste biomass samples in dry basis (w.b.)

Biomass sample	C (%)	H (%)	N (%)	S (%)	O (%)
Jatoba	52.62	5.71	0.23	0.03	41.10
Garapa	51.16	5.60	0.23	0.02	39.97

C – Carbon; H – Hydrogen; N – Nitrogen; S – Sulphur; O – Oxygen.

As the specific value indicates, it is obvious that the level of ash content  $A_c$  (%) occurred at a high level (required result) in cases of both samples. Moreover, in the case of Jatoba wood waste biomass, the values proved extremely good results. The mandatory technical standard states maximal level of ash content  $A_c < 10\%$  in case of bio-briquette fuel intended for commercial sale (EN 15234-1. (2011)).

The values of net calorific values NCV ( $\text{MJ kg}^{-1}$ ) proved the extremely high level of materials energy potential. Such results are highly recommended if consider the mandatory technical standard of the level of net calorific value  $\text{NCV} > 15 \text{ MJ kg}^{-1}$  within the commercial bio-briquette production (EN ISO 17225-1. (2015)).

Within the elementary composition of fuel intended for direct burning, the content of Oxygen O (%) is considered as an important indicator of materials ability to burn; the higher content of Oxygen O ( $> 40\%$ ) can cause problems during the fuel burning. The evaluation of obtained data indicates a satisfactory level of the monitored chemical parameter in both cases; in case of Jatoba, the values occurred only slightly above the theoretical maximal level. The expression of the chemical analysis in dry ash Free State form was also used (noted in Table 5) for the evaluation of precise values of selected chemical parameters without the influence of the presence of the ash.

**Table 5.** Chemical composition of investigated wood waste biomass samples in dry ash free state (d.a.f.)

Biomass sample	C (%)	H (%)	N (%)	S (%)	O (%)	GCV ( $\text{MJ kg}^{-1}$ )	NCV ( $\text{MJ kg}^{-1}$ )
Jatoba	52.79	5.73	0.23	0.03	41.22	20.23	18.98
Garapa	52.75	5.77	0.24	0.02	41.21	20.22	18.97

C – Carbon; H – Hydrogen; N – Nitrogen; S – Sulphur; O – Oxygen; GCV – gross calorific value; NCV – net calorific value; d.a.f. – dry ash free state.

### Mechanical quality indicators

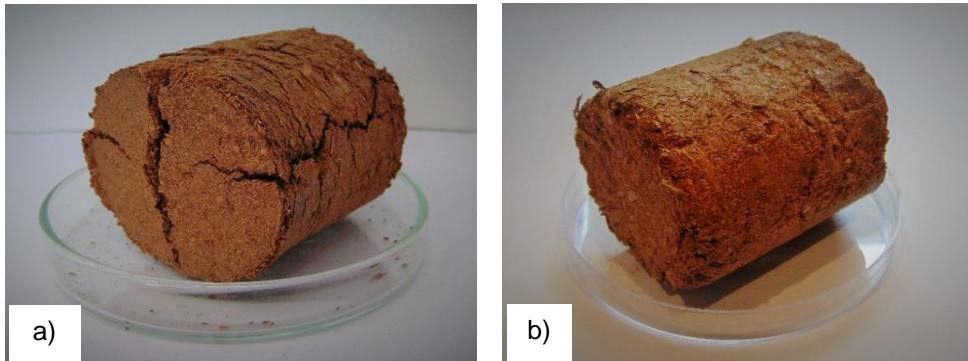
Present chapter provides the evaluation of the mechanical quality of investigated bio-briquette samples produced from Jatoba wood waste biomass; detail values of specific experimental measurements are noted in Table 6.

**Table 5.** Mechanical quality indicators of Jatoba bio-briquette samples

Bio-briquette sample	$W_c$ (%)	$\rho$ ( $\text{kg m}^{-3}$ )	RF ( $\text{N mm}^{-1}$ )	DU (%)
Jatoba	5.61	$896.34 \pm 105.93$	$47.05 \pm 18.78$	77.60

$W_c$  – moisture content;  $\rho$  – volume density; RF – rupture force; DU – mechanical durability;  $\pm$  – standard deviation The values in Table 5 represent only average values.

Volume density  $\rho$  ( $\text{kg}\cdot\text{m}^{-3}$ ) of investigated bio-briquette samples occurred at a satisfactory level (ISO 13061-2, 2014) which indicated the suitability of such material for densification process and proved the efficiency of such bio-briquette production. Results of rupture force RF ( $\text{N mm}^{-1}$ ) and mechanical durability DU (%) described the strength of the bio-briquette samples and their resistance to the handling, transportation or long-term storage. The conditions of the bio-briquette samples after mentioned tests are expressed in Fig. 6. It ought to be mentioned, that both of the tests are destructive, thus, the destruction of tested bio-briquette samples is necessary for obtaining the required data.



**Figure 6.** Jatoba wood waste bio-briquette samples after testing: a) Rupture force RF; b) Mechanical durability DU.

Namely, the specific result of rupture force RF indicated a satisfactory level of such indicator if compare with other previously published results of bio-briquette samples produced from the wood waste biomass under the same conditions with the same diameter (Brožek, 2013; Brožek, 2016). However, the values of mechanical durability DU represented negative results of present research. The minimum level of mechanical durability DU required for commercial bio-briquette production must be  $> 90\%$  and is stated by mandatory technical standard EN ISO 17831-2 (2015). Obtained result of mechanical durability DU equal to 77.60% is insufficient. Nevertheless, such result can be improved by changing of one or more specific production factors related to the densification process; e.g. increasing of briquetting press pressure, decreasing of feedstock materials moisture content or mixing of Jatoba wood waste biomass with other feedstock materials or external binders. All of those factors can positively influence final mechanical durability DU produced bio-briquette samples.

## CONCLUSIONS

Overall evaluation of obtained data within the chemical and mechanical parameters of investigated wood waste biomass from Jatoba and Garapa tree species proved its suitability for direct combustion, thus, its suitability for energy generation. The main advantages of their utilization for such purposes were the great results of ash content  $A_c$  (%) and their high level of energy potential expressed by the NCV ( $\text{MJ kg}^{-1}$ ). Focused on the efficiency of bio-briquette samples produced from Jatoba wood waste biomass,

their volume density  $\rho$  ( $\text{kg m}^{-3}$ ) and rupture force RF ( $\text{N mm}^{-1}$ ) indicated that it is high-quality biofuel, but the result of mechanical durability DU (%) expressed unsatisfactory level. Nevertheless, as was mentioned in ‘Result and Discussion’ chapter, such result can be easily influenced by changing of production parameters of such bio-briquette production. The energy use of wood waste products for biomass combustion should start from harvesting process and follow the all chain of custody to avoid the unnecessary burning of these waste materials. The harvesting plans of these species should contain a specific chapter that state how the wood waste post-harvest will be processed to guarantee that sawmills are sustainably harvesting and make the use of the wood waste as energy potential.

**ACKNOWLEDGEMENTS.** The research was supported by Internal Grant Agency of the Faculty of Engineering, Czech University of Life Sciences Prague, 2018 grant: ‘Energetické využití zemědělských residuí (odpadní biomasy) pomocí procesu densifikace (produkce bio-briket) v domácích i komerčních podmínkách.’ and by the Internal Grant Agency of the Czech University of Life Sciences Prague, grant number 20173005 (31140/1313/3108). The research was performed in cooperation with Kovocite A.S. company in Czech Republic, which provided the processed investigated wood waste biomass materials.

## REFERENCES

- Altafini, C.R., Wander, P.R. & Barreto, R.M. 2003. Prediction of the working parameters of a wood waste gasifier through an equilibrium model. *Energy Conversion and Management* **44**(17), 2763–2777.
- Bergeron, F.C. 2016. Energy and climate impact assessment of waste wood recovery in Switzerland. *Biomass and Bioenergy* **94**, 245–257. Repéré à <http://dx.doi.org/10.1016/j.biombioe.2016.09.009>
- Bittencourt, S.A.M., Sellitto, M.A., Gabbi, A., Schimith, C.D., Ferreira, A.R., Basso, R.L. & Scavarda, A. 2015. Waste management practices adopted by brazilian companies members of the corporate sustainability index of the stock exchange. *Chemical Engineering Transactions* **45**, 769–774. Repéré à <http://dx.doi.org/10.3303/CET1545129>
- Brožek, M. 2013. Study of briquette properties at their long-time storage. *Journal of Forest Science* **59**(3), 101–106.
- Brožek, M. 2015. Evaluation of selected properties of briquettes from recovered paper and board. *Research in Agricultural Engineering* **61**(2), 66–71.
- Brožek, M. 2016. the Effect of Moisture of the Raw Material on the Properties. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* **64**(5), 1453–1458.
- Brožek, M., Nováková, A. & Kolářová, M. 2012. Quality evaluation of briquettes made from wood waste. *Research in Agricultural Engineering* **58**(1), 30–35.
- Daian, G. & Ozarska, B. 2009a. Wood waste management practices and strategies to increase sustainability standards in the Australian wooden furniture manufacturing sector. *Journal of Cleaner Production*, **17**(17), 1594–1602. <http://dx.doi.org/10.1016/j.jclepro.2009.07.008>
- Daian, G. & Ozarska, B. 2009b. Wood waste management practices and strategies to increase sustainability standards in the Australian wooden furniture manufacturing sector. *Journal of Cleaner Production* **17**(17), 1594–1602. <http://dx.doi.org/10.1016/j.jclepro.2009.07.008>
- Emerhi, E.A. 2011. Physical and combustion properties of briquettes produced from sawdust of three hardwood species and different organic binders. *Adv. Appl. Sci. Res.* **2**(6), 236–246.
- European Commission. (2010). *Being wise with waste: the EU 's approach to waste management. Publications Office of the European Union.* Repéré à [http://ec.europa.eu/environment/waste/pdf/WASTE\\_BROCHURE.pdf](http://ec.europa.eu/environment/waste/pdf/WASTE_BROCHURE.pdf)

- Giuntoli, J., Boulamanti, A.K., Corrado, S., Motegh, M., Agostini, A. & Baxter, D. 2013. Environmental impacts of future bioenergy pathways: The case of electricity from wheat straw bales and pellets. *GCB Bioenergy* **5**(5), 497–512.
- Hiramatsu, Y., Tsunetsugu, Y., Karube, M., Tonosaki, M. & Fujii, T. 2002. Present state of wood waste recycling and a new process for converting wood waste into reusable wood materials. *Materials Transactions* **43**(3), 332–339.
- Huron, M., Oukala, S., Lardière, J., Giraud, N. & Dupont, C. 2017. An extensive characterization of various treated waste wood for assessment of suitability with combustion process. *Fuel* **202**, 118–128.
- Israel, D.C., & Bunao, D.F.M. 2017. Value Chain Analysis of the Wood Processing Industry in the Philippines.
- Joshi, O., Grebner, D.L. & Khanal, P.N. 2015. Status of urban wood-waste and their potential use for sustainable bioenergy use in Mississippi. *Resources, Conservation and Recycling*, **102**, 20–26. Repéré à <http://dx.doi.org/10.1016/j.resconrec.2015.06.010>
- Jungmeier, G., Werner, F., Jarnehammar, A., Hohenthal, C. & Richter, K. 2002. Allocation in LCA of Wood-based Products: Experiences of Cost Action E9, Part I. Methodology. *The International Journal of Life Cycle Assessment* **7**(5), 369–375.
- Kaplinsky, R., Memedovic, O., Morris, M. & Readman, J. 2003. *THE GLOBAL WOOD FURNITURE VALUE CHAIN : What Prospects for Upgrading by Developing Countries THE GLOBAL WOOD FURNITURE VALUE CHAIN : What Prospects for Upgrading by Developing Countries The case of South Africa*. Vienna.
- Kim, M.H. & Song, H.B. 2014. Analysis of the global warming potential for wood waste recycling systems. *Journal of Cleaner Production* **69**, 199–207. Repéré à <http://dx.doi.org/10.1016/j.jclepro.2014.01.039>
- Knauf, M. 2015. Waste hierarchy revisited – an evaluation of waste wood recycling in the context of EU energy policy and the European market. *Forest Policy and Economics* **54**(1), 58–60. Repéré à <http://dx.doi.org/10.1016/j.forpol.2014.12.003>
- Marchesan, R. 2012. *Rendimento e qualidade de madeira serrada de três espécies tropicais*. Universidade Federal do Paraná. Repéré à [http://www.floresta.ufpr.br/defesas/pdf\\_ms/2012/d592\\_0826-M.pdf](http://www.floresta.ufpr.br/defesas/pdf_ms/2012/d592_0826-M.pdf)
- Meier, E. 2015. *WOOD! Identifying and Using Hundreds of Wood Worldwide*. (S.l.) : (s.n.).
- Ming, T., Richter, R. De, Liu, W. & Caillol, S. 2014. Fighting global warming by climate engineering : Is the Earth radiation management and the solar radiation management any option for fighting climate change ? *Renewable and Sustainable Energy Reviews* **31**, 792–834. Repéré à <http://dx.doi.org/10.1016/j.rser.2013.12.032>
- Moreno, A.I. & Font, R. 2015. Pyrolysis of furniture wood waste: Decomposition and gases evolved. *Journal of Analytical and Applied Pyrolysis* **113**, 464–473. Repéré à <http://dx.doi.org/10.1016/j.jaap.2015.03.008>
- Owoyemi, J.M., Zakariya, H.O. & Elegbede, I.O. 2016. Sustainable wood waste management in Nigeria. *Environmental & Socio-economic Studies* **4**(3), 1–9. Repéré à <https://www.degruyter.com/view/j/environ.2016.4.issue-3/environ-2016-0012/environ-2016-0012.xml>
- Patel, N. 2014. Environmental and economical effects of fossil fuels. *Journal of Recent Research in Engineering and Technology* **1**, 2349–2252.
- Raud, M., Kesperi, R., Oja, T., Olt, J. & Kikas, T. 2014. Utilization of urban waste in bioethanol production: Potential and technical solutions. *Agronomy Research* **12**(2), 397–406.
- Ribeiro Massote, C.H. & Moura Santi, A.M. 2013. Implementation of a cleaner production program in a Brazilian wooden furniture factory. *Journal of Cleaner Production*, **46**, 89–97. Repéré à <http://dx.doi.org/10.1016/j.jclepro.2012.09.004>

- Röder, M. & Thornley, P. 2017. Waste wood as bioenergy feedstock. Climate change impacts and related emission uncertainties from waste wood based energy systems in the UK. *Waste Management*.
- Röder, M., Whittaker, C. & Thornley, P. 2014. How certain are greenhouse gas reductions from bioenergy? Life cycle assessment and uncertainty analysis of wood pellet-to-electricity supply chains from forest residues. *Biomass and Bioenergy* **79**, 50–63.
- Schulze, M., Grogan, J., Landis, R.M. & Vidal, E. 2008. How rare is too rare to harvest?. Management challenges posed by timber species occurring at low densities in the Brazilian Amazon. *Forest Ecology and Management* **256**(7), 1443–457.
- Singh, D., Cabbage, F., Gonzalez, R. & Abt, R. 2016. Locational determinants for wood pellet plants: A review and case study of North and South America. *BioResources* **11**(3), 7928–7952. Repéré à <https://www.scopus.com/inward/record.uri?eid=2-s2.0-84988659920&doi=10.15376%2Fbiores.11.3.Singh&partnerID=40&md5=df4a883ddb035972b3d3e4ccb688d860>
- Tatano, F., Barbadoro, L., Mangani, G., Pretelli, S., Tombari, L. & Mangani, F. 2009. Furniture wood wastes: Experimental property characterisation and burning tests. *Waste Management* **29**(10), 2656–2665. Repéré à <http://dx.doi.org/10.1016/j.wasman.2009.06.012>
- Top, Y. 2015. Waste generation and utilisation in micro-sized furniture-manufacturing enterprises in Turkey. *Waste Management* **35**, 3–11. Repéré à <http://dx.doi.org/10.1016/j.wasman.2014.09.028>
- van Dam, J. 2013. Competition in wood waste: inventory of policies and markets, 55.
- Warnken, J. 2008. *Wood recovery and recycling : A source book for Australia* **61**, (SI), 45 pp.



## **Yeast as a production platform in biorefineries: conversion of agricultural residues into value-added products**

A. Cristobal-Sarramian\* and D. Atzmüller

University of Applied Sciences Upper Austria, School of Engineering, Stelzhamerstraße 23, AT4600 Wels, Austria

\*Correspondence: [alvaro.cristobal@fh-wels.at](mailto:alvaro.cristobal@fh-wels.at)

**Abstract.** In contrast to a petroleum-based economy, which relies on the unlimited presence of fossil fuels, a biobased economy utilizes a broad spectrum of natural crops and biomass as raw substrates for the production of valuable materials. Biorefineries represent a promising approach for the co-production of bioenergy (biofuels, biogas) and value-added products (biochemicals, biomaterials, food). Within Europe, wheat straw represents the major crop residue and has been extensively considered as a promising feedstock in the biorefining process. Firstly, wheat straw is hydrolysed to obtain a sugar solution that is further converted into the desired product in a biocatalytic manner. Microbial fermentation is the core component of biorefineries and yeast, as for instance *Candida guilliermondii*, is an effective production platform for both, biofuels and biochemicals. One limiting aspect in using yeast in the biorefinery approach is the presence of inhibitors in lignocellulosic hydrolysates, such as acetic acid or furfural, influencing cellular growth and diverse metabolic processes. In order to overcome this problem, several genetic engineering approaches are used to increase yeast resistance towards these inhibitors and to enhance the overall production. In this paper, we summarized: 1) the pretreatment technologies for wheat straw bioconversion; 2) the *Candida guilliermondii* genetic engineering technologies and their biotechnological potential. In conclusion, biorefineries are a crucial factor in the transition towards a biobased and circular economy, and the implementation of yeast into this system offers a great opportunity to develop innovative strategies for a sustainable production in an environmentally friendly and economically feasible manner.

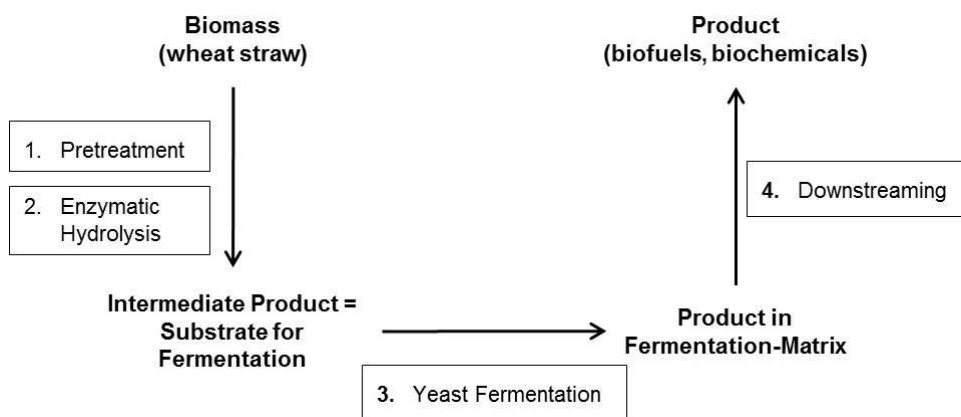
**Key words:** biorefinery, wheat straw, genetic engineering, yeast, *Candida guilliermondii*.

### **INTRODUCTION**

Climate change, depletion of natural resources and an increased worldwide population are three actual and future challenges faced by political leaders. The replacement of a petroleum-based economy by a global biological-based economy (bioeconomy) has become of great importance for European countries in the last decades and is currently accepted as an economically feasible option to address these worldwide issues. In order to achieve long term environmental and economical sustainability, the European Commission set up a goal to implement a low carbon economy by 2050 (Scarlat et al., 2015). Bioeconomy is described as the part of an economy where renewable biological sources are used for the production of vital compounds, biofuels and bioenergy. During the last decades, many organizations contributed to raise public

acceptance of these products and it is expected that by 2025, 15% of the chemicals produced worldwide will derive from biological sources (Savvanidou et al., 2010; Scarlat et al., 2015; Amin et al., 2017). The development of cost-effective technologies for biomass processing and conversion into value-added products are key factors in the implementation of a bio-based economy. Biorefinery systems, which are successfully being used for the co-production of biofuels and biochemicals, are a great example of the potential contribution of biotechnological processes to worldwide waste management and a reduced energy demand (Almeida et al., 2012).

Within lignocellulosic feedstocks, wheat straw, corn fiber, corn stover, switchgrass and barley straw are examples of biomass that has been successfully used as substrates for the production of biochemicals and biofuels (Qureshi et al., 2013). In European countries, wheat straw represents the major crop residue with an overall production of  $170 \times 10^6$  tons per year, and can therefore serve as an easy accessible raw material for its bioconversion in biorefineries (Dias et al., 2010). Additionally, its high carbohydrate/sugar content makes it a promising substrate for microbial growth. The three main components of lignocellulosic residues are hemicellulose (heteropolysaccharide composed of glucose, galactose, xylose and mannose), cellulose (polysaccharide from glucose) and lignin (aromatic polymer composed of three different phenyl propane monomers: coumaryl alcohol, coniferyl alcohol and syringyl alcohol) (Marcos et al., 2013). The common process to convert wheat straw into valuable compounds consists of four major steps: biomass pretreatment, enzymatic hydrolysis, fermentation and purification of the product (downstream processing) (Fig. 1) (Silveira et al., 2015; A. Kumar et al., 2016). In order to get an efficient biomass (e.g. wheat straw) reutilization, innovative technologies combining two key processes have to be developed: 1) Biomass pretreatment technologies and 2) Optimization of yeast as a whole-cell biocatalyst.



**Figure 1.** Biorefinery circular system. Wheat straw as a substrate for the production of biochemicals and biofuels in a four steps process that involves biomass pretreatment, enzymatic hydrolysis, fermentation (by mean of yeast, e.g. *C. guilliermondii*) and product purification.

The goal of the first two steps is to obtain a sugar solution, by pretreating wheat straw and hydrolysing it enzymatically, which can then be utilized during the third step as a fermentable substrate for yeast cells. In the last step, the synthesized product is recovered and purified via downstream processing. During this cycle, several by-products are released, (e.g. glycerol during biodiesel production) and can be re-introduced into this circular system to a certain extent, providing an excellent opportunity to generate a cost-effective system for continuous microbial growth.

During evolution, yeast has been an essential component of human societies, as it is used for human activities, such as the making of bread, wine, beer or other distilled beverages. Additionally, it is also a research model organism used in many laboratories worldwide to elucidate the molecular mechanisms behind cellular processes relevant to biotechnology (Mortimer, 2000). In the actual bioeconomy era, biotechnological processes associated with yeast are believed to be a key factor for the establishment of a circular economy, due to its potential to generate industrially relevant compounds from natural sources and waste streams in a cost-effective and environmentally friendly manner (Moore et al., 2017). Thus, exploiting yeast genetic diversity offers a great opportunity to search for additional ways of increasing production yields, as well as the spectrum of biochemicals and biofuels produced in biorefineries. For this aim, researchers have developed numerous technologies to elucidate the molecular basis of cellular metabolic processes in these microorganisms and applied them for the bioprocessing of agricultural residues (Campbell et al., 2017).

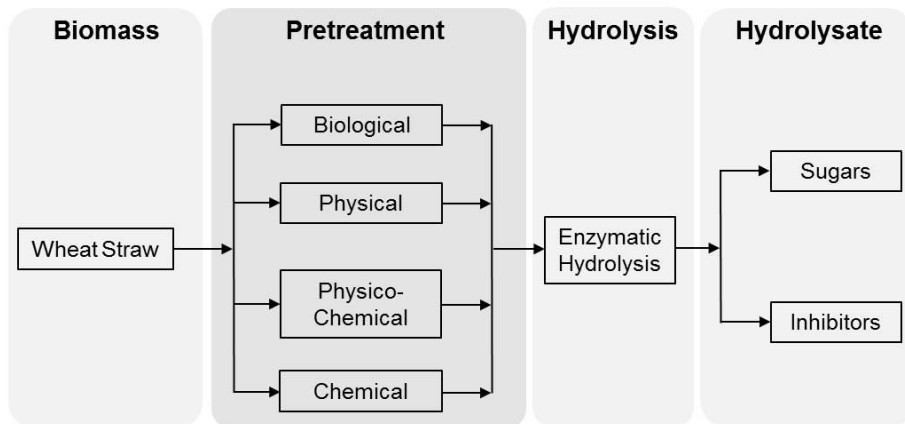
*Saccharomyces cerevisiae* is the most commonly used yeast for biotechnological applications and it is regularly used at industrial scale for the production of biochemicals and biofuels (Mattanovich et al., 2014; Kavšček et al., 2015; Kwak & Jin, 2017). Other non-conventional yeasts represent a great biocatalytic alternative to be used as an economically feasible whole cell production platform, as for instance *Candida guilliermondii* for the production of xylitol, the oleaginous yeast *Yarrowia lipolytica* for the production of lipids and *Ashbya gossypii* for the production of riboflavin (Vitamin B<sub>2</sub>) (Rodrigues et al., 2006; Revuelta et al., 2017; Niehus et al., 2018). Recent advances in genetic engineering (GE) technologies, such as the discovery of the CRISPR-Cas9 system and 'omics' approaches, offer a great opportunity to explore the biotechnological potential these non-conventional yeasts (Raschmanová et al., 2018).

In the first part of this paper, we summarize the main pretreatment methods applied to wheat straw, as an example of a potential biomass substrate that can be used in biorefineries. Then, specific GE approaches designed for *C. guilliermondii* are discussed, as well as examples of potential industrially relevant compounds synthesized by this yeast.

## WHEAT STRAW PRETREATMENT TECHNOLOGIES

The first step in using wheat straw as a biomass source for the production of industrially relevant compounds is the extraction of its contained sugars, which will then be further utilized during microbial fermentation. The optimization of the different pretreatment technologies is the main challenge for biorefineries, due to their high energy demands and costs, as well as the negative effects of inhibitors released during this process on microbial growth (Cardona & Sánchez, 2007).

Regarding the pretreatment of lignocellulose, four different technologies are mostly used: biological, physical, physico-chemical and chemical (Fig. 2). Independently of the chosen pretreatment technology, wheat straw is chopped to increase the yield of the subsequent steps (Eisenhuber et al., 2013). Overall, the described methods aim to increase the enzymes' accessibility to the hemicellulosic and cellulosic polymers.



**Figure 2.** Pretreatment methods used to facilitate wheat straw sugar polymers hydrolysis during enzymatic hydrolysis and production of a fermentable hydrolysate solution.

*Biological* – Biological pretreatment is done by different fungi, as for instance the white rot fungus from the *Trametes* species (Knežević et al., 2016). The special characteristic of this fungi is its ability to produce a lignin-degrading enzyme system, containing a laccase, a lignin-peroxidase and a Mn-dependent peroxidase (Dias et al., 2010). Biological pretreatment has been proven to be a cost-effective manner to delignify wheat straw, thus representing a promising environmentally friendly method to be used in biorefineries. Due to its high lignin and low nitrogen content, the decomposition of wheat straw is a rather slow process (Dias et al., 2010). Lignin is a recalcitrant polymer that offers mechanical resistance during its disruption and makes the subsequent enzymatic hydrolysis step harder.

*Physical* – Mechanical processing of wheat straw, such as milling, grinding, ultrasound or chipping, is a commonly used pretreatment method that confers a cheap and rapid way to increase wheat straw surface area and facilitate the enzymes accessibility to the substrate (Eisenhuber et al., 2013; Silveira et al., 2015). Due to its inability to completely remove the lignin and hemicellulose, it is mostly used in combination with the other pretreatment methods, which then increases enzymatic hydrolysis efficiency.

*Physico-Chemical* – Physico-Chemical pretreatments combine chemical and physical techniques to disrupt the structure of the lignocellulosic materials, as for instance combination of water at high temperatures and high pressures (Liquid Hot Water - LHW) or combination of liquid anhydrous ammonia, high pressure and moderate temperature (Ammonia Fiber Explosion – AFEX) (Brodeur et al., 2011). Another pretreatment technique is Steam Explosion (SE), which has been successfully applied to wheat straw and other agricultural crops and wood residues (Guerrero et al., 2017).

During this process, wheat straw is incubated at high steam temperatures and high pressures during a defined time period. The rapid release of pressure provokes a decompression of the steam and induce structural changes and partial disruption of wheat straw fibers, but also transforms the lignin into other chemicals, e.g. acetic acid, formic acid, furfural and 5-hydroxymethylfurfural (HMF), that can inhibit microbial growth (Auxenfans et al., 2017). Optimization of SE parameters (temperature/time) was shown to be an effective way to recover the maximum amount of the desired sugar (Marcos et al., 2013; Alvira et al., 2016). Additionally, during this process different liquid and solid fractions are recovered and can be used in the subsequent steps, depending on the desired outcome (Alvira et al., 2016). On the one hand, SE is an attractive option requiring no chemicals and having a low energy demand (García-Aparicio et al., 2006). During this process, different inhibitory compounds influencing microbial growth are formed and released (Palmqvist & Hahn-Hägerdal, 2000). Further optimization of this process, for example using N<sub>2</sub> gas instead of compressed air, showed greater results in the overall glucose recovery from wheat straw biomass (Raud et al., 2016; Tutt et al., 2016)

*Chemical* – The alkaline pretreatment process aims to remove the lignin and partially degrade the hemicellulose to facilitate the enzymatic access to cellulose and hemicellulose. This can be achieved for instance by using sodium hydroxide (NaOH). It was shown that a higher concentration of this base positively influences the enzymatic hydrolysis step (Han et al., 2012). Acidic pretreatment is performed at temperatures from 140 °C to 200 °C and involves the addition of acids, such as hydrochloric acid (HCl) or sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), in a diluted or undiluted form, which hydrolyze and remove the hemicellulose (Silveira et al., 2015). The main disadvantages of this method are the requirement of special equipment, due to the high corrosive power of the acids, and the production of a high concentration of microbial growth inhibitors (Sun & Cheng, 2005).

### ***Candida guilliermondii* – GENETIC ENGINEERING APPROACHES AND BIOTECHNOLOGICAL POTENTIAL**

During metabolic engineering approaches, the knowledge of different disciplines, such as systems biology, synthetic biology and ‘*omics*’ (genomics, proteomics, transcriptomics and metabolomics) technologies are combined to enable the production of a desired compound. Enzymatic reactions are improved by modulating specific activities, or expanded by integrating heterologous genes using synthetic biology tools. In this case, where a compound is not naturally produced by a host organism, synthetic biology tools significantly reduced the time to generate genetically optimized production strains. Recent advances in GE technologies, such as the discovery of the CRISPR-Cas9 system, provided an essential tool to rapidly integrate genetic modifications in non-conventional yeast strains, as opposed to the genetic modifications performed using the cellular DNA repair system (Raschmanová et al., 2018).

Using the inherent DNA repair mechanisms, researchers developed tools to integrate foreign DNA into the yeast genome by using the natural DNA homologous recombination (HR) and non-homologous end joining systems (NHEJ). Although different yeast species usually prefer one of the systems, both of them have been successfully used to genetically modify *Candida guilliermondii* (Papon et al., 2015). Additionally, the novel CRISPR-Cas9 technology has been applied to perform genetic

modifications on other *Candida* species, such as *Candida albicans* and *Candida glabrata* (Vyas et al., 2015; Enkler et al., 2016).

*Candida guilliermondii* (teleomorph *Meyerozyma guilliermondii*) is an ascomycetous yeast found in a wide number of environmental sources. During the last decades, researchers studied the biotechnological potential of this non-conventional yeast, including its ability to convert xylose to xylitol and efficiently utilize hemicellulosic hydrolysates as an energy source (Canilha et al., 2003; Rodrigues et al., 2006; Papon et al., 2013).

The complete sequenced genome of the *C. guilliermondii* reference strain ATCC6260 allowed a more detailed study of *Candida*'s biology (Butler et al., 2009). In contrast to other species from the subphylum *Saccharomycotina*, *C. guilliermondii* belongs to the CTG fungal clade, where the CTG codon encodes serine instead of leucine. Therefore, specific efforts have been made in order to design a standardized and versatile genetic toolbox for genetic engineering of yeasts from this clade (Papon et al., 2012; Defosse et al., 2018). This includes different drug-resistance cassettes, as for instance nourseothricin- and hygromycin B-resistance markers, which enable the genetic transformation of wild type strains (Millerioux et al., 2011a; Foureau, et al., 2013b). Additionally, a *C. guilliermondii* mutant strain, named NP566U (*ura5*), was selected during a screening to identify uracil auxotrophic strains and enabled the development of a URA5 integrative cassette which can be used in combination with the so-called URA5 blaster system (Millerioux, et al., 2011b). In this method, the cassette is introduced at a desired position in the genome via homologous recombination and allows the generation of mutant strains in which specific genes are overexpressed, knocked out or proteins are tagged with fluorescence markers such as GFP (Courdavault et al., 2011). Using this system, an optimized recipient strain (KU141F1) derived from the reference strain ATCC6260, which presents an increased homologous recombination rate, has been successfully used to knock out genes and elucidate factors responsible of *C. guilliermondii* virulence in mouse models or for the production of long-chain  $\alpha,\omega$ -dicarboxylic acids (Foureau, et al., 2013a; Navarro-Arias et al., 2016; Werner et al., 2017).

These new technologies offer an excellent opportunity to further explore the biotechnological potential of *C. guilliermondii*. GE approaches, as well as growth optimization processes for wild type strains, are strategies that are exploited by the research community to optimize the production of valuable compounds within this yeast, as for example:

*Xylitol* – Xylitol is a five-carbon polyol industrially produced by a chemical hydrogenation reaction from the five-carbon sugar xylose, a main component of lignocellulosic feedstocks. However, it is also synthesized naturally by some microorganisms (Pal et al., 2016). This rare sugar alcohol is used by the food industry as a sugar substitute for people suffering from diabetes, and it has shown beneficial properties for human health (Granström et al., 2007). Over the last decades, biotechnology research on *C. guilliermondii* was performed aiming to increase the levels of xylitol produced by this yeast. For this, optimization of fermentation parameters or influencing cell permeabilization were the strategies used to efficiently bioconvert xylose to xylitol by wild type strains (De Albuquerque et al., 2014; Cortez et al., 2016). Interestingly, microbial synthesis of xylitol was optimized using metabolic engineering approaches on other yeasts belonging to the *Candida* genus. For instance, in *Candida*

*tropicalis*, xylitol metabolism was modulated by knocking out the gene encoding a xylitol dehydrogenase (XDH), an enzyme catalysing the conversion of xylitol to D-xylulose, or expressing *C. parapilopsis* xylitol reductase (XR) in *C. tropicalis*. This increased xylitol productivity by 25% and 33%, respectively (Lee et al., 2003; Ko et al., 2006).

**Riboflavin** – Among other yeast species, *C. guilliermondii* belongs to the group of flavinogenic yeasts, which are capable of overproducing riboflavin (vitamin B2) under iron limitation conditions (Tanner et al., 1945). Interestingly, it was shown that this yeast is capable of producing riboflavin when grown on media with xylose as a sole carbon source. By using an insertion mechanism approach, the genes responsible for the accumulation of this compound were recently identified (Leathers & Gupta, 1997; Boretsky et al., 2011).

**Ethanol** – *S. cerevisiae* is the most commonly used yeast strain in the bioethanol industry for the production of ethanol (Meijnen et al., 2016). Within the non-conventional yeasts, an osmotolerant *C. guilliermondii* strain was isolated and showed the ability to obtain in 48 hours an ethanol yield of 0.46 g ethanol g<sup>-1</sup> of sugar, using soybean hull hydrolysates as feedstock (Schirmer-Michel et al., 2008).

**Biodiesel** – due to their ability to accumulate high concentrations of lipids within the cell, oleaginous yeasts are the most efficient microorganisms used for biodiesel production. In a recent study, using a high-throughput method to identify strains with a high-lipid content, a novel *C. guilliermondii* oleaginous strain (BI281A) was identified and characterized (Ramírez-Castrillón et al., 2017). Similarly to other oleaginous yeast, such as *Yarrowia lipolytica*, this new strain is able to utilize raw glycerol, derived from a biodiesel refinery, as a carbon source and represents a new possibility for biodiesel production.

**Citric acid and  $\alpha$ -amylase** – taking advantage of its ability to utilize glucose and galactose, citric acid was produced by a *C. guilliermondii* UV mutagenized strain grown on hydrolysed whey permeate as a substrate (Tisnadajaja et al., 1996). Additionally, date wastes, containing high levels of starch, have been described as a possible growth substrate for the production of  $\alpha$ -amylase by this yeast (Acourene & Ammouche, 2012).

**Inulinase** – Inulin is a storage carbohydrate widely found in plants, consisting of a mixture of fructose oligo- and polysaccharides of different lengths. The enzyme hydrolysing these complex carbohydrates into fructose is called inulinase. Interestingly, a *C. guilliermondii* strain isolated from the surface of marine algae showed the ability to produce 60 U mL<sup>-1</sup> of inulinase and was further optimized by UV-mutagenesis to increase the production to 115 U mL<sup>-1</sup> of inulinase (Sirisansaneeyakul et al., 2007; Guo et al., 2009).

## CONCLUSION

Biorefineries are a promising alternative for the synthesis of chemicals and fuels in an environmentally friendly manner. Agricultural crops, e.g. wheat straw, are used as a biomass substrate and are converted into value-added products. To achieve this, several biomass pretreatment technologies are used, as for instance steam explosion. Future development of less energy intensive methods, including the combination of different biomass processing technologies, will contribute to an economically more feasible production within biorefineries.

Yeast has been used over many years for the synthesis of products used by humans on a daily basis. Exploration of yeast diversity offers a great chance to search and increase the spectrum of industrially relevant compounds produced by microorganisms, such as yeast. Recent advances in ‘omics’ technologies, such as proteomics and lipidomics, might facilitate the rapid and efficient analysis of biological samples. Additionally, new technologies for genome analysis and DNA editing greatly facilitated the genetic optimization of production strains.

The yeast *Candida guilliermondii*, which was mostly studied for its ability to produce xylitol, has been successfully modified genetically using drug-resistance cassettes. Thus, the development and implementation of this yeast by a marker-free genetic modification method, such as CRIPSR-Cas9, might contribute to broaden the range of compounds that can be produced by this yeast and exploit its biotechnological potential.

**ACKNOWLEDGEMENTS.** We thank Dr. Manuel Selg for critical reading the manuscript and helpful suggestions. Dr. Alvaro Cristobal-Sarramian’s and Denise Atzmüller’s research for this work was funded within the scope of the European Union Program ‘IWB/EFRE-Regionalprogramm 2014-2020’ (Project name: Combined Agro-Forest Biorefinery – CAFB) from the purse of the European Regional Development Fund (ERDF) and the Federal State of Upper Austria.



## REFERENCES

- Acourene, S. & Ammouche, A. 2012. Optimization of ethanol, citric acid, and  $\alpha$ -amylase production from date wastes by strains of *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Candida guilliermondii*. *J. Ind. Microbiol. Biotechnol.* **39**(5), 759–766.
- Almeida, J.R.M., Fávoro, L.C.L. & Quirino, B.F. 2012. Biodiesel biorefinery: Opportunities and challenges for microbial production of fuels and chemicals from glycerol waste. *Biotechnol. Biofuels* **5**, 1–16.
- Alvira, P., Negro, M. J., Ballesteros, I., González, A. & Ballesteros, M. 2016. Steam Explosion for Wheat Straw Pretreatment for Sugars Production. *Bioethanol* **2**(1), 66–75.
- Amin, L., Hashim, H., Mahadi, Z., Ibrahim, M. & Ismail, K. 2017. Determinants of stakeholders’ attitudes towards biodiesel. *Biotechnol. Biofuels* **10**(1), 219.
- Auxenfans, T., Crônier, D., Chabbert, B. & Paës, G. 2017. Understanding the structural and chemical changes of plant biomass following steam explosion pretreatment. *Biotechnol. Biofuels* **10**(1), 1–16.
- Boretsky, Y.R., Pynyaha, Y.V., Boretsky, V.Y., Fedorovych, D.V., Fayura, L.R., Protchenko, O., Philpott, C.C. & Sibirny, A.A. 2011. Identification of the genes affecting the regulation of riboflavin synthesis in the flavinogenic yeast *Pichia guilliermondii* using insertion mutagenesis. *FEMS Yeast Res.* **11**(3), 307–14.
- Brodeur, G., Yau, E., Badal, K., Collier, J., Ramachandran, K.B. & Ramakrishnan, S. 2011. Chemical and Physicochemical Pretreatment of Lignocellulosic Biomass: A Review. *Enzyme Res.* **2011**, 1–17.



- Butler, G., Rasmussen, M.D., Lin, M.F., Santos, M.A.S., Sakthikumar, S., Munro, C.A., Rheinbay, E., Grabherr, M., Forche, A., Reedy, J.L., Agrafioti, I., Arnaud, M.B., Bates, S., Brown, A.J.P., Brunke, S., Costanzo, M.C., Fitzpatrick, D.A., de Groot, P.W.J., Harris, D., Hoyer, L.L., Hube, B., Klis, F.M., Kodira, C., Lennard, N., Logue, M.E., Martin, R., Neiman, A.M., Nikolaou, E., Quail, M.A., Quinn, J., Santos, M.C., Schmitzberger, F.F., Sherlock, G., Shah, P., Silverstein, K.A.T., Skrzypek, M.S., Soll, D., Staggs, R., Stansfield, I., Stumpf, M.P.H., Sudbery, P.E., Srikantha, T., Zeng, Q., Berman, J., Berriman, M., Heitman, J., Gow, N.A.R., Lorenz, M.C., Birren, B.W., Kellis, M. & Cuomo, C.A. 2009. Evolution of pathogenicity and sexual reproduction in eight *Candida* genomes. *Nature* **459**(7247), 657–662.
- Campbell, K., Xia, J. & Nielsen, J. 2017. The Impact of Systems Biology on Bioprocessing. *Trends Biotechnol.* **35**(12), 1156–1168.
- Canilha, L., Silva, J., Felipe, M. & Carvalho, W. 2003. Batch xylitol production from wheat strawhemicellulosic hydrolysate using *Candida guilliermondii* in a stirred tank reactor. *Lett* **25**, 1811–4.
- Cardona, C.A. & Sánchez, Ó.J. 2007. Fuel ethanol production: Process design trends and integration opportunities. *Bioresour. Technol.* **98**(12), 2415–2457.
- Cortez, D.V., Mussatto, S.I. & Roberto, I.C. 2016. Improvement on d-xylose to Xylitol Biotransformation by *Candida guilliermondii* Using Cells Permeabilized with Triton X-100 and Selected Process Conditions. *Appl. Biochem. Biotechnol.* **180**(5), 969–979.
- Courdavault, V., Millerioux, Y., Clastre, M., Simkin, A.J., Marais, E., Crèche, J., Giglioli-Guivarc'h, N. & Papon, N. 2011. Fluorescent protein fusions in *Candida guilliermondii*. *Fungal Genet. Biol.* **48**(11), 1004–1011.
- De Albuquerque, T.L., Da Silva, I.J., De Macedo, G.R. & Ponte Rocha, M.V. 2014. Biotechnological production of xylitol from lignocellulosic wastes: A review. *Process Biochem.* **49**(11), 1779–1789.
- Defosse, T.A., Courdavault, V., Coste, A.T., Clastre, M., de Bernonville, T.D., Godon, C., Vandeputte, P., Lanoue, A., Touzé, A., Linder, T., Droby, S., Rosa, C.A., Sanglard, D., D'Enfert, C., Bouchara, J.P., Giglioli-Guivarc'h, N. & Papon, N. 2018. A standardized toolkit for genetic engineering of CTG clade yeasts. *J. Microbiol. Methods* **144**(November 2017), 152–156.
- Dias, A.A., Freitas, G.S., Marques, G.S.M., Sampaio, A., Fraga, I.S., Rodrigues, M.A.M., Evtuguin, D.V. & Bezerra, R.M.F. 2010. Enzymatic saccharification of biologically pretreated wheat straw with white-rot fungi. *Bioresour. Technol.* **101**(15), 6045–6050.
- Eisenhuber, K., Krennhuber, K., Steinmüller, V. & Jäger, A. 2013. Comparison of Different Pretreatment Methods for Separating Hemicellulose from Straw During Lignocellulose Bioethanol Production. *Energy Procedia* **40**, 172–181.
- Enkler, L., Richer, D., Marchand, A.L., Ferrandon, D. & Jossinet, F. 2016. Genome engineering in the yeast pathogen *Candida glabrata* using the CRISPR-Cas9 system. *Sci. Rep.* **6**(1), 35766.
- Foureau, E., Courdavault, V., Rojas, L.F., Dutilleul, C., Simkin, A.J., Crèche, J., Atehortúa, L., Giglioli-Guivarc'h, N., Clastre, M. & Papon, N. 2013. Efficient gene targeting in a *Candida guilliermondii* non-homologous end-joining pathway-deficient strain. *Biotechnol. Lett* **35**(7), 1035–1043.
- Foureau, E., Courdavault, V., Simkin, A.J., Sibirny, A.A., Crèche, J., Giglioli-Guivarc'h, N., Clastre, M. & Papon, N. 2013. Transformation of *Candida guilliermondii* wild-type strains using the *Staphylococcus aureus* MRSA 252 ble gene as a phleomycin-resistant marker. *FEMS Yeast Res.* **13**(3), 354–358.
- García-Aparicio, M.P., Ballesteros, I., González, A., Oliva, J.M., Ballesteros, M. & Negro, M.J. 2006. Effect of inhibitors released during steam-explosion pretreatment of barley straw on enzymatic hydrolysis. *Appl. Biochem. Biotechnol.* **129**(1–3), 278–288.

- Granström, T.B., Izumori, K. & Leisola, M. 2007. A rare sugar xylitol. Part I: The biochemistry and biosynthesis of xylitol. *Appl. Microbiol. Biotechnol.* **74**(2), 277–281.
- Guerrero, A.B., Ballesteros, I. & Ballesteros, M. 2017. Optimal conditions of acid-catalysed steam explosion pretreatment of banana lignocellulosic biomass for fermentable sugar production. *J. Chem. Technol. Biotechnol.* **92**(9), 2351–2359.
- Guo, N., Gong, F., Chi, Z., Sheng, J. & Li, J. 2009. Enhanced inulinase production in solid state fermentation by a mutant of the marine yeast *Pichia guilliermondii* using surface response methodology and inulin hydrolysis. *J. Ind. Microbiol. Biotechnol.* **36**(4), 499–507.
- Han, L., Feng, J., Zhang, S., Ma, Z., Wang, Y. & Zhang, X. 2012. Alkali pretreated of wheat straw and its enzymatic hydrolysis. *Brazilian J. Microbiol.* **43**(1), 53–61.
- Kavšček, M., Stražar, M., Curk, T., Natter, K. & Petrovič, U. 2015. Yeast as a cell factory: current state and perspectives. *Microb. Cell Fact.* **14**(1), 94.
- Knežević, A., Stajić, M., Jovanović, V.M., Kovačević, V., Čilerdžić, J., Milovanović, I. & Vukojević, J. 2016. Induction of wheat straw delignification by *Trametes* species. *Sci. Rep.* **6**(1), 26529.
- Ko, B.S., Kim, J. & Kim, J.H. 2006. Production of xylitol from D-xylose by a xylitol dehydrogenase gene-disrupted mutant of *Candida tropicalis*. *Appl. Environ. Microbiol.* **72**(6), 4207–4213.
- Kumar, A., Gautam, A. & Dutt, D. 2016. Biotechnological Transformation of Lignocellulosic Biomass in to Industrial Products: An Overview. *Adv. Biosci. Biotechnol.* **7**(3), 149–168.
- Kumar, A.K. & Sharma, S. 2017. Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. *Bioresour. Bioprocess* **4**(1), 7.
- Kwak, S. & Jin, Y.S. 2017. Production of fuels and chemicals from xylose by engineered *Saccharomyces cerevisiae*: A review and perspective. *Microb. Cell Fact.* **16**(1), 1–15.
- Leathers, T.D. & Gupta, S.C. 1997. Xylitol and riboflavin accumulation in xylose-grown cultures of *Pichia guilliermondii*. *Appl. Microbiol. Biotechnol.* **47**(1), 58–61.
- Lee, J.-K., Koo, B.-S. & Kim, S.-Y. 2003. Cloning and Characterization of the *xy11* Gene, Encoding an NADH-Preferring Xylose Reductase from *Candida parapsilosis*, and Its Functional Expression in *Candida tropicalis*. *Appl. Environ. Microbiol.* **69**(10), 6179–6188.
- Marcos, M., García-Cubero, M.T., González-Benito, G., Coca, M., Bolado, S. & Lucas, S. 2013. Optimization of the enzymatic hydrolysis conditions of steam-exploded wheat straw for maximum glucose and xylose recovery. *J. Chem. Technol. Biotechnol.* **88**(2), 237–246.
- Mattanovich, D., Sauer, M. & Gasser, B. 2014. Yeast biotechnology: Teaching the old dog new tricks. *Microb. Cell Fact.* **13**(1), 1–5.
- Meijnen, J.-P., Randazzo, P., Foulquié-Moreno, M.R., van den Brink, J., Vandecruys, P., Stojiljkovic, M., Dumortier, F., Zalar, P., Boekhout, T., Gunde-Cimerman, N., Kokošar, J., Štajdohar, M., Curk, T., Petrovič, U. & Thevelein, J.M. 2016. Polygenic analysis and targeted improvement of the complex trait of high acetic acid tolerance in the yeast *Saccharomyces cerevisiae*. *Biotechnol. Biofuels* **9**, 5.
- Millerioux, Y., Clastre, M., Simkin, A.J., Courdavault, V., Marais, E., Sibirny, A.A., Noël, T., Crèche, J., Giglioli-Guivarc'h, N. & Papon, N. 2011. Drug-resistant cassettes for the efficient transformation of *Candida guilliermondii* wild-type strains. *FEMS Yeast Res.* **11**(6), 457–463.
- Millerioux, Y., Clastre, M., Simkin, A.J., Marais, E., Sibirny, A.A., Noël, T., Crèche, J., Giglioli-Guivarc'h, N. & Papon, N. 2011. Development of a *URA5* integrative cassette for gene disruption in the *Candida guilliermondii* ATCC 6260 strain. *J. Microbiol. Methods* **84**(2), 355–358.

- Moore, R.H., Thornhill, K.L., Weinzierl, B., Sauer, D., D'Ascoli, E., Kim, J., Lichtenstern, M., Scheibe, M., Beaton, B., Beyersdorf, A.J., Barrick, J., Bulzan, D., Corr, C.A., Crosbie, E., Jurkat, T., Martin, R., Riddick, D., Shook, M., Slover, G., Voigt, C., White, R., Winstead, E., Yasky, R., Ziemba, L.D., Brown, A., Schlager, H. & Anderson, B.E. 2017. Biofuel blending reduces particle emissions from aircraft engines at cruise conditions. *Nature* **543**, 411.
- Mortimer, R.K. 2000. Evolution and variation of the yeast (*Saccharomyces*) genome. *Genome Res.* **10**(4), 403–409.
- Navarro-Arias, M.J., Defosse, T.A., Dementhon, K., Csonka, K., Mellado-Mojica, E., Dias Valério, A., González-Hernández, R.J., Courdavault, V., Clastre, M., Hernández, N.V., Pérez-García, L.A., Singh, D.K., Vizler, C., Gácsér, A., Almeida, R.S., Noël, T., López, M.G., Papon, N. & Mora-Montes, H.M. 2016. Disruption of Protein Mannosylation Affects *Candida guilliermondii* Cell Wall, Immune Sensing, and Virulence. *Front. Microbiol.* **7**(December), 1951.
- Niehus, X., Crutz-Le Coq, A.-M., Sandoval, G., Nicaud, J.-M. & Ledesma-Amaro, R. 2018. Engineering *Yarrowia lipolytica* to enhance lipid production from lignocellulosic materials. *Biotechnol. Biofuels* **11**(1), 11.
- Pal, S., Mondal, A.K. & Sahoo, D. K. 2016. Molecular strategies for enhancing microbial production of xylitol. *Process Biochem.* **51**(7), 809–819.
- Palmqvist, E. & Hahn-Hägerdal, B. 2000. Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition. *Bioresour. Technol.* **74**(1), 25–33.
- Papon, N., Boretsky, Y.R., Courdavault, V., Clastre, M. & Sibirny, A.A. 2015. Genetic Manipulation of *Meyerozyma guilliermondii*. *Genet. Transform. Syst. Fungi, Vol. 2* pp. 245–262.
- Papon, N., Courdavault, V., Clastre, M., Simkin, A.J., Crèche, J. & Giglioli-Guivarc'h, N. 2012. Deus ex *Candida* genetics: Overcoming the hurdles for the development of a molecular toolbox in the CTG clade. *Microbiology* **158**(3), 585–600.
- Papon, N., Savini, V., Lanoue, A., Simkin, A.J., Crèche, J., Giglioli-Guivarc'h, H.N., Clastre, M., Courdavault, V. & Sibirny, A.A. 2013. *Candida guilliermondii*: Biotechnological applications, perspectives for biological control, emerging clinical importance and recent advances in genetics. *Curr. Genet.* **59**(3), 73–90.
- Qureshi, N., Saha, B.C., Cotta, M.A. & Singh, V. 2013. An economic evaluation of biological conversion of wheat straw to butanol: A biofuel. *Energy Convers. Manag.* **65**, 456–462.
- Ramírez-Castrillón, M., Jaramillo-García, V.P., Rosa, P.D., Landell, M.F., Vu, D., Fabricio, M.F., Ayub, M.A.Z., Robert, V., Henriques, J.A.P. & Valente, P. 2017. The Oleaginous Yeast *Meyerozyma guilliermondii* BI281A as a New Potential Biodiesel Feedstock: Selection and Lipid Production Optimization. *Front. Microbiol.* **8**(SEP), 1776.
- Raschmanová, H., Weninger, A., Glieder, A., Kovar, K. & Vogl, T. 2018. Implementing CRISPR-Cas technologies in conventional and non-conventional yeasts: Current state and future prospects. *Biotechnol. Adv.*
- Raud, M., Rooni, V. & Kikas, T. 2016. Explosive decompression pretreatment: Nitrogen vs. compressed air. *Agron. Res.* **14**(2), 569–578.
- Revuelta, J.L., Ledesma-Amaro, R., Lozano-Martinez, P., Díaz-Fernández, D., Buey, R.M. & Jiménez, A. 2017. Bioproduction of riboflavin: a bright yellow history. *J. Ind. Microbiol. Biotechnol.* **44**(4–5), 659–665.
- Rodrigues, R.C.L.B., Sene, L., Matos, G.S., Roberto, I.C., Pessoa, A. & Felipe, M.G.A. 2006. Enhanced xylitol production by precultivation of *Candida guilliermondii* cells in sugarcane bagasse hemicellulosic hydrolysate. *Curr. Microbiol.* **53**(1), 53–59.
- Savvanidou, E., Zervas, E. & Tsagarakis, K.P. 2010. Public acceptance of biofuels. *Energy Policy* **38**(7), 3482–3488.

- Scarlat, N., Dallemand, J., Monforti-ferrario, F. & Nita, V. 2015. The role of biomass and bioenergy in a future bioeconomy : Policies and facts. *Environ. Dev.* **15**, 3–34.
- Schirmer-Michel, Â.C., Hickmann Flôres, S., Hertz, P.F., Matos, G.S. & Ayub, M.A.Z. 2008. Production of ethanol from soybean hull hydrolysate by osmotolerant *Candida guilliermondii* NRRL Y-2075. *Bioresour. Technol.* **99**(8), 2898–2904.
- Silveira, M.H.L., Morais, A.R.C., da Costa Lopes, A.M., Oleksyszzen, D.N., Bogel-Lukasik, R., Andreas, J. & Pereira Ramos, L. 2015. Current Pretreatment Technologies for the Development of Cellulosic Ethanol and Biorefineries. *ChemSusChem* **8**(20), 3366–3390.
- Sirisansaneeyakul, S., Worawuthiyanan, N., Vanichsiratana, W., Srinophakun, P. & Chisti, Y. 2007. Production of fructose from inulin using mixed inulinases from *Aspergillus niger* and *Candida guilliermondii*. *World J. Microbiol. Biotechnol.* **23**(4), 543–552.
- Sun, Y. & Cheng, J.J. 2005. Dilute acid pretreatment of rye straw and bermudagrass for ethanol production. *Bioresour. Technol.* **96**(14), 1599–1606.
- Tanner, F.W., Vojnovich, C. & Van Lanen, J.M. 1945. Riboflavin production by *Candida* species. *Science.* **101**(2616), 180–181.
- Tisnadjaja, D., Gutierrez, N.A. & Maddox, I.S. 1996. Citric acid production in a bubble-column reactor using cells of the yeast *Candida guilliermondii* immobilized by adsorption onto sawdust. *Enzyme Microb. Technol.* **19**(5), 343–347.
- Tutt, M., Raud, M., Kahr, H., Pointner, M., Olt, J. & Kikas, T. 2016. Nitrogen explosion pretreatment of lignocellulosic material for bioethanol production. *Energy Sources, Part A Recover. Util. Environ. Eff.* **38**(12), 1785–1789.
- Vyas, V.K., Barrasa, M.I. & Fink, G.R. 2015. A *Candida albicans* CRISPR system permits genetic engineering of essential genes and gene families. *Sci. Adv.* **1**(3), e1500248–e1500248.
- Werner, N., Dreyer, M., Wagner, W., Papon, N., Rupp, S. & Zibek, S. 2017. *Candida guilliermondii* as a potential biocatalyst for the production of long-chain  $\alpha,\omega$ -dicarboxylic acids. *Biotechnol. Lett.* **39**(3), 429–438.

## **The influence of dietary inclusion of peas, faba bean and lupin as a replacement for soybean meal on pig performance and carcass traits**

L. Degola and D. Jonkus

Latvia University of Life Sciences and Technologies, Institute of Agrobiotechnology, Liela 2, LV3001 Jelgava, Latvia

Correspondence: [lilija.degola@llu.lv](mailto:lilija.degola@llu.lv); [daina.jonkus@llu.lv](mailto:daina.jonkus@llu.lv)

**Abstract.** The effect of peas, faba bean and lupin seed inclusion in growing and finishing pig diets was evaluated. The control diet included soybean meal at 15%, but in the trial groups diets peas were 15 or 28%, faba bean 20 or 25%, lupin seed 12 or 15%, completely replacing soybean meal. Diets formulated to be isoenergetic for ME and with the same crude protein content. The faba bean and, especially, lupin seed meal inclusion in pig diets for growing period significantly reduced ADG ( $P = 0.02$  and  $0.01$ ), and G : F was also significantly influenced ( $P = 0.02$ ) for pigs in lupin seed meal groups. There were no effects on finisher pigs average daily gain, inclusion peas or faba bean, daily gain were, respectively  $892 \pm 19$  and  $915 \pm 11$ ,  $867 \pm 12$  and  $828 \pm 11$ , except lupin seed meal ( $P = 0.04$ ) inclusion. There were no significant effects on carcass quality and to pork chemical content, but pigs fed the diets with peas 28% and faba bean 25% had less of lean meat content, greater backfat thickness and internal fat than other groups which have a similar results. The muscle chemical content show that inclusion of pulses increased the total fat content in pork. In conclusion, results from this experiment suggest that pigs fed peas and faba bean have equal or slightly lower growth performance and carcass traits than pigs fed soybean meal, except lupin seed meal.

**Key words:** peas, faba bean, lupin, growing and fattening pigs, nutrition.

### **INTRODUCTION**

The cereal grains commonly used as feed in pig diets contain insufficient of several of the indispensable amino acids such as lysine, threonine and the sulfur containing amino acids to meet the amino acid requirements of the rapidly growing pig (Sauer et al., 1977). Therefore, it is essential that pig's diet contain a supplementary source of these limiting amino acids.

Soybean meal is the most widely used as the main protein supplement in pig feed (Jezierny et al., 2010) and it is generally a consistent, high quality product (Swick, 1994). In temperate environments, soybeans are difficult to cultivate and pig industry relies heavily on imported soybean meal (Fearnside, 2001). Thus, there is need to find a viable alternative protein source for pig diets.

The grain legumes such as pea, faba bean and also lupin are potential protein sources that could be considered for pig feed in temperate regions. The peas and faba beans have been increasingly grown in Europe during the last 15 years. Lupin (*Lupinus spp.*) have been traditionally grown as a nitrogen-fixation crop in rotation with cereals. Grain legumes are used in different quantities as dietary protein sources because the levels of all nutrients can vary depending on the variety, location and growing conditions.

The peas are composed of two major components, the hull, which consists primarily of non-starch polysaccharides, and the kernel, which consists mainly of protein and starch, with some ash, crude fat, fibre and sugars (Castell & Guenter, 1994). The pea hull is largely indigestible fibre which has low nutritional value and may dilute the nutrients in peas thereby lowering their nutritional value. The pea hull also contains tannins which are known to interfere with protein digestion (Gdala et al., 1992). The second grain legume which mostly used in pig diets is faba bean.

There are two major types of faba beans: those from white-flower varieties and those from colourful-flower varieties. Their chemical composition and nutritive value is about the same, but the colourful-flower varieties contain more tannins. Tannins (usually about 0.3 to 0.5 percent in faba beans) reduce animal feed intake and depress digestibility of protein and energy. The other major anti-nutritional factors in faba beans include trypsin inhibitors and hemagglutinins. Faba beans have extremely low concentration of methionine and cystine (Partanen et al., 2003).

The low content of methionine (2.2 g kg<sup>-1</sup>DM) and tryptophan (3.2 g kg<sup>-1</sup>DM) also found in lupin cultivars comparison to soybean meal (6.7 and 6.9 g kg<sup>-1</sup>DM) (Jezierny et al., 2011). The amino acid profile in lupin is characterized also by a lower content of threonine (Simon & Köhn, 2004) and by a much higher content of arginine (43.9 g kg<sup>-1</sup>DM) which is often deficient in animal diets, comparison to soybean meal (39.8 g kg<sup>-1</sup>DM) (Jezierny et al., 2011).

Lupins seeds is use in the animal feed industry was limited because of the high concentration of alkaloids causing unacceptable taste. Consequently, plant breeders have developed low-alkaloid lupins that are suitable for animal feeding.

The sweet lupin are currently utilised as a valuable protein source in pig diets. Lupin seed is classified as a leguminous plant, a crop with high content of crude protein. Its use as an alternative source of protein in pig diets is gradually increasing.

The seeds of sweet lupin contain 28 to 48% of crude protein relative to the variety and climatic conditions (Linnemann & Dijkstra, 2002). However, considerable amounts of anti-nutritional factors, such as non-starch poly-saccharides and oligosaccharides that are known to influence physiological characteristics of digestive tract and nutrient digestibility, are also present.

In the present study, we assessed the effects of including different levels of pea, faba bean and lupin seeds meal in pig diets as a replacements of soybean meal, on performance of pigs in the growing and finishing periods, and carcass quality.

## MATERIALS AND METHODS

**Animals and housing.** A total of 70 local Yorkshire × Landrace growing-finishing pigs with initial body weight 30 kg were selected from a commercial pig herd and used in the experiment.

Pigs were previously fed commercial soybean meal based diets and were allocated to 1 of 7 dietary treatments for both grower (30 to 60 kg) and finisher (60 to 100 kg) periods, balanced for body weight and sex. They were placed by 10 pigs per pen (5 female and 5 castrated male). Pigs were housed on concrete floors with shavings and access to drinking water at all times. A 2-hole feeder and nipple drinker were installed in each pen. Ambient room temperature in the commercial animal house used ranged between 18 and 22 °C.

**Dietary treatments and Performance Measures.** Commercial sources of peas ('Almara'), faba bean (colourful-flowered spring bean 'Fuego'), lupin seed ('Sonet') and soybean meal were obtained for experiment (Table 1).

**Table 1.** Analyzed composition of main variable ingredients in the diets (on dry matter)

Indices	Pea 'Almara'	Faba bean 'Fuego'	Soybean meal	Lupin seed 'Sonet'
ME, <sup>1</sup> MJ kg <sup>-1</sup>	13.31	12.72	15.85	12.73
Nutrients, &				
DM	92.25	91.84	92.21	92.64
CP	22.83	27.1	29.91	32.8
CF	6.2	7.55	10.89	14.79
Fat	1.02	0.97	19.64	5.32
Ash	1.66	2.43	4.83	3.07
Ca	0.09	0.1	0.21	0.34
P	0.24	0.42	0.95	0.58
Indispensable AA, g kg <sup>-1</sup>				
Arginine	20.83	19.45	20.05	39.22
Histidine	8.78	9.1	9.47	13.53
Isoleucine	10.31	10.32	13.17	14.29
Leucine	16.47	17.2	19.76	22.82
Lysine	15.92	15.07	19.12	14.03
Methionine	2.86	3.12	5.24	4.15
Phenylalanine	10.66	9.9	12.27	12.46
Threonine	8.6	8.19	11.39	12.0
Tryptophan	1.8	2.3	6.7	7.3
Valine	10.97	10.81	12.1	13.18

<sup>1</sup>Calculated as: digestible energy (DE) MJ kg<sup>-1</sup> dry matter DM = 17.47 + 0.0079 × CP + 0.0158 × EEAH – 0.0331 × Ash – 0.0140 × NDF, where EEAH is ether extraction with an organic solvent after acid hydrolysis (Mc Donald et al., 2002) and ME averaged 81.9% of DE for protein feeds (Morgan, 1975).

The seven dietary treatments were formulated for both periods (growing and finishing) in a dose response feeding trial (Table 2). The control diet included soybean meal at 15%, but in the trial groups diets peas were 15 or 28%, faba bean 20 or 25%, lupins 12 or 15%, completely replacing soybean meal. Other ingredients were kept constant and included barley, wheat, triticale, canola oil, salt and trace element – vitamin premix. Diets formulated to be isoenergetic for metabolizable energy (ME) and with the same crude protein (CP) content.

**Table 2.** Composition of experimental diets for both (growing and fattening) periods (as-fed basis)

Indices	Soybean meal, %	Pea, %		Faba bean, %		Lupin seed meal, %	
Ingredients, %							
Pea 'Almara'	-	15	28	-	-	-	-
Faba bean 'Fuego'	-	-	-	20	25	-	-
Lupin seed meal 'Sonet'	-	-	-	-	-	12	15
Soybean meal	15	-	-	-	-	-	-
Wheat	36.7	36.7	23.7	31.7	26.7	39.7	36.7
Barley	30	30	30	30	30	30	30
Triticale	13	13	13	13	13	13	13
Vitamin-mineral premix	3.3	3.3	3.3	3.3	3.3	3.3	3.3
Canola oil	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Calculated analysis							
ME <sup>1</sup> MJ kg <sup>-1</sup>	13.58	13.53	13.39	13.37	13.27	13.75	13.67
Lys, &	0.73	0.65	0.69	0.71	0.70	0.65	0.68
Analyzed composition							
Nutrients, %							
DM	88.2	87.9	87.8	87.8	87.8	88.3	88.2
CP	14.94	14.38	14.08	14.70	15.03	14.43	15.10
CF	4.15	3.01	3.38	3.70	4.05	3.65	3.95
Fat	6.24	3.45	3.27	3.4	3.38	4.37	4.56
Ash	5.26	4.53	4.58	4.88	4.99	4.74	4.61
Ca	0.83	0.81	0.80	0.82	0.87	0.86	0.85
P	0.56	0.55	0.51	0.52	0.6	0.63	0.62

<sup>1</sup> Calculated as: digestible energy (DE) MJ kg<sup>-1</sup> dry matter DM = 17.47 + 0.0079 × CP + 0.0158 × EEAH – 0.0331 × Ash – 0.0140 × NDF, where EEAH is ether extraction with an organic solvent after acid hydrolysis (Mc Donald et al., 2002) and ME averaged 81.9% of DE for protein feeds (Morgan, 1975).

Each diets was available on an ad libitum basis to pens with 10 pigs per pen for period from weaning age till pigs reach 100 kg liveweight.

Every month the bodyweight (BW) for individual pigs and every week pen feed intake recorded to assess average daily gain (ADG), average daily feed intake (ADFI) and liveweight gain from one kg feed (G : F).

**Slaughter and Carcass Quality Measurements.** The finisher pigs at the 100 kg liveweight, slaughtered at a commercial slaughter house. Hot carcass weights (HCW) were obtained and backfat depth was measured at a specific site [i.e., the head of the last rib, 6 cm from the mid back line (P2), using a probe (Introscope Optimal Probe; SFK, Kolding, Denmark)]. For each individual pig, the percentage of lean meat (% Lean) was calculated as:  $66.6708 - 0.3493 \times P2$  (Latvia reg.of the Cabinet of Ministers Nr. 307), and killing out percentage was calculated as:  $HCW/BW \times 100\%$ . The length 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. The internal fat was removed from carcass and weighed. One side of carcass was divided into fractions for determination ham. The pork samples only of one carcass were taken from the *musculus longissimus lumborum et thoracis* 24 hours *post mortem* and subsequently subjected to the chemical analysis to assess water, fat and crude protein content, ph and cholesterol.



**Analytical Methods.** Diets and ingredients were milled through a 1-mm screen before analysis. The chemical content of feed samples were determined in Scientific laboratory of Agronomic analysis of Latvia. The dry matter (DM), crude protein (CP), crude fibre (CF), fat, ash, Ca and P contents were analysed based on standard methodology, respectively DM with ISO 6496: 1999, CP with LVS EN ISO 5983-2: 2009, Ca with LVS EN ISO 6969: 2002, P with ISO 6491: 1998, crude fibre (CF) with ISO 5498: 1981, fat ISO 6492: 1999, ash with ISO 5984: 2002/Cor1: 2005. The metabolizable energy (ME) were calculated according to equation (Morgan, 1975; Mc Donald et al., 2002). The amino acids contents in pea, faba bean, lupin seed and soybean meal were determined by near infrared reflectance spectroscopy (NIRS). The chemical analysis in pork were determined by LVS ISO 1442:1997 for water, LVS ISO 1443:1973 for fat and LVS ISO 937:1978 for crude protein content, LVS ISO 2917:2004 for ph and BIOR-T-012-132-2011 for cholesterol content in laboratory of Food and Environmental Investigations (BIOR) in Latvia.

**Statistical analysis.** All data were statistically processed to determine the differences between protein sources. Statistical analysis was performed according to the General linear Model procedure of SAS/STAT 9.22 software package (2010). Most data was reported as arithmetic means with the pooled SEM. The treatment means were compared using Student's *t*-test. Statements of statistical significance were based upon  $P < 0.05$ .

## RESULTS AND DISCUSSION

The mean of ADG, ADFI and G:F for pigs in growing period were  $532 \pm 37$  g,  $1947 \pm 69$  and  $0.27 \pm 0.02$  g g<sup>-1</sup>, but for pigs in finishing period were  $854 \pm 64$ ,  $2681 \pm 178$  and  $0.32 \pm 0.02$ , respectively (Table 3). The faba bean and, especially, lupin seed meal inclusion in pig diets for growing period significantly reduced ADG ( $P = 0.02$  and  $0.01$ ), and G : F was also significantly influenced ( $P = 0.02$ ) for pigs in lupin seed meal groups. There were no effects on finisher pigs average daily gain, inclusion peas or faba bean, daily gain were, respectively  $892 \pm 19$  and  $915 \pm 11$ ,  $867 \pm 12$  and  $828 \pm 11$ , except lupin seed meal ( $P = 0.04$ ) inclusion (Table 3). The use of peas and faba bean as an alternative protein source in pig diets in early trials indicated that greater than 20% inclusion levels reduced performance. (Castell, 1976; Gatel & Grosjan, 1990). In other research (Smith et al., 2013) the ADG for grower and finishing pigs were more higher than in our research, respectively in growing period  $869 \pm 41$  g and in finishing  $1,000 \pm 40$  g. It should be noted that the diets in our studies were not formulated to ensure deficiencies of methionine or tryptophan. In previous studies using pea diets supplemented with crystalline methionine or tryptophan to correct for this deficiency have shown pig performance comparable to soybean meal (Gatel & Grosjan, 1990). Similarly, a small number of trials supplementing faba bean diets with crystalline amino acid demonstrate improved performance (Crepon, 2006). Thus, better peas, faba bean and lupin seed inclusion results may be attainable provided diets are balanced for the limiting amino acids. The results of our current study confirm this.

**Table 3.** Effect of dietary treatment on performance of pigs

Dietary treatment	In growing period			In finishing period		
	ADG, g	ADFI, g	G:F, g g <sup>-1</sup>	ADG, g	ADFI, g	G:F, g g <sup>-1</sup>
Soybean meal	555	1,848	0.300	897	2,560	0.351
Pea						
15%	550	1,970	0.279	892	2,900	0.347
28%	554	1,831	0.303	915	2,980	0.335
Faba bean						
20%	545	1,975	0.275	867	2,630	0.330
25%	540	1,995	0.270	828	2,580	0.321
Lupine seed meal						
12%	528	2,000	0.264	853	2,680	0.318
15%	450	2,011	0.223	726	2,440	0.298
SEM	37	69	0.0246	64	178	0.0168
<i>P-value</i>						
Soybean meal vs. pea	0.39	0.17	0.48	0.26	0.13	0.15
Soybean meal vs. faba bean	0.02	0.82	0.21	0.06	0.11	0.09
Soybean meal vs. lupine seed meal	0.01	0.57	0.02	0.04	0.36	0.07

Table 4. shows the slaughter measures: P2, lean meat , killing out , the length of carcass, muscle-eye area, internal fat and ham weight. Dietary treatment did not significantly affect carcass traits. The mean of backfat, lean meat, killing out, the length of carcass, muscle-eye area, internal fat and ham weight were  $12.7 \pm 2.3$  mm,  $62.2 \pm 0.71\%$ ,  $76.0 \pm 4.17\%$ ,  $101.7 \pm 12.11$  cm,  $52.5 \pm 1.54$  cm<sup>2</sup>,  $1.72 \pm 0.37$  kg,  $11.31 \pm 0.97$  kg, respectively.

**Table 4.** Effect of dietary treatment on carcass traits

Dietary treatment	P2, mm	Lean, %	Killing out, %	Carcass length, cm	Muscle-eye area, cm <sup>2</sup>	Internal fat, kg	Ham weight, kg
Soybean meal	10	63.1	79.0	89	54.8	1.3	10.5
Pea							
15%	15	61.4	83.6	109	51.9	2.3	11.6
28%	12	62.5	75.6	110	50.8	2.0	10.3
Faba bean							
20%	14	61.8	70.7	114	52.2	1.5	12.5
25%	11	62.8	73.2	90	53.8	1.4	10.4
Lupine seed meal							
12%	16	61.1	72.1	115	50.4	2.1	12.9
15%	11	62.8	78.0	85	53.9	1.5	11.0
SEM	2.3	0.71	4.17	12.11	1.54	0.37	0.97
<i>P-value</i>							
Soybean meal vs. pea	0.22	0.28	0.18	0.22	0.19	0.10	0.34
Soybean meal vs. faba bean	0.49	0.42	0.13	0.49	0.37	0.19	0.44
Soybean meal vs. lupine seed meal	0.25	0.31	0.39	0.44	0.39	0.41	0.25

The chemical composition of meat was shown in Table 5. The values of water content in trial groups were in the range of 69.4–75.7%, the highest value of water was found in pork which received 12% lupine seed meal in diet. The other authors also presented the same water content in pork from 72.0–74.0% (Correa et al., 2006). The fat content were higher in pork samples of all pig groups which received pulses in diets, especially with 28% pea and 25% of faba bean, respectively 1.9 and 1.8 times more than of soybean meal pig group. The content of crude protein also was very different in the groups (20.1–24.1%) and also the cholesterol concentration of pigs ranged in large, from 60.6 till 108.3 mg 100 g. In the researchers Kim et al. (2016) studies, a higher protein content resulted in pork lower pH<sub>24h</sub> value, pH 5.7 (n = 90) 24.37%, but pH 6.1 (n = 56) 23.16% crude protein content (Kim et al., 2016). A number of previous studies reported levels of cholesterol in *longissimus* muscle with 57 mg 100 g (Dorada et al., 1999) and 59 mg 100 g (Moss et al., 1983). Similarly Bohac & Rhee (1988) reported cholesterol content of 55.7 mg 100 g. These figures are considerably lower than our current results. Apparently, the obtained figures depended on the feeding of pigs, breed and rearing environment.

**Table 5.** The chemical analyses of the *musculus longissimus lumborum et thoracis*

Item	Soybean meal	Pea		Faba bean		Lupine seed meal	
		15%	28%	20%	25%	12%	15%
Water, &	72.4	70.1	69.4	71.7	70.1	75.7	72.2
Fat content, &	3.8	6.5	7.2	5.3	6.7	3.6	4.4
Crude protein content, &	24.1	23.6	22.4	21.8	22.8	20.1	22.4
pH	5.48	5.38	5.41	5.49	5.28	5.62	5.47
Cholesterol, mg 100g	108.3	105.7	78.0	77.6	103.3	60.6	69.1

The pH of muscle in the living pigs is around 7.0 till 7.2. After slaughter, it drops to around 5.5. Pork already reaches its lowest pH value of 5.4 to 5.8 at 6–10 hours after slaughter. Glycogen is broken down to lactic acid when muscle turns into meat. The pH of pork can range from 5.2 to 7.0. The highest quality products tend to fall in the range of 5.7 to 6.0 (Kim et al., 2016). The pH of pork muscle in our research was from 5.28–5.62.

## CONCLUSIONS

The results from the present study indicate that 20% and 25% faba bean, 12% and 15% lupine seed meal inclusion without crystalline amino acids may for pigs in growing period significantly reduced average daily gain. The liveweight gain from one kg feed was also significantly influenced for pigs in lupine seeds meal groups in growing period. There were no effects of inclusion of peas or faba bean on pigs average daily gain in finishing period, except lupine seed meal inclusion. There were no significantly effects on carcass quality and to pork chemical content. But pigs fed the diets with peas 28% and faba bean 25% had less of lean meat content, greater backfat thickness and internal fat than other groups which have a similar results. The muscle chemical content show that inclusion of pulses increased the total fat content in pork.

ACKNOWLEDGEMENTS. This research was part of the project "Pulses as alternative protein feeds instead of soybean meal, their production, agricultural and economic evaluation in Latvia conditions" funded by Ministry of agriculture, Republic of Latvia, but the chemical content of pork is supported by second project 'VPP- 20142017 AgroBioRes Project No. 3 LIVESTOCK'.

## REFERENCES

- Bohac, C.E. & Rhee, K.S. 1988. Influence of animal diet and muscle location on cholesterol content of beef and pork muscles. *Meat.Sci.* **23**, 71–75.
- Castell, A.G. 1976. Comparison of faba beans (*Vicia faba*) with soubean meal or field peas (*Pisum sativum*) as protein supplements in barley diets for growing –finishing pigs. *Can. J. Anim.Sci.* **432**, 425–432.
- Castell, A.G. & Guenter, W.1994. Nutritive value of peas for monogastric diets. In *Proceedings of the 15<sup>th</sup> Western Nutrition Conference*, Winnipeg, Manitoba, pp. 21–38.
- Crepon, K. 2006. Nutritional value of legumes (pea and faba bean) and economics of their use. In: Garnsworthy, P.C. & Wisenman, J., editors, *Recent Advances in Animal Nutrition*. Nottingham Univ. Press, Nottingham, UK, 331–366.
- Correa, J.A., Faucitano, L., Laforest, J.P., Rivest, J., Marcoux, M. & Gariépy, C. 2006. Effects of slaughter weight on carcass composition and meat quality in pigs of two different growth rates. *Meat sci.* **51**, 215–219.
- Dorado, M., Martin Gomez, E.M., Jimenez-Colmenero, F. & Masoud, T.A. 1999. Cholesterol and fat contents of spanish commercial pork cuts. *Meat Sci.* **51**, 321–323.
- Fearnside, P.M. 2001. Soybean cultivation as a threat to the environment in Brazil. *Environ.Conserv.* **28**, 23–38.
- Gatel, F. & Grosjean, F. 1990. Composition and nutritive value of peas for pigs: a review of European results. *Livestock Production Science* **26**(3), 155–175.
- Gdala, J., Buraczewska, L. & Grala, W. 1992. The chemical composition of different types and varieties of pea and digestion of their protein in pigs. *J. Anim.Feed Sci.* **1**, 71–79.
- ISO 6496:1999. Animal feeding stuffs. Determination of moisture and other volatile matter content. ISO/TC34/SC10, ICS: 65.120, ed.2, 7 pp.
- ISO 6491:1998. Animal feeding stuffs. Determination of phosphorus content. ISO/TC34/SC10, ICS: 65.120, ed.2, 7 pp.
- ISO 5498:1999. Animal feeding stuffs. Determination of fat content. ISO/TC34/SC10, ICS: 65.120, ed.1, 9 pp.
- ISO 5498:1981. Agricultural food products. Determination of crude fibre content. ISO/TC34, ICS: 67.050, ed.1, 8 pp.
- ISO 5984: 2002/Cor.1:2005. Animal feeding stuffs. Determination of crude ash. ISO/TC34, Food products SC 10 ed.3, 10 pp.
- Kim, T.W., Kim, C.W., Yang, M.R., No, G.R., Kim, S.W. & Kim, I.S. 2016. Pork Quality Traits According to Postmortem pH and Temperature in Berkshire. *Korean J. Food Sci. Anim. Resour.* **36**(1), 29–36.
- Jezierny, D., Mosenthin, R. & Bauer, E. 2010. The use of grain legumes as a protein source in pig nutrition: A review. *Anim. Feed Sci. Technol.* **157**, 111–128.
- Jezierny, D., Mosenthin, R., Sauer, N., Roth, S., Piepho, H.P., Rademacher, M. & Eklund, M. 2011. Chemical composition and standardised ileal digestibilities of crude protein and amino acids in grain legumes for growing pigs. *Livestock Science* **138**, 229–243.
- Linnemam, R.A. & Dijkstra, S.D. 2002. Toward Sustainable Production of Protein-Rich Foods: Appraisal of Eight Crops for Western Europe. PART I. Analysis of the Primary Links of the Production Chain. *Food. Sci.And Nutrition.* **42**(4), 377–401.

- LVS EN ISO 5983-2:2009. Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content. ISO 5983-2:2009, ISO/TC34/SC10, ICS: 65.120, ed. 2. part 2, 15pp.
- LVS EN ISO 6869:2002. Animal feeding stuffs. Determination of calcium, magnesium and sodium. ISO 6869:2000, ISO/TC34/SC10, ed.1,15 pp.
- LVS ISO 1442:1997. Meat and meat products. Determination of moisture content. ISO 1442:1997,ISO/TC34/SC6, ICS: 67.120.10, ed. 2, 4 pp.
- LVS ISO 1443:1973. Meat and meat products. Determination of total fat content. ISO 1442:1973, ISO/TC34/SC6, ICS: 67.120.10, ed.1, 2 pp.
- LVS ISO 937:1978. Meat and meat products. Determination of nitrogen content. ISO 937:1978, ISO/TC34/SC6, ICS: 67.120.10, ed.1, 3 pp.
- LVS ISO 2917:2004. Meat and meat products. Measurement of pH content. ISO 2917:1999, ISO/TC34/SC6, ICS: 67.120.10, ed.2, 6 pp.
- Mc Donald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A. 2002. *Animal nutrition*. 6th ed. Pearson Education, Harlow, UK, 665 pp.
- Morgan, D.J., Cole, D.J.A. & Lewis, D. 1975. Energy value in pig nutrition. 1. The relationship between digestible energy, metabolizable energy and total digestible nutrient values of a range of feedstuffs. *J. Agric. Sci.* **84**, 7–17.
- Moss, M., Holden, J.M., Ono, K., Cross, R., Slover, H., Berry, B., Lanza, E., Thompson, R., Wolf, W., Vanderslice, J., Johnson, H. & Stewart, K. 1983. Nutrient composition of fresh retail pork. *J. Food Sci.* **48**, 1767–1771.
- Partanen, K., Alaviuhkola, T., Siljander-Rasi, H. & Suomi, K. 2003. Faba bean in diets for growing –finishing pigs. *Agricultural and Food Sci.* **12**, 35–47 (in Finland).
- Sauer, W.C, Stothers, S.C. & Phillips, G.D. 1977. Apparent availabilities of amino acids in corn, wheat and barley for growing pigs. *Can. J. Anim. Sci.* **57**, 585–597.
- Shelton, J., Hermann, M. D., Strode, R.M., Brashear, G.L., Ellis, M., McKeith, F.K., Bidner, T.D. & Southern L.L. 2001. Effect of different protein sources on growth and carcass traits in growing – finishing pigs. *J. Anim. Sci.* **79**, 2428–2435.
- Simon, A. & Köhn, W. 2004. Influence of variety and location on the crude protein content and amino acid composition of grain legumes *Proc.Soc. Nutr. Physiol.* **13**, 42.
- Smith, L.A., Houdijk, J.G.M., Homer, D. & Kyriazakis, I. 2013. Effects of dietary inclusion of pea and faba bean as are placement for soybean meal on grower and finisher pig performance and carcass quality. *J. of Anim. Sci.* **91**, 3733–3741.
- Swick, R.A. 1994. Soybean meal quality. American Soybean Association Technical Bulletin PO11.

## Low temperature BMP tests using fish waste from invasive Round goby of the Baltic Sea

A. Gruduls\*, K. Balina, K. Ivanovs and F. Romagnoli

<sup>1</sup>Riga Technical University, Institute of Energy Systems and Environment, Azenes street 12-K1, LV-1048 Riga, Latvia

\*Correspondence: Arturs.Gruduls@rtu.lv

**Abstract.** Round goby (*Neogobius melanostomus*) is an invasive fish species in the Baltic Sea. While meat can be used for human consumption, fish processing residues are considered as a waste. Within circular economy and bio-economy perspectives fish waste could be used as a valuable feedstock for biogas production. However, the research is mostly focused on evaluating biogas yield at mesophilic conditions (i.e. 37 °C). In this study the impact of low temperature on Biochemical Methane Potential (BMP) tests has been investigated. Round goby's processing leftovers - heads, intestines and skin/bone mixture were tested in codigestion with sewage sludge. Anaerobic digestion (AD) was carried out in 100 mL batch tests at low temperature 23 °C and 37 °C conditions, over an incubation period of 31 days. The results show that AD at low temperature occurs twice as slowly as under 37 °C conditions. However, after 31 days the BMP values for 23 °C samples were only 2% lower than for high temperature samples. Heads and skins showed similar BMP values reaching on average 502 L CH<sub>4</sub> kg<sub>VS</sub><sup>-1</sup> and 556 L CH<sub>4</sub> kg<sub>VS</sub><sup>-1</sup> respectively. BMP for fish intestines was higher, reaching on average 870 L CH<sub>4</sub> kg<sub>VS</sub><sup>-1</sup>. Average BMP for mixes of fish heads, skins, intestines and bones was 660 L CH<sub>4</sub> kg<sub>VS</sub><sup>-1</sup>. Acquired BMPs were further compared with the theoretical BMPs from Buswell's formula. Research results suggests that anaerobic digestion of fish waste under low temperature conditions could be feasible as the process still efficiently occurs, in fact opening a new opportunity to explore the overall sustainability of technologies based on these conversion processes.

**Key words:** Biomethane, low temperature, fish waste, anaerobic digestion, *Neogobius melanostomus*.

### INTRODUCTION

In last decades' the population of round goby (*Neogobius melanostomus*) has spread into the Baltic Sea. Coming from Caspian Sea, this fish in Latvian coastline has been firstly observed in 2004 and since then, the amount of it has increased significantly reaching 25 tons in the year 2013 and more than 700 tons in year 2017 (Riekstiņš, 2014; 2017). Currently in the nearshore waters of the Baltic Sea this is the second most caught fish species after the Baltic herring. Distribution area is still expanding and has become a huge problem regarding both environmental and economic aspects. This fish species has become invasive in Latvia due to easy adaption to surrounding environment (Charlebois et al., 2001). Since the amount of fish has been growing, it can represent a valuable economic opportunity.

Physiology of round goby allows using only 40% of it as a meat for food, creating large amounts of waste. Waste biomass includes parts like skin, head, bones, fins and intestines (Eiroa et al., 2012). In recent years potential use of this fish waste has become a popular research topic. Melvere et al. (2017) describe many options for use of round goby's processing waste in bioeconomy. The author suggests using it as raw material to produce a wide range of products including also high value-added end products like enzymes, proteins and fish oil. Salam et al. (2009) claims that fish waste can also be successfully used for energy production producing biogas in anaerobic fermentation processes. However the high content of ammonia nitrogen might negatively affect fermentation processes thus one of the best ways for fish waste biomethanation is co-digestion (Tomczak-Wandzel et al., 2013).

Anaerobic fermentation has been used for waste treatment and biogas recovery from many types of organic waste. Its numerous advantages, such as the recovery of a renewable energy, waste volume and odour reduction are well documented (Gunaseelan, 1997; Wu et al., 2009). Anaerobic treatment of fish waste not only reduces unpleasant odour but also gives the opportunity to regain some energy used for the production processes. However, until recently, research has mainly been focused on anaerobic digestion (AD) at mesophilic (25–45 °C) or thermophilic (45–65 °C) temperatures. It is believed that a lower temperature in the psychrophilic range (< 25 °C) reduces microbial activity and in fact is lowering the biogas yield (Connaughton et al., 2006; Saady & Massé, 2013). One of the main advantages of psychrophilic temperatures would be the lower energy input required for heating the reactor, consequently reducing the overall operating cost (Smith et al., 2013). The most recent results on microbiological activity in psychrophilic conditions show that lower temperatures require a longer fermentation time and lead to higher methane content and lower accumulation of volatile fatty acids compared to mesophilic conditions, although still keeping a similar cumulative biomethane yield in both conditions (Wei & Guo, 2018).

In this study experiments on biogas production at mesophilic and lower temperatures were carried out and the data have been compared. The aim of this study was to assess the process performance of two BMP test setups inoculated with the same sewage sludge for treatment of fish waste. One setup of 100 mL bioreactors was operated at 37 °C, while the second was maintained at room temperature 21–23 °C. Comparative investigations of biomethane production in both temperature ranges would allow evaluation of the overall economic feasibility. In fact, it would be a key aspect to assess the potential benefits in operational costs in terms of lower energy input required for heating, reduction of the amount of waste in fish processing plants, energy recovery capability within the production processes, although bigger digester volume may be necessary.

## MATERIALS AND METHODS

### **Substrate (collection, pre-treatment, and storage)**

The *Neogobius melanostomus* used within the batch tests for the BMP evaluation were freshly caught on Baltic sea costal area in August 2015 (biomass 2) and April 2017 (biomass 1), near the city of Liepaja, West Latvia. Whole fish samples were transported within plastic bags to the Biosystem Laboratory at the Riga Technical University, separated in smaller portions and then frozen at –18 °C. Prior experiments biomass was

defrosted at room temperature. Then fish were skinned, gutted, deboned and beheaded. Processing waste products – heads, intestines and skin/bone mixture were used for further BMP testing. Each fish waste fraction was separately homogenized using 1,500 W kitchen blender and given to total solids (TS) and volatile solids (VS) content analyses. Homogenized samples were frozen again at  $-18\text{ }^{\circ}\text{C}$ , and defrosted a day before the start of BMP tests.

TS and VS values were determined prior to the experiments based on ISO Standards (ISO 14780:2017, ISO 18134–2:2017, ISO 18134–3:2015). TS were obtained by placing a sample into an oven for 18 hours at  $105\text{ }^{\circ}\text{C}$ , and then the dry sample was finely ground and placed into an oven for 5 hours at  $105\text{ }^{\circ}\text{C}$ . VS were obtained by placing 5g of totally dry sample into an oven for 11 hours with a heating step  $50\text{ }^{\circ}\text{C h}^{-1}$  and then kept at  $550\text{ }^{\circ}\text{C}$  for 3 hours to be able to obtain the VS content as a fraction of TS (% of TS). The results are presented in Table 1.

**Table 1.** TS and VS content of inoculum and fish waste fractions

Substrate	TS, %	VS, % of TS
Inoculum 1	2.0	60.5
Inoculum 2	1.9	60.5
Inoculum 3	1.9	60.5
Heads <sup>1</sup>	20.5	76.5
Skin/bone mix <sup>1</sup>	22.2	75.3
Intestines <sup>1</sup>	36.7	82.6
Heads <sup>2</sup>	19.8	76.5
Skin/bone mix <sup>2</sup>	19.4	75.3
Intestines <sup>2</sup>	30.1	82.6

Inoculums 1, 2, 3 – inoculums for experiment 1, 2 and 3; <sup>1</sup> – biomass 1; <sup>2</sup> – biomass 2.

### Inoculum

Sewage sludge was collected from local waste water treatment plant ‘Daugavgriva’ (Riga district, Latvia) directly from biogas bioreactors. Prior to the BMP experiments, the inoculum was incubated for 6 days at  $37\text{ }^{\circ}\text{C}$ , with regular degassing. Inoculum was always evaluated for TS and VS content using ISO standards (ISO 14780:2017, ISO 18134–2:2017, ISO 18134–3:2015, ISO 18122:2015).

### BMP test method

BMP tests were used to define the amount of methane produced per kilogram of VS, for an inoculum to substrate ratio (ISR) equal to 3 based on a TS basis. Generally, BMP measuring methods are based on liquid displacement or the displacement of a syringe piston. An alkaline solution for cleaning the biogas (by absorbing the  $\text{CO}_2$  fraction) is added in both methods. The method is a well-known approach, but still lacking true standardization (Esposito et al., 2012; Edward et al., 2015). A pH range from 6.5 to 8.2 (Ağdağ & Sponza, 2005; Chandra et al., 2012; Esposito et al., 2012) is optimal for most anaerobic bacteria, including methanogens. Therefore, an alkaline compound is normally added within the solution as a buffer capacity (i.e. sodium hydroxide, sodium (bi)carbonate or sodium sulphide) (Chynoweth et al., 2000), in our case a  $0.7\text{M NaHCO}_3$  solution was specifically prepared.



BMP is a sensitive method, influenced by the conditions for the anaerobic bacteria to grow. In this light, the analysis of the results can be difficult due to the amount of potentially influential factors, resulting in likely possible errors and/or inaccuracies (Angelidaki & Sanders, 2004, Wellinger et al., 2013). Moreover, sometimes the same substrates don't show the same BMPs based on the tests' conditions (Del Borghi et al., 1999).

### **Experimental set-up**

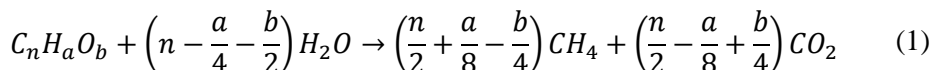
BMP tests were conducted in a batch mode using 100 mL crimp neck ND20 vials with a working volume of 50 mL. Each bottle was filled with 30 mL of distilled water, 20 mL of inoculum and 1 mL of 0.7M NaHCO<sub>3</sub> buffer basal solution to maintain neutral the pH. Different amount (fresh weight) of different fish waste fraction was added to specific samples based on TS content and to maintain ISR around 3. Additionally, reference samples (blanks) containing only inoculum were prepared both for high and low temperature conditions to account for the methane production solely from the fish waste biodegradation. Sample headspace was flushed with N<sub>2</sub> for 30 seconds at flow rate around 2 L min<sup>-1</sup> before sealing them with butyl rubber stoppers and aluminium crimps. The tests were carried out in dark conditions at a mesophilic temperature (37 °C) in the EcoCell LSIS-B2V / EC 111 incubator and at 23 °C, and lasted for 31 days. The batches were manually shaken one time per day on average. All batch tests were prepared in triplicates.

In total, three experiments were performed. In first experiment fish waste from year 2017 (biomass 1) was used. Tested samples contained heads, skin/bone mixture and intestines. For second and third experiment fish waste from year 2015 (biomass 2) was used. These samples also contained heads, skin/bone mixture, intestines and additional biomass mixes (consisting of all waste fractions in different shares). First mix (M1) contained all waste fractions in equal share based on TS. Second mix (M2) contained all waste fractions in equal share based on wet weight. Third mix (M3) contained all waste fractions in wet weight ratios: 2 parts heads, 2 parts skin/bone mixture, 1 part intestines (based on practical fish processing approach when intestines make up only one fifth of total waste amount). Experiments were performed with one-month time shift between them, thus also having slightly different inoculum for each test setup. In total 90 samples were analysed for 6 different feedstock's and two AD temperature conditions.

A volumetric measuring method was used by measuring the biomethane amount through the displacement of a 20 mL syringe piston connected to a batch bottle. For triplicates three best syringes were selected (with lowest friction) and slightly modified (cutting off excess piston rubber to minimize friction). Each syringe was dedicated to specific triplicate in consistent order, thus giving opportunity to see if piston friction changes and affects measurements. To determine the methane concentration without the CO<sub>2</sub> fraction, 5 mL of 3M NaOH alkaline solution was filled into the measuring syringes before each measurement. For extra confidence some of measured samples periodically were left overnight in closed syringes to see if all CO<sub>2</sub> has been absorbed during measurement.

### Theoretical BMP according to Buswell's formula

Depending on the type of biomass, the assessment of BMP can eventually require time of up to 90 days (Hansen et al., 2004; Angelidaki et al., 2009; Kafle & Kim, 2013). For a more rapid estimation, a theoretical biomethane potential ( $BMP_{theo}$ ) can be used from the Buswell equation (Allen et al., 2013) – see formula 1. Once the biomass' chemical compositions of C, H, O are known, it is possible to calculate the  $BMP_{theo}$  (Angelidaki & Sanders, 2004) and the correspondent  $CH_4$  fraction as  $BMP_{theo}$ .



where n – carbon atoms in biomass; a – hydrogen atoms in biomass; b – oxygen atoms in biomass.

The methane yield ( $BMP_{theo}$ ) from the Buswell's equation can be recalculated with a reference to the unit of gram (i.e. g-VS) in standard condition (i.e. STP) (Raposo et al., 2011), see Formula 2.

$$BMP_{theo,yield} = \frac{\left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) \cdot 22.4}{12n + a + 16b} \cdot \left(STP \frac{lCH_4}{g - VS}\right) \quad (2)$$

where n – carbon atoms in biomass; a – hydrogen atoms in biomass; b – oxygen atoms in biomass.

Experimental yields are usually lower but knowing the theoretical yield value allows to calculate the efficiency of digestion.

Chemical composition of fish waste fractions was analysed by a Latvian State Institute of Wood Chemistry. Results are presented in Table 2.

## RESULTS AND DISCUSSION

### Inoculum and substrate characterization

TS and VS content for all three inocula were similar, however, slightly different methanogenic activity was observed referring to the methane volume produced from the blanks (data not shown) and the total accumulated methane amount from samples. Sludge was most active in the second experiment and especially at high temperature conditions. However, that did not have a relevant impact on the final BMP values acquired from batch tests.

**Table 2.** Chemical composition of different fish waste fractions (for biomass 2)

Substrate	% of TS					
	Carbon (C)	Hydrogen (H)	Oxygen (O)	Nitrogen (N)	Sulphur (S)	Ash
Heads	37.82	4.72	22.51	11.14	0.29	23.51
Skin/bone mix	40.30	5.06	17.37	12.16	0.35	24.75
Intestines	57.17	6.78	12.12	6.17	0.34	17.43
M1	43.55	5.44	19.32	9.53	0.32	21.85
M2	46.89	5.83	16.09	9.64	0.33	21.22
M3	41.51	5.51	20.62	9.77	0.32	22.27

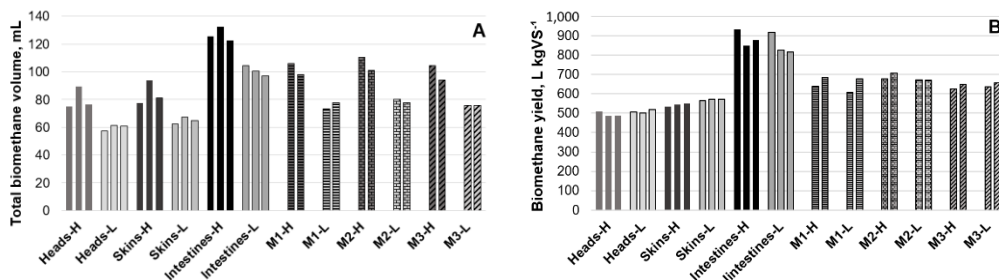
TS and VS content for fish heads and skin/bone mixture (furthermore also referred as ‘skins’) was similar both for the biomass 1 and biomass 2 (Table 1). TS were around 20% and VS were 75–76% of TS. Although homogenized intestine samples seemed more liquid, they showed the highest TS content varying between 36% for biomass 1 and 30% for biomass 2. This could be explained with high lipid content that is not lost during TS drying operation.

Furthermore, this high lipid concentration is affecting BMP test results, showing the highest methane yield for samples with intestines both for high and low temperature conditions. Similar effect was observed by Nges et al., 2012. VS content for round goby’s intestines was similar for both biomass sources reaching 82.6% of TS.

### Biochemical methane potential

BMP testing was done with slightly modified 20 mL rubber piston syringes containing 5 mL of 3M NaOH solution for CO<sub>2</sub> absorption. Piston’s friction was constantly monitored and no significant change was detected during all three experiments. Periodically, accumulated gas samples were left overnight in closed syringes to check NaOH solution’s CO<sub>2</sub> absorption efficiency during slow biogas collection. Fortunately, no visible change in gas volume was ever detected. Consequently, the measured biogas values pertain to the methane content produced.

Regarding to **total accumulated** biomethane volume per test vial, significant difference can be seen between low temperature and high temperature batch samples. Overall, for the samples that were incubated at 23 °C an average 23% reduction can be observed in total accumulated biomethane volumes (Fig. 1, A). This matches with trends reported in literature stating that lowering temperature by 10 °C biogas production slows down approximately two times (Seadi et al., 2008; Zhu & Kumar, 2014).



**Figure 1.** Total accumulated biomethane amount (A) and BMP per 1 kg VS (B) during experiments 1, 2, and 3. Index –H stands for 37 °C, –L stands for 23 °C.

After calculating the net biomethane volumes (by subtracting blank sample volumes from the total accumulated biomethane volumes), the difference between low and high temperature samples occurs to be very low. Furthermore, after calculating the final BMP values (always based on the net biomethane volumes) per kg of VS, the overall average BMP results for low temperature samples are only 2% lower than for 37 °C (Fig. 1, B).

In total, the BMP difference per 1 kg of VS among the two sets of temperature conditions was only 2%. Nevertheless, it must be clarified that the overall difference in total accumulated biomethane amount is 23% (see Fig. 1, A). This result may be due to an extra 23% of total biomethane volume that was contributed by the sewage sludge inoculum at higher temperature. Methanogenic bacteria activity and growth is much lower at low incubation temperature conditions, thus resulting in a slower augmentation and decay (dead biomass methanation) of the microorganism consortium, thereby lowering the amounts of total produced biomethane. This should be taken into account when designing bioreactor for fish waste and sewage sludge co-digestion at low temperature conditions in terms of bigger digester's size. Nevertheless, results of this study suggest that lowered temperature does not have a strong impact on fish waste digestion efficiency and final BMP, however, it affects digestion kinetics.

During all three experiments the highest BMP values were obtained from batch samples containing fish intestines both for high and low temperature conditions (Fig. 1, B). Average biomethane yield from all three experiments at 37 °C was 887 L CH<sub>4</sub> kgVS<sup>-1</sup> and 853 L CH<sub>4</sub> kgVS<sup>-1</sup> at 23 °C. These high values are reached because of high lipid and protein content, especially in gonads and fish eggs that were present in Round Goby's abdomens. The theoretical BMP yield for lipids is about 1000 L CH<sub>4</sub> kgVS<sup>-1</sup>, while the theoretical yield for protein is about 490 L CH<sub>4</sub> kgVS<sup>-1</sup> (Nges et al., 2012). BMP values of first experiment are higher than those of second and third, reaching 933 L CH<sub>4</sub> kgVS<sup>-1</sup> at 37 °C and 917 L CH<sub>4</sub> kgVS<sup>-1</sup> at 23 °C. In comparison, results from second and third experiment were only 850–878 L CH<sub>4</sub> kgVS<sup>-1</sup> for high and 816–826 L CH<sub>4</sub> kgVS<sup>-1</sup> for low temperature. Despite similar VS content (82.6%) of round goby's both biomasses this difference in results could be explained due to the fact that for first experiment used fish biomass was caught in spring season (April). In spring time fish are ready for new spawning season and have larger gonads and contain more mature fish eggs, thus increasing overall lipid and protein relative share in viscera.

These results are slightly higher than reported 500 L CH<sub>4</sub> kgVS<sup>-1</sup> for perch (*Perca fluviatilis*) intestines (Tomczak-Wandzel et al., 2013), however, this could be attributed to the fact that relative share of gonads in perch abdomen is much smaller (if present at all in different seasons).

The overall average BMPs acquired from three experiments for fish heads at high temperature and low temperature was 494 L CH<sub>4</sub> kgVS<sup>-1</sup> and 508 L CH<sub>4</sub> kgVS<sup>-1</sup> respectively. Skin and bone mix showed slightly higher results, therefore average BMP at 37 °C was 542 L CH<sub>4</sub> kgVS<sup>-1</sup> but at 23 °C was 570 L CH<sub>4</sub> kgVS<sup>-1</sup>. It can be seen that at lower temperatures average BMP values are slightly higher than at 37 °C both for heads and skin/bone mixture. This could be explained due to the fact that for several high temperature samples after 20 days' biomethane production was delayed and a slight inhibition of methane production was observable, as blank reference samples on daily basis produced more gas than samples containing fish waste. This in fact resulted in negative daily net biomethane values, indicating the start of inhibition which is consequential after digestion of high organic content substrates and rapid VFA accumulation, as can be observed also during dairy product anaerobic digestion (Labatut et al., 2011). This also is in line with literature where it is suggested that AD under lower temperature conditions is more stable and less volatile fatty acids are accumulated

(Appels et al., 2008). However, no great change in pH was observed at the end of all experiments, only for few samples lowering from pH8 to pH 7.7.

Summary of BMP values acquired during this research for different fish waste samples can be seen in Table 3.

**Table 3.** Summary of estimated yields from Buswell’s equation and experimental CH<sub>4</sub> yields

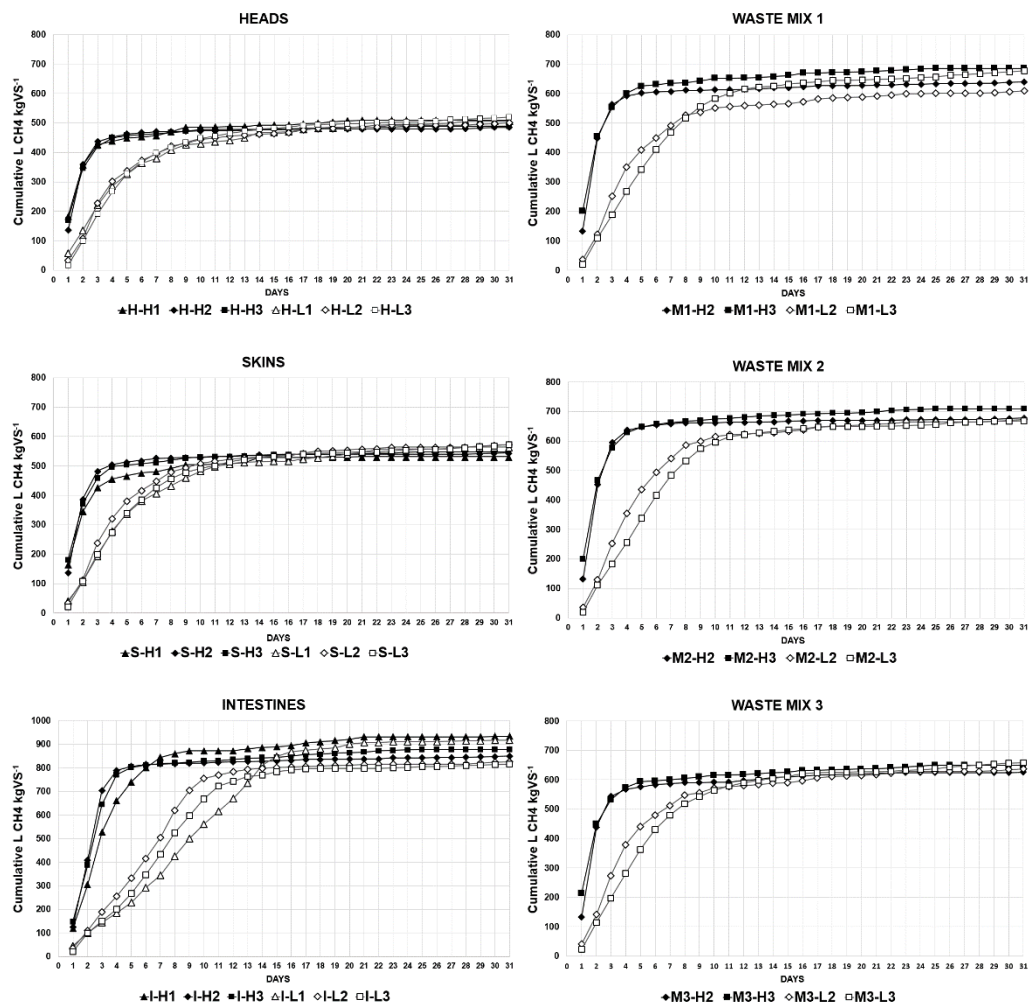
Substrate	BMP <sub>theo</sub> (L CH <sub>4</sub> kgVS <sup>-1</sup> )	BMP at 37 °C (L CH <sub>4</sub> kgVS <sup>-1</sup> )	BMP at 23 °C (L CH <sub>4</sub> kgVS <sup>-1</sup> )
Heads <sup>1</sup>	–	509.2 ± 29.5	506.3 ± 1.0
Skin/bone mix <sup>1</sup>	–	533.0 ± 17.8	565.4 ± 110.8
Intestines <sup>1</sup>	–	933.1 ± 60.9	916.9 ± 39.7
Heads <sup>2</sup>	625.0	485.4 ± 20.2	500.8 ± 14.9
Skin/bone mix <sup>2</sup>	728.9	544.9 ± 25.5	572.6 ± 26.3
Intestines <sup>2</sup>	895.7	849.8 ± 15.4	826.1 ± 26.0
M1 <sup>2</sup>	719.4	639.1 ± 4.8	609.2 ± 11.6
M2 <sup>2</sup>	791.8	677.6 ± 18.0	672.4 ± 11.0
M3 <sup>2</sup>	769.0	626.3 ± 24.5	636.7 ± 2.5
Heads <sup>3</sup>	625.0	488.8 ± 18.6	519.6 ± 19.1
Skin/bone mix <sup>3</sup>	728.9	548.8 ± 24.4	572.2 ± 22.9
Intestines <sup>3</sup>	895.7	877.7 ± 41.8	816.3 ± 51.9
M1 <sup>3</sup>	719.4	685.7 ± 17.4	676.5 ± 27.0
M2 <sup>3</sup>	791.8	709.2 ± 37.5	668.6 ± 30.7
M3 <sup>3</sup>	769.0	649.5 ± 10.3	657.6 ± 18.4

<sup>1</sup> – experiment 1 (biomass 1); <sup>2</sup> – experiment 2 (biomass 2); <sup>3</sup> – experiment 3 (biomass 2).

Three different fish waste fraction mixes were also prepared. First mix (M1) contained all waste fractions in equal share based on TS. Second mix (M2) contained all waste fractions in equal share based on wet weight. Third mix (M3) contained all waste fractions in wet weight ratios: 2 parts heads, 2 parts skin/bone mixture, 1 part intestines (based on practical fish processing approach). M1 average BMP at 37 °C and 23 °C was 662 L CH<sub>4</sub> kgVS<sup>-1</sup> and 642 L CH<sub>4</sub> kgVS<sup>-1</sup> respectively. M2 average BMP at high temperature was 693 L CH<sub>4</sub> kgVS<sup>-1</sup> and 670 L CH<sub>4</sub> kgVS<sup>-1</sup> at low temperature. M3 average BMP at high temperature was 638 L CH<sub>4</sub> kgVS<sup>-1</sup> and 647 L CH<sub>4</sub> kgVS<sup>-1</sup> at 23 °C. No significant difference can be seen regarding to anaerobic digestion of these three mixes, thus any of these three compositions can be successfully used for biomethane production. As expected, average BMP was around 660 L CH<sub>4</sub> kgVS<sup>-1</sup>, that is similar to mathematical average from heads, skins and intestines BMPs’. Other authors report similar results for Pacific saury, Nile perch, mackerel and cuttlefish wastes, ranging between 562–777 L CH<sub>4</sub> kgVS<sup>-1</sup> (Kassuwi et al., 2012; Kafle et al., 2013). BMP for cod meat and intestine mix was reported to be 503–533 L CH<sub>4</sub> kgVS<sup>-1</sup> after 14 days long incubation period (Almkvist, 2012; Shi, 2012). Regarding to 14-day period BMP from round goby’s waste mix is slightly higher reaching approximately 640 L CH<sub>4</sub> kgVS<sup>-1</sup>. In this light, it would be advisable to measure BMP for more extended time period, as far as it is reasonable, to obtain fully total BMP of biomass.

## Dynamics of biomethane production

Cumulative curves and dynamics of biomethane production are shown in Fig. 2. For high temperature samples the main production was observed during the first 7–9 days, accounting for 95% of the total BMP. In turn for low temperature conditions main biomethane production was observed during first 14–16 days, accounting for 94% of the total BMP.



**Figure 2.** Averaged triplicate methane production dynamics trough experiments 1, 2, and 3. Indexes –H stands for 37 °C; –L stands for 23 °C; 1, 2, 3 stands for experiments 1, 2, 3.

Similar pattern regarding to fish waste highest production rate time shift was reported by (Chen et al., 2010), where highest biogas production rate under thermophilic conditions (50 °C) was achieved on day 10, in comparison to 17 days at mesophilic (35 °C) conditions. Moreover, this great difference could be also attributed to type of inoculum that was used in this research, because sewage sludge was gathered from bioreactors that normally operate at 37 °C. Shift to low temperature conditions put extra

stress on microorganism consortium. It is also suggested that more appropriate microbial consortium can be developed and adapted for fish waste AD by sequential addition of fish based feedstock, thus making optimized inoculum for substrates with low C:N ratios (Quinn et al., 2016).

Nevertheless, slower biomethane production rate had no significant impact on final BMP results. In addition, slower digestion time means that substrate needs longer hydraulic retention time (HRT) in digester (Dhaked et al., 2010; Zhu et al., 2014), thus slowing down biogas production or forcing to increase digesters size. On average, lowering fermentation temperature by 10 °C required anaerobic digester's size increases 2–2.5 times (Balasubramaniyam et al., 2008). However, digester's size can be reduced if shorter HRT is selected. In respect to this research results, it would be more reasonable to use a HRT of 15 days instead of 30 days for low temperature fish waste anaerobic digestion, as more than 94% of BMP is achieved during this short time.

## CONCLUSIONS

The results of this research show that AD of round goby's processing waste at 23 °C is twice as slow as under 37 °C conditions. Thus prolonging hydraulic retention time (HRT) needed for complete biomethanation of feedstock, in turn increasing necessary size of digester. However, costs of digesters size increase should be compared to savings on insulation materials and heat energy input. Thus most feasible approach regarding to ratios of digesters size, HRT and fermentation temperature could be found.

For low temperature conditions an overall 23% reduction in total produced biomethane volume was observed. However, this difference is attributable to the inoculums specific activity at different temperatures and counteracting the contribution to the total biomethane volume, rather than to feedstock's biomethanation efficiency. Despite the fact, that several fish waste fractions such as heads and skins showed higher BMP values at lower temperature, based on overall averaged results, in general only a 2% reduction in total BMP outcome was observed for low temperature samples after 25 days, thus showing that biomethanation is still efficient also at lowered temperatures.

Round goby's processing wastes could be successfully used for biogas production in co-digestion, especially if containing intestines, however in-depth research is still needed to find out possible inhibitory effects and mechanisms. Also volatile fatty acid accumulation and inhibitory effect during continuous low temperature fermentation should be researched. Furthermore, AD of *Neogobius melanostomus* under psychrophilic conditions should be explored.

Research results suggests that anaerobic digestion of fish waste under low temperature conditions could be feasible as the process still occurs with 98% efficiency in respect to 37°C, in fact opening a new opportunity to explore the overall sustainability of technologies based on these conversion processes.

**ACKNOWLEDGEMENTS.** The work has been supported by the National Research Program 'Energy efficient and low-carbon solutions for a secure, sustainable and climate variability reducing energy supply (LATENERGI)'. Latvian State Institute of Wood Chemistry is acknowledged for chemical composition testing of biomass.

## REFERENCES

- Ağdağ, O.N. & Sponza, D.T. 2005. Effect of alkalinity on the performance of a simulated landfill bioreactor digesting organic solid wastes. *Chemosphere* **59**(6), 871–879.
- Allen, E., Browne, J., Hynes, S. & Murphy, J.D. 2013. The potential of algae blooms to produce renewable gaseous fuel. *Waste Manag.* **33**(11), 2425–2433.
- Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P. & Van Lier, J.B. 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. *Water Sci. Technol.* **59**(5), 927–934.
- Angelidaki, I. & Sanders, W. 2004. Assessment of the anaerobic biodegradability of macropollutants. *Rev. Environ. Sci. Biotechnol.* **3**(2), 117–129.
- Appels, L., Baeyens, J., Degreè, J. & Dewil, R. 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. *Prog. Energy Combust. Sci.* **34**(6), 755–781.
- Balasubramaniam, U., Zisengwe, L.S., Meriggi, N. & Buysman, E. 2008. Biogas production in climates with long cold winters. (May), 68.
- Del Borghi, A., Converti, A., Palazzi, E. & Del Borghi, M. 1999. Hydrolysis and thermophilic anaerobic digestion of sewage sludge and organic fraction of municipal solid waste. *Bioprocess Eng.* **20**(6), 553–560.
- Chandra, R., Vijay, V.K., Subbarao, P.M.V. & Khura, T.K. 2012. Production of methane from anaerobic digestion of jatropha and pongamia oil cakes. *Appl. Energy* **93**, 148–159.
- Charlebois, P.M., Corkum, L.D., Jude, D.J. & Knight, C. 2001. The Round Goby (*Neogobius melanostomus*) Invasion: Current Research and Future Needs. *J. Great Lakes Res.* **27**(3), 263–266.
- Chen, X., Romano, R.T. & Zhang, R. 2010. Anaerobic digestion of food wastes for biogas production. *Int. J. Agric. Biol. Eng.* **3**(4), 61–72.
- Chynoweth, D.P., Owens, J.M. & Legrand, R. 2000. Renewable methane from anaerobic digestion of biomass. *Renew. Energy* **22**(1–3), 1–8.
- Connaughton, S., Collins, G. & O'Flaherty, V. 2006. Psychrophilic and mesophilic anaerobic digestion of brewery effluent: A comparative study. *Water Res.* **40**(13), 2503–2510.
- Dhaked, R.K., Singh, P. & Singh, L. 2010. Biomethanation under psychrophilic conditions. *Waste Manag.* **30**(12), 2490–2496.
- Edward, M., Edwards, S., Egwu, U. & Sallis, P. 2015. Bio-methane potential test (BMP) using inert gas sampling bags with macroalgae feedstock. *Biomass and Bioenergy* **83**, 516–524.
- Eiroa, M., Costa, J.C., Alves, M.M., Kennes, C. & Veiga, M.C. 2012. Evaluation of the biomethane potential of solid fish waste. *Waste Manag.* **32**(7), 1347–1352.
- Esposito, G., Frunzo, L., Panico, A. & Pirozzi, F. 2012. Enhanced bio-methane production from co-digestion of different organic wastes. *Environ. Technol.* (May 2015), 1–8.
- Gunaseelan, V.N. 1997. Anaerobic digestion of biomass for methane production: A review. *Biomass and Bioenergy* **13**(1–2), 83–114.
- Hansen, T.L., Schmidt, J.E., Angelidaki, I., Marca, E., Jansen, J.L.C., Mosbæk, H. & Christensen, T.H. 2004. Method for determination of methane potentials of solid organic waste. *Waste Manag.* **24**(4), 393–400.
- Kafle, G.K. & Kim, S.H. 2013. Anaerobic treatment of apple waste with swine manure for biogas production: Batch and continuous operation. *Appl. Energy* **103**, 61–72.
- Kafle, G.K., Kim, S.H. & Sung, K.I. 2013. Ensiling of fish industry waste for biogas production: A lab scale evaluation of biochemical methane potential (BMP) and kinetics. *Bioresour. Technol.* **127** 326–336.



- Kassuwi, S.A.A., Mshandete, A.M. & Kivaisi, A.K. 2012. Anaerobic Co-Digestion of Biological Pre-Treated Nile Perch Fish Solid Waste With Vegetable Fraction of Market Solid Waste. *J. Agric. Biol. Sci.* **7**(12), 1016–1031.
- Labatut, R.A., Angenent, L.T. & Scott, N.R. 2011. Biochemical methane potential and biodegradability of complex organic substrates. *Bioresour. Technol.* **102**(3), 2255–2264.
- LVS–EN ISO 14780:2017, Solid biofuels – Sample preparation.
- LVS–EN ISO 18134–2:2017, Solid biofuels – Determination of moisture content – Oven dry method – Part 2: Total moisture – Simplified method.
- LVS–EN ISO 18134–3:2015, Solid biofuels – Determination of moisture content – Oven dry method – Part 3: Moisture in general analysis sample.
- LVS–EN ISO 18122:2015 Solid biofuels – Determination of ash content.
- Melvere, M., Ivanovs, K., Pubule, J. & Blumberga, D. 2017. Use of round goby (*Neogobius melanostomus*) processing waste in bioeconomy. *Sci. direct* **0**(128), 484–490.
- Nges, I.A., Mbatia, B. & Björnsson, L. 2012. Improved utilization of fish waste by anaerobic digestion following omega-3 fatty acids extraction. *J. Environ. Manage.* **110**, 159–165.
- Quinn, B.M., Apolinario, E.A., Gross, A. & Sowers, K.R. 2016. Characterization of a microbial consortium that converts mariculture fish waste to biomethane. *Aquaculture* **453**, 154–162.
- Raposo, F., Fernández-Cegr í, V., de la Rubia, M.A., Borja, R., Béline, F., Cavinato, C., Demirer, G., Fernández, B., Fernández-Polanco, M., Frigon, J.C., Ganesh, R., Kaparaju, P., Koubova, J., Méndez, R., Menin, G., Peene, A., Scherer, P., Torrijos, M., Uellendahl, H., Wierinck, I. & de Wilde, V. 2011. Biochemical methane potential (BMP) of solid organic substrates: Evaluation of anaerobic biodegradability using data from an international interlaboratory study. *J. Chem. Technol. Biotechnol.* **86**(8), 1088–1098.
- Riekstiņš, N. 2014. LATVIJAS ZIVSAIMNIECĪBAS GADAGRĀMATA 2014 The Latvian Rural Advisory and Training Centre.
- Riekstiņš, N. 2017. LATVIJAS ZIVSAIMNIECĪBAS GADAGRĀMATA 2017 The Latvian Rural Advisory and Training Centre.
- Saady, N.M.C. & Massé, D.I. 2013. Psychrophilic anaerobic digestion of lignocellulosic biomass: A characterization study. *Bioresour. Technol.* **142**, 663–671.
- Salam, B., Islam, M. & Rahman, M.T. 2009. Biogas from the anaerobic digestion of fish waste. *Int. Conf. Mech. Eng.* **2009** (December), 26–28.
- Seadi, T.A., Rutz, D., Prassl, H., Köttner, M., Finsterwalder, T., Volk, S. & Janssen, R. 2008. *Biogas Handbook*.
- Shi, C. 2012. P Otential B Iogas P Roduction From F Ish W Aste and S Ludge. (August),.
- Smith, A.L., Skerlos, S.J. & Raskin, L. 2013. Psychrophilic anaerobic membrane bioreactor treatment of domestic wastewater. *Water Res.* **47**(4), 1655–1665.
- Tomczak-Wandzel, Levlin, & Östen, E. 2013. Biogas production from fish wastes in co - digestion with sewage sludge, pp. 7–14.
- Wei, S. & Guo, Y. 2018. Comparative study of reactor performance and microbial community in psychrophilic and mesophilic biogas digesters under solid state condition. *J. Biosci. Bioeng.* **xx**(xx),.
- Wellinger, A., Murphy, J. & Baxter, D. 2013. *The biogas handbook*. Wellinger, A., Murphy, J. and Baxter, D. (eds) Woodhead Publishing Limited.
- Wu, G., Hu, Z., Healy, M.G. & Zhan, X. 2009. Thermochemical pretreatment of meat and bone meal and its effect on methane production. *Front. Environ. Sci. Eng. China* **3**(3), 300–306.
- Zhu, G., Li, J. & Kumar Jha, A. 2014. Anaerobic Treatment of Organic Waste for Methane Production under Psychrophilic Conditions. *ISSN Online* 1560–8530.

## **Improvement of monitoring of cattle in outdoor enclosure using IQRF technology**

V. Hartová<sup>1,\*</sup> and J. Hart<sup>2</sup>

<sup>1</sup>Czech University of Life Sciences Prague, Faculty of Engineering, Department of Vehicles and Ground Transport, Kamýcká 129, CZ165 00 Prague, Czech Republic

<sup>2</sup>Czech University of Life Sciences Prague, Department of Technological Equipment of Buildings, Kamýcká 129, CZ165 00 Prague, Czech Republic

\*Correspondence: nverca@seznam.cz

**Abstract.** Monitoring of cattle in the outdoor enclosure is a very important issue. Currently, the increasingly stealing cows and other cattle from the pasture. For businesses that manage it, this is a very lossy business. For thieves, the electric fences, which are currently the most widespread, are easily surmountable. And the owner does not have a chance early on to learn the fact theft of cattle. For this reason, a system for monitoring cattle on outdoor enclosure using IQRF (intelligent local network topology) technology has been developed. We have been dealing with this issue for a long time and the biggest problem was the monitoring of only the inner part of the enclosure. Previously, there was an overlap of monitoring in the area beyond grazing. This problem has been solved by using a shielded base for mounting IQRF receivers.

**Key words:** IQRF technology, wireless transmission, interference, livestock.

### **INTRODUCTION**

Wireless technologies are increasingly being used. Whether it is wireless transmission in surveillance systems, Wi-Fi networks, Bluetooth transmissions or various RC models, their transmissions are ubiquitous. All in all, they basically define the modern time and our civilization as such. Of course there are also many risks and imperfections related to wireless networks that need to be considered. One of the most frequent risks of wireless transmission is natural interference or a limited range of wireless transmitters. Though being serious, these problems have their realistic solutions provided by an IQRF MESH (intelligent local network topology) network which can at least partially eliminate them (Dong et al., 2013; Elmasry, 2013; Behkami et al., 2017).

As for the above mentioned technologies, ISM bands (industrial, scientific and medical) are mostly used for wireless transmissions. They are amply used in a variety of industrial transmissions. Officially, these bands should only be used for industrial, medical or scientific purposes. The Federal Communications Commission and the European Telecommunications Standards Institute established just the ISM bands as licence-free and given their licence-free usage they are also heavily preferred for commercial purposes (Tahir & Shah, 2008; Hartová & Hart, 2017).

It is therefore favourable to use these modern technologies in the licence-free ISM bands to protect livestock in such a way that attempts at their theft are detected in time. Although its purpose is clearly defined, following small modifications the resulting technology could also help in monitoring animal welfare (Kucera et al., 2015; Lopes & Carvalho, 2016; Hartová & Hart, 2017).

The problem was mainly to solve the directivity of the used receivers. It is necessary to limit the reception of the signal from the area outside the supervised area. This research builds on the already solved problem that was solved by Bluetooth: 'Livestock monitoring system using bluetooth technology' (Hartová & Hart, 2017).

Currently, the increasingly stealing cows and other cattle from the pasture. For businesses that manage it, this is a very lossy business. For thieves, the electric fences, which are currently the most widespread, are easily surmountable. And the owner does not have a chance early on to learn the fact theft of cattle. For this reason, a system for monitoring cattle on outdoor enclosure using IQRF technology has been developed.

IQRF technology is one of the other communications representatives on radio frequencies and a potential participant in IoT combat. It was published in 2004 and is mainly devoted to Czech developers (Kuchta et al., 2009; Sulc et al., 2009; Bazydlo et al., 2015; Martin & Radovan, 2016).

The aim of the investigation was to design a livestock monitoring system. Based on the discovery of the deficiencies in the system's predecessor design, the detection technology was changed. At the same time a shielded pad was developed to correct the detected area.

## MATERIALS AND METHODS

Following in-depth market research a technology conforming to exacting criteria for monitoring of livestock theft was selected. Due to the experience of the past, more flexible technology has been chosen than it was in the first place in preliminary studies (Hartová & Hart, 2017). IQRF technology (see Fig. 1) has replaced Bluetooth technology as it better meets the requirements of the overall system. The main demands placed on the transmitters were their low energy demands. In addition, their range of coverage was evaluated and therefore the IQRF technology was selected, which enables transfer on ISM868 bands (industrial, scientific and medical) (Hartová & Hart, 2017; Bazydlo et al., 2015; Kuchta et al., 2009; Sulc et al., 2009).

For LPWAN (Low-Power Wide-Area Network), it uses an atypical mesh topology instead of a star topology that is common for Sigfox or LoRa technologies. This choice has its plus and minus. It uses a specially created IQRF MESH protocol, which is integrated into the operating system's system for better and more efficient routing. This is a packet-oriented technology where the payload is at most 64 B but

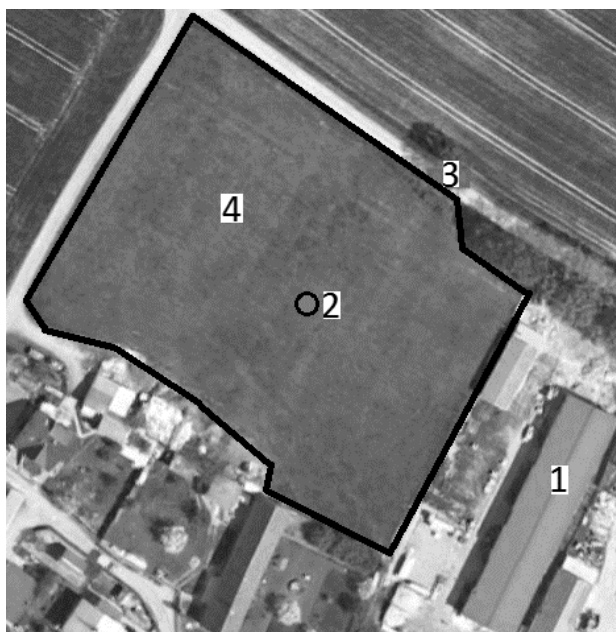


**Figure 1.** IQRF elements.

where the actual size is given by the specific device requirements. The resulting range of such a network ranges in tens of kilometers (Kuchta et al., 2009; Sulc et al, 2009; Bazydlo et al., 2015; Martin & Radovan, 2016).

In the frequency spectrum, the technology is specified at frequencies of 868, 916 and 433 MHz. In these bands it uses a number of channels, which are different for all three frequencies. It also varies with the maximum transmit power at 433 MHz 5 mW and at 868 and 916 MHz at 3.5 mW (5.44 dBm). In the band around 868 MHz, which is most discussed here, the frequencies range from 863.15 to 869.25 MHz, corresponding to the European band g with subgroups g1 and g2. In the Czech Republic, it falls within band h with subgroups h1 and h2 under General Authorization. There are 62 channels with numbers from 0 to 61 with a standard 100 kHz bandwidth. Very often, channel 52 is used, which is a frequency of 868.35 MHz. These channels work at a transfer rate of 19,836 kbit s<sup>-1</sup>, which is the most common transfer rate for IQRF system (Kuchta et al., 2009; Sulc et al., 2009; Bazydlo et al., 2015; Martin & Radovan, 2016).

A small farm was chosen for the purpose of designing the system, with an adjoining pasture for cattle – see Fig. 2. The same farm was selected as in the previous proposal. (Hartová & Hart, 2017)



**Figure 2.** The chosen farm (1 – barn for housing cattle; 2 – location of centralized drinking troughs; 3 – fenced pastures; 4 – pasture).

The pasture was 100 m long and 70 m wide. There were 50 cows on grazing. Measurements took place throughout the year 2017. In our design of location of the receivers we have chosen the circuit procedure. By doing that we reached the optimal range of transmission of across the area, however with an effective wireless coverage even outside the supervised area. The distance of this effective transmission is referred to as the declared distance, which is considered a distance over which the transmission

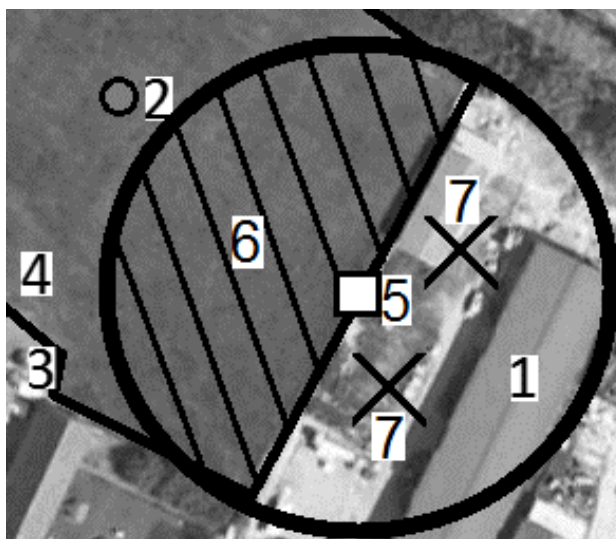
should take place almost under any conditions. To reduce the declared distance of the receivers, a shielding socket was created (Hartová & Hart, 2017).

Detection of an animal loss is based on a simple principle. When an IQRF nod regularly sends data and the IQRF control unit receives regular messages of its presence from the given IQRF nod, this indicates that things are in order and no alarm is launched. There was a time window determined during which an IQRF nod must send a message at least once, and this was set to 5 seconds. On a standard basis it should report five times within this time, but an interference may occur thus this safeguard is set to prevent false alarms. As in previous research on the topic: ‘Livestock monitoring system using bluetooth technology’ (Hartová & Hart, 2017).

The size of the monitored areas is limited by the maximum number of nodes in the network. Each knot has a range of up to 500 m in free space. These networks may be displayed as several superstructures on top of each other, provided that frequencies are not disturbed. The area can take up to several tens of kilometers, depending on the structure.

## RESULTS AND DISCUSSION

A problem of past research in the appeared when it came to outdoor grazing where animals can move beyond the determined area without raising alarm – see Fig. 2. This problem has remained unresolved in past research (Hartová & Hart, 2017).

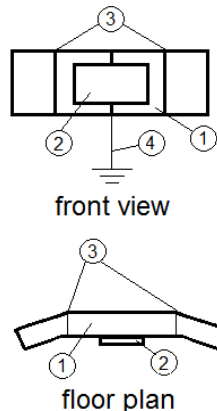


**Figure 2.** Section of chosen farm (1 – barn for housing cattle; 2 – location of centralized drinking troughs; 3 – pasture fencing; 4 – pasture; 5 – IQRF unit; 6 – monitored area; 7 – unmonitored area).

Due to the change of centralized monitoring to perimeter, technology change, and shielded pad development (see Fig. 3), we have made some changes. With these changes, we have achieved a minimum overlap outside the monitored area. We electrically ground and separate the IQRF knot from the pad. This eliminated reception of the signal from the unmonitored area (Hartová & Hart, 2017).

Although the use of Bluetooth technology was initially considered, as described in the article on ‘Livestock monitoring system using bluetooth technology’, it was given up for reasons associated with effectiveness of the detection method (Hartová & Hart, 2017).

As the selected method was proven an optimal solution, just fine-tune a few details and verify in real terms for a long time., since as claimed by the authors of the article ‘Livestock Low Power Monitoring System’, the development of systems to monitor livestock theft is a necessity today (Lopes & Carvalho, 2016; Hartová & Hart, 2017). Long-term testing will run from 2018 to 2020.



**Figure 3.** Scheme of shielding pad (1 – shielded pad; 2 – space for placement of the IQRF unit; 3 – shielding flaps with adjustable angle; 4 – electrical grounding).

## CONCLUSIONS

Testing and improvement of existing technologies is very important. Due to the continuous development in the field of livestock technology is always important to continue to develop new and better systems.

The originally proposed system for livestock theft monitoring has been only partially successful so far. Outdoor detection almost did not work. Currently has been achieved on the system modification to achieve monitoring of an exactly defined area without its spill-over to undesirable areas.

The centralized method was replaced by a perimeter method. Where the receivers were placed on the perimeter of the guarded area. Additionally, it had to develop shielded pad to limit the reception of signals from the unsecured area.

Wireless transmissions are unfortunately very susceptible to interference – both interference caused by the environment, and interference caused by a targeted jammer. It is important to have an overview of the reliability and functionality of each wireless transmissions. Natural interference which may affect a wireless transmission occurs rather rarely, still it is important for wireless transmissions to be able to either identify such interference or replace the path of transfer. IQRF MESH systems do possess this feature and therefore are more secure in real life operation than standard wireless systems.

ACKNOWLEDGEMENTS. It is a project supported by the CULS IGA TF ‘The University Internal Grant Agency’ The Examination of the Influence of Blended Biofuels on Operating Parameters of CI Engines, 2017:31150/1312/3116.

## REFERENCES

- Bazydło, P., Dąbrowski, S. & Szewczyk, R. 2015. Wireless temperature measurement system based on the IQRF platform. In: *Advances in Intelligent Systems and Computing*, vol. **317**, pp. 281–288.
- Behkami, S., Zain, S.M., Gholami, M. & Bakirdere, S. 2017. Isotopic ratio analysis of cattle tail hair: A potential tool in building the database for cow milk geographical traceability. *FOOD CHEMISTRY* **217**, 438–444.
- Dong, Q., Liu, D.G. & Wright, M. 2013. Mitigating jamming attacks in wireless broadcast systems, *WIRELESS NETWORKS* **19**(8), 1867–1880.
- Elmasry, G.F. 2013. The Progress of Tactical Radios from Legacy Systems to Cognitive Radios, *IEEE COMMUNICATIONS MAGAZINE* **51**(10), 50–56.
- Hartová, V. & Hart, J. 2017. Livestock monitoring system using bluetooth technology. *Agronomy Research* **15**(3), 707–712.
- Kucera, L., Bradna, J. & Malatak, J. 2015. Use of Molasses Stillage Utilization as Fertilizer for Organically. *Listy Cukrovarnické a Reparské* **131**(12), 373–376.
- Kuchta, R., Vrba, R. & Sulc, V. 2009. IQRF smart wireless platform for home automation: A case study. In: *5th International Conference on Wireless and Mobile Communications*, pp. 168–173.
- Lopes, H.F. & Carvalho, N.B. 2016. Livestock Low Power Monitoring System. In: *IEEE Topical Conference on Wireless Sensors and Sensor Networks (WiSNet)*, pp. 15–17.
- Martin, P. & Radovan, H. 2016. Use of IQRF technology for detection of construction inclination. In: *International Conference of Numerical Analysis and Applied Mathematics 2015*, vol. **1738**, art.no. 480121.
- Sulc, V., Kuchta, R. & Vrba, R. 2009. IQMESH implementation in IQRF wireless communication platform. In: *2nd International Conference on Advances in Mesh Networks*, pp. 62–65.
- Tahir, H. & Shah, S.A.A. 2008. Wireless Sensor Networks – A Security Perspective. In: *INTERNATIONAL MULTITOPIC CONFERENCE*, pp. 189–193.

## **Differential thermal regulation of the growth of the bee colonies in the early spring period**

T. Jehlička\* and J. Sander

Czech University of Life Sciences Prague, Faculty of Engineering, Department of Technological Equipment of Buildings, Kamýcká 129, CZ165 21 Prague 6 – Suchbátka, Czech Republic

\*Correspondence: [jehlickat@tf.czu.cz](mailto:jehlickat@tf.czu.cz)

**Abstract.** This paper addresses the issue of the control of activity and growth of the bee colonies (brooding) in the early spring period. The bees are brooding up already in the late winter, and the intensity of brooding in this period is determined by daytime temperatures and sunshine hours that increase the temperature of the inner hive space. The objective is to design and verify a technology that would ensure the conditions for the optimal brooding in the early spring period and thus the numerically strong bee colonies. The experimental part was based on the temperature regulation in the inner hive space. A preset temperature was maintained inside the hive by an electric heating system with regulation. A differential thermal regulation which enabled the optimal growth of the bee colonies in accordance with the phenophases was chosen for verification. To verify the proposed method, two groups of the bee colonies were assembled. One group of the bee colonies had a heating system with regulation installed inside the hive. The second group of the bee colonies was in the hives without the heating system installed. The dependence of the brood area on time was monitored for the evaluation of both groups of the bee colonies. It was proven that the differential thermal regulation enables the optimal growth of the bee colonies in the early spring period in accordance with the phenophases. The brood area increased evenly for the group of the bee colonies with a regulated temperature of the hive space, reaching a larger area.

**Key words:** bee colonies development, brood area, hive thermal regulation.

### **INTRODUCTION**

This paper addresses the issue of the control of activity and growth of the bee colonies (brooding) in the early spring period in commercial beekeeping. For commercial beekeeping, the early spring development is a prerequisite for gaining numerically strong bee colonies and efficient beekeeping with high production capability. The objective is to design and verify a technology that will ensure the conditions for the optimal brooding in the early spring period and thus the numerically strong bee colonies in the main production period. The optimal brooding conditions were simulated by the regulation of the temperature inside the hive. The experiment was focused on the locations with less appropriate climate conditions (low number of sunshine hours in the early spring period and altitude above 500 metres).



Intensive agriculture is increasingly dependent on commercial beekeeping. Beekeeping ensures honey production and pollination of agricultural crops by commercially provided service (Williams, 1994). It is estimated that in case of cross-pollination plants the bees participate in 80–90% increase in yield (Aizen et al., 2009). For agricultural crops such as winter rape, fruit orchards or clovers, yields are increased by 30–50% by bee pollination in comparison with the self-pollination (Klein et al., 2007). The effectiveness of commercial beekeeping is thus determined by the seasonal yield of honey and the ability to maximally pollinate agricultural plants (Gallai et al., 2009). In both cases, the effectiveness of the beekeeping is directly impacted by the number of bees that ensure nectar collection and thus pollination of plants.

In the main production period, a strong bee colony has more than 50–60 thousand bees (Jaffé et al., 2010). In order to achieve this population density, the optimal development (brooding) in the early spring period is necessary (Seeley, 1985). Brooding is the ability to lay eggs by the queen. In the early spring period, the development of bee colonies is influenced by several factors. Intense brooding capacity is affected by the health condition of the queen and the bee colony, supply of pollen, honey, and climate conditions (Herbert & Simanuki, 1978). In a broader sense, the intensity of the early spring development depends on the successful overwintering of the bee colony and on the climate conditions during the early spring (Eckert et al., 1994). Climate conditions are a significant factor influencing bee colony development during the early spring period. The bees are brooding up already at the end of the winter and the intensity of brooding during this period is determined by daytime temperatures and sunshine hours, which increase the temperature of the inner hive space.

### **Overwintering of bees and its influence on the early spring development**

Overwintering is a perfectly developed physiological ability of bees. Successful overwintering is not impacted directly by the climate conditions. The basic factors influencing the success of overwintering are the bee colony's health conditions and sufficient food supplies. Climate conditions only influence the activity of winter cluster (its expansion and shrinkage depending on ambient temperature) and have a direct impact on the consumption of food supplies (Matilla & Otis, 2006). At stable nonfluctuating temperatures, the bee colony is calm (inactive) and the consumption of supplies low (Kronenberg & Heller, 1982). On the contrary, with significant temperature fluctuations or during a long above average temperature winter period, bee activity is increased and leads to higher consumption of supplies (Jones et al., 2004).

In commercial and hobby beekeeping, a technology of brooding in the insulated hives is expanded. The insulated hives eliminate (offset) the impact of climate changes on the hive temperature. From this point of view, the technology of the beekeeping in the insulated hives has an impact on the overall consumption of the supplies in the winter (Stabentheiner et al., 2010). It has therefore an economic importance. On the contrary, in the early spring and spring period, the insulated hives prevent the increase of the daily temperature inside the hive and has a direct impact on the slower development of the bee colonies in the early spring period (Severson & Erickson, 1990).

### **Climate conditions and their impact on the early spring development**

The life cycle of the bee colonies and their activity in each season is closely related to plant phenophases and climate conditions. Climate conditions and hence plant

phenophases do not occur every year the same way. Plant phenophases may show a certain shift in time against the calendar period (Gordo & Sanz, 2006). Even in areas between which there is no great distance, there may be major differences due to, for example, altitude or differences between the open country and the urban area. Central Europe is characterized by great climate diversity. During the spring development, cold and above average hot periods can occur. Vegetation responds to fluctuation between cold and hot periods by slowing or accelerating its development, but nectar production in its amount remains usually the same. Only the phenophases of the flower change. The bee colonies can easily cope with the fluctuations in cold and hot periods during the early spring period, but the cold periods reduce the activity of brooding and thus such bee colonies enter into the main production period (to main brooding) with the lower population density (Todd & Reed, 1970). Such bee colonies have lower production capacity.

## **MATERIALS AND METHODS**

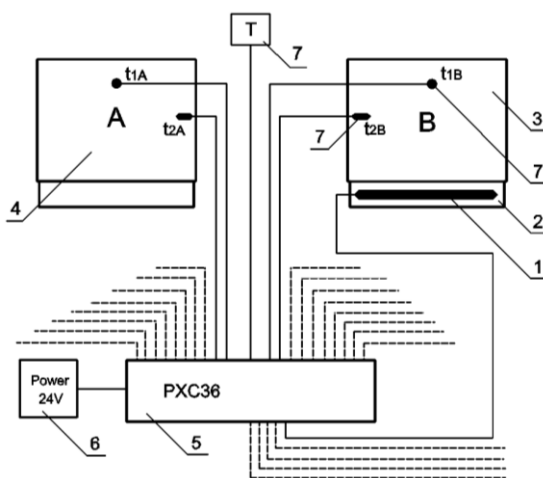
The experimental part of the work was based on the measurement of the activity of the early spring development of the bee colonies in dependence on the changing temperature inside the hive. To verify the procedure whether it is possible to influence the early spring development of the bee colonies, ten bee colonies were monitored. The bee colonies were divided into two groups of five bee colonies. The first group of five bee colonies (group A) was in the hives without the heating system and the natural development was retained throughout the experiment. The second group of five bee colonies (group B) was equipped with the heating system inside the hives. The heating system has increased the temperature of the inner hive space by a set value. The difference in the early spring development was continuously monitored in both groups of bee colonies by the measurement of the brood area. The whole experiment ran from 23 January 2017 to 31 March 2017. The experiment included bee colonies with an equal quantity and comparable supply of glycid and pollen. The term ‘glycid’ supply means the sum of the original winter supply and the honey added by the insertion of the honeycombs at the beginning of the experiment.

### **Construction of hives and their location**

All bee colonies (group A and B) were in constructively identical hives. The hives were double-spaced, not insulated all-wooden construction with an insulated cap. The size of the frames 240 x 390 mm. The hives were located in the area with the altitude 520 metres. They were located in an open agricultural landscape. The position of the hives was set so that the front side headed south into the open landscape (i.e. all day sunshine exposure of the hive) and from behind they were protected against the northwest winds by the forest. All hives were fitted with two temperature sensors. The location of the sensors was the same for all hives (one sensor near the inner side wall and the other near the inner back wall) so that they were out of reach of the winter cluster. Both sensors were located about 200 mm under the insulated cap. The sensor on the side wall was located between the first and second frames, i.e. about 40 mm from the inner wall, the second sensor was located also about 40 mm from the inner back wall. In the group B bee colonies, the heating system connected to a programmable control unit was installed. The heating system was placed on the bottom board of the hive.

### Heating system and programmable control unit of thermal regulation

The diagram of the heating system connection and the programmable control unit is shown in Fig. 1. The heat source was an electric heating system (1) located on the bottom board (2) of each hive of the group B (3). In the hives of the group A (4), the heating system was not installed. The heating system (1) consisted of two commercially produced heating plates Solar TF03 of size 320 x 1,236 mm connected consecutively. The total heating area then had a dimension of 320 x 272 mm. The rated output of the heating system was 2 x 36 W. The heating system (1) was supplied with pulse voltage via the programmable control unit (5). A 24 V safe voltage source was used for power supply (6). The heating system of this design has been chosen with regard to both construction and operational simplicity (easy manipulation, maintenance, low purchase costs, no need for adjustment of the hive for use). Switching on the heating system was ensured by the Siemens Desigo PXC36 programmable control unit (5). Siemens Desigo PXC36 is the freely programmable control unit designed for controlling and regulation of the technical equipment of buildings (heating, ventilation and air conditioning of buildings). Temperature sensors Siemens QAP22 (7) were used as temperature sensors in both inner and outdoor hive spaces. The sensor for the measurement of the outdoor temperature was placed in a sealed plastic box (wind protection) located under the hives (sunshine protection).



**Figure 1.** Diagram of the heating system connection and the programmable control unit: 1–heating system; 2–bottom board of the hive; 3–hive of the group B; 4–hive of the group A; 5–programmable control unit; 6–power supply; 7–temperature sensors.

### Adjustment of the programmable control unit and measurement methodology

The setting of the programmable control unit was accomplished so that the heating system installed in the group B bee colonies simulated by its thermal power and time switch the thermal power of the solar radiation, which would fall on the walls of the hive during sunny day. This setting should ensure the basic condition for successful verification of the proposed procedure. The heat supplied by the heating system into the hive must increase the activity of the bee colony, encourage the brooding, but at the same

time there must be no discrepancy between the increased activity of the bees and the phenophases of vegetation in the given locality.

The solar radiation simulation was determined by the operating time and heat power of the heating system and was performed only during the early spring period from 23 January 2017 to 31 March 2017 for the group B bee colonies. The programmable control unit switched on the heating system daily between 9 am and 3 pm. The temperature at which the inner hive space was warmed up during this time interval was set to be 5 °C higher than the average temperature of the same hive at night between 9 pm and 3 am. At the same time, the heating system was put out of operation once the inner hive space temperature reached 10 °C. The average night temperature was evaluated by the programmable control unit on the basis of the values measured by the temperature sensors located inside the hive. With this setting of the programmable control unit, it was ensured with an acceptable error that the bee colony was exposed to the natural climate conditions in the given locality for a substantial part of the day (between 3 pm and 9 am). The temperatures at which the inner hive space was warmed up between 9 am and 3 pm followed the changes in the outdoor climate conditions, their development during the whole early spring period and corresponded to the phenophases of the surrounding vegetation.

### **Methodology for the monitoring of the early spring bees development**

Bee colonies activity was evaluated by the size of the brood area on the honeycombs. A commonly used measurement method so-called frame grid was used to evaluate the brood area. By the frame grid, the brood area is measured in dm<sup>2</sup>, therefore this unit is used in the following text. The measurement was performed irregularly with an interval of approximately 14 days. The irregularity of the measurement interval was due to two reasons: the requirement for optimal climate conditions for measurement (windless sunny day, outdoor temperature at least 8 °C) and the requirement for minimal disturbance to the bee colony.

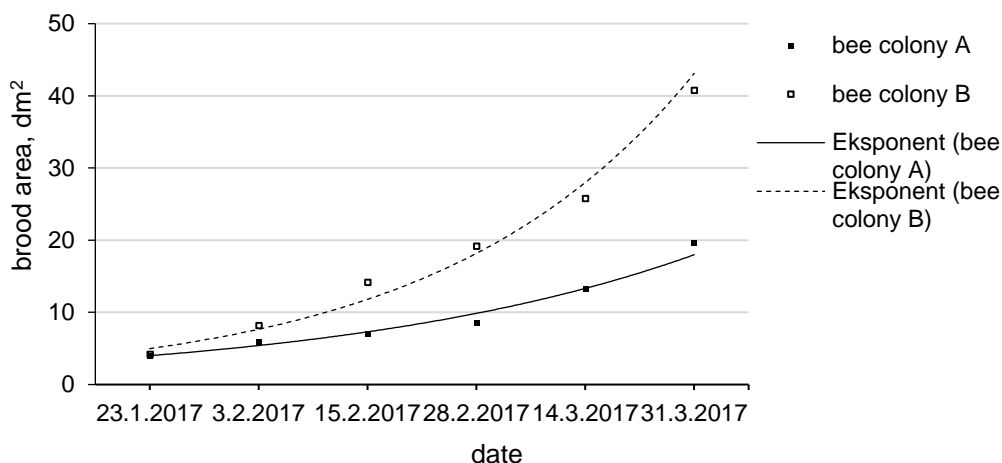
## **RESULTS AND DISCUSSION**

The suitable climate conditions to conduct an early spring control of the bee colonies overwintering occurred on 23 January 2017. All monitored bee colonies were checked for glycid and pollen supply, their health conditions were assessed subjectively, and the initial values of the brood areas were recorded, see Table 1. In the hives from the group B bee colonies, the heating system was put into operation and the programmable control unit was set up. In the following period, the brood area was measured at approximately 14 day interval (whenever appropriate climate conditions for the opening of the hives occurred). The brood area was measured in all five bee colonies from the group A (A1 to A5) as well as the group B (B1 to B5). The measured values are recorded in Table 1. The programmable control unit continuously recorded the average outdoor temperatures, the average temperatures of the inner hive space of the group A and B bee colonies, and the maximum temperatures of the inner hive space of the group A and B bee colonies.

The growth of the brood area in the monitored period is shown in Fig. 2. To create a chart, the average brood areas in the monitored period were used, see Table 1. The exponential trendline was used to plot the dependence of the growth of the brood area

on time as it most closely reflected such dependence. Correlation reliability for dependencies was above 0.95.

The brood area grew faster in the group B bee colonies during the monitored period, where the daily temperatures of the inner hive space were increased by 5 °C compared to the average night temperatures of the inner hive space. As shown in Fig. 2, at the end of the monitored period the total brood area of the group B bee colonies reached approximately double the values of the group A bee colonies, where the daily temperatures of the inner hive space were not increased by the heating system. Thus, the plotted course on dependency of the both groups of bee colonies indicate that the growth of the brood area is temperature–dependent.



**Figure 2.** The brood area in the monitored period.

**Table 1.** The brood area of each bee colony in the monitored period

	Brood area, dm <sup>2</sup>					
	23.1.2017	3.2.2017	15.2.2017	28.2.2017	14.3.2017	31.3.2017
Bee colony A1	3	6	7	9	12	20
Bee colony A2	4	7	8	9	13	19
Bee colony A3	5	6	7	9	13	18
Bee colony A4	4	6	7	8	14	21
Bee colony A5	4	5	6	8	14	20
The average brood area of the group A bee colonies, dm <sup>2</sup>	<b>4</b>	<b>6</b>	<b>7</b>	<b>8,6</b>	<b>13,2</b>	<b>19,6</b>
Bee colony B1	5	7	13	18	28	44
Bee colony B2	4	9	14	19	26	40
Bee colony B3	5	8	15	20	25	36
Bee colony B4	3	8	14	18	26	42
Bee colony B5	4	9	15	21	24	42
The average brood area of the group B bee colonies, dm <sup>2</sup>	<b>4.2</b>	<b>8.2</b>	<b>14.2</b>	<b>19.2</b>	<b>25.8</b>	<b>40.8</b>

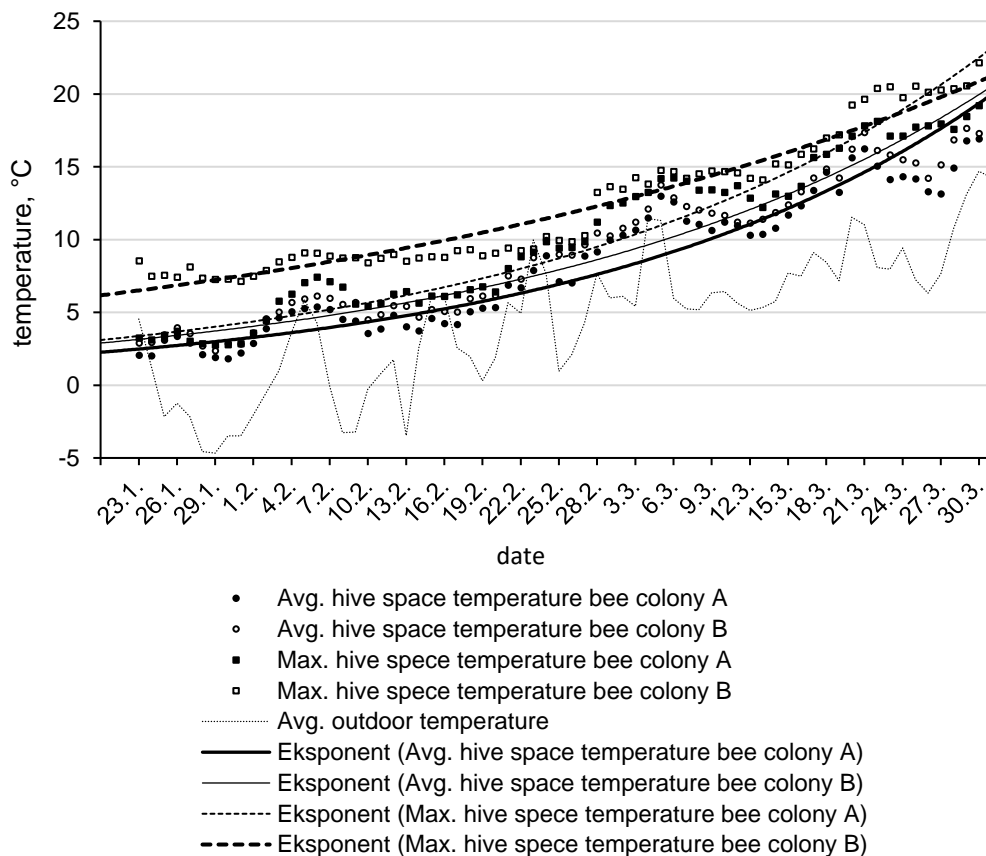
During the experiment the condition of equal glycid and pollen supply was fulfilled. At the same time, all monitored bee colonies were numerically balanced and were in the equal health conditions based on the subjective assessment. The only objective variable was the hive temperature. The temperatures at which the inner hive space of the group B bee colonies were warmed up were not constant throughout the experiment, but reflected the changes of the outdoor temperatures. As outdoor temperatures gradually increased during the upcoming spring, the temperatures in the hives increased as well. Once the temperature of the inner hive space reached 10 °C, the heating system was disconnected. After disconnecting the heating system, the higher temperatures of the inner hive space could only be achieved in a natural way (as a result of external climate conditions). These climate conditions affected both groups of the monitored bee colonies the same way, i.e. the temperature above 10 °C was in this case also in the inner hive space of the group A bee colonies. This condition occurred during some warm days at the end of the monitored period (March).

Nevertheless, for the group A bee colonies, the size of the brood area was delayed behind the group B bee colonies. It can be estimated that the brood area depends not only on the temperatures reached but also on the length of the period with balanced optimal temperatures for the growth of the brood area. Although the programmable control unit in the group B bee colonies in the second half of March regularly switched off the heating system (higher temperatures above 10 °C were reached naturally) and thus the conditions were equal for both groups of the bee colonies (A and B), the group B bee colonies showed almost twice larger brood area.

In Fig. 3 the temperatures that were measured during the bee colonies' monitoring are plotted. The measured average outdoor temperatures, the average temperatures of the inner hive space for both groups of the monitored bee colonies (A and B) and the maximum inner hive space temperatures for both groups of the bee colonies are plotted. The average outdoor temperatures as well as the average temperatures in the inner hive space were calculated using the method of the hourly measurement. Measured temperatures (average outdoor, average inner hive space and maximum inner hive space) for each day of the monitored period represent a considerably large set of data. Therefore, measured temperatures are not listed in a separate table, but are only plotted as the points in Fig. 3. The average outdoor temperatures are plotted as a stacked line precisely displaying temperature fluctuations in the monitored period. The average and maximum inner hive space temperatures are for greater clarity plotted as a trendline. The exponential course of the trendline with a correlation greater than 0.95 was used for the plot.

From the plotted average temperatures of the inner hive spaces for all bee colonies of the group A and group B is clear that they have a similar course but different values of average temperatures in the monitored period. The average temperatures of the inner hive spaces in the group B bee colonies (equipped with the heating system) were on average 0.9 °C higher. It can be concluded that in the absence of the 0.9 °C measured average temperature difference, the group B bee colonies was exposed to similar climate conditions as the group A bee colonies (without the heating system) during the monitored period and developed in accordance with the phenophases of the surrounding vegetation. This assumption is based on a similar course of the average temperatures of the inner hive space of the both groups of bee colonies. Thus, the heating system had little impact on the average inner hive space temperature and had no influence on the achieved

temperature course. The difference in the average inner hive space temperatures of an average value of 0.9 °C is unlikely to significantly affect the activity of bees and the growth of the brood area during this period. However, as shown in Fig. 2, all of the group B bee colonies monitored showed the growth in the brood area. The reason for this growth will not be average inner hive space temperatures but fluctuation in daytime and nighttime temperatures. This would correspond to the course of the measured maximal temperatures of the inner hive space.



**Figure 3.** Temperatures measured in the inner and outdoor hive space.

The maximum temperatures' course of the inner hive space is also plotted in Fig. 3. At the beginning of the monitored period (January, February), the differences in the maximum temperatures were reached by the heating system setup and in the group B bee colonies reached values higher up to 5 °C. At the end of the monitored period (March), as with the average inner temperatures, the difference gradually decreased. It was again a result of the rising outdoor temperatures, which were the signal to disconnect the heating system. It can be assumed that the difference in the maximum achieved temperatures within the inner hive spaces between the bee colonies of both groups is the factor that influences the activity of the bee colonies and affects the growth of the brood area.

## CONCLUSIONS

The result of the experiment is the design of differential thermal regulation technology of bee activities. The design of the technology is based on the increase of the daily temperatures of the inner hive space to simulate the climate conditions of sunny days (thermal power of solar radiation). During the early spring period, the outdoor temperature varies greatly in sunny days. This is manifested by the great difference between daytime and nighttime temperatures. Simulating the effect of solar radiation by raising the temperature in the hives means increasing the temperature of the inner hive space during the day, but allowing natural climate conditions to occur at night. The bees are considered to be thermosensitive organisms. It can be assumed that they will actively respond to the maximum daily temperatures that simulate solar radiation. The proposed technology, its technical design and experimental verification confirm this assumption.

The realization of the experiment confirmed that the early spring bee development, as measured by the growth of the brood area, is temperature dependent. By the differential regulation of the temperature of the inner hive space was at the end of the monitored period for the group B bee colonies (with a heating system) achieved about twice the size of the brood area in comparison to the group A bee colonies, where the daily temperatures of the inner hive space were not increased by the heating system. From a detailed analysis of the measured temperatures of the inner hive spaces of the two monitored groups of bee colonies (A and B), it is obvious that the average hive temperatures have a similar course in the monitored period, but different values of the average temperatures reached. The difference in average temperatures in the inner hive spaces was about 0.9 °C. Such a small difference is unlikely to significantly affect the activity of the bees and the growth of the brood area during the early spring period. The reason for increased activity of bee colonies and the growth of the brood area will not be the average temperatures of the inner hive space but fluctuations in daytime and nighttime temperatures. This would correspond to the course of the measured maximum daytime temperatures of the inner hive space, where the differences between the group A and B bee colonies were about 5 °C. It can be assumed that the difference in the maximum achieved temperatures within the inner hive spaces between the bee colonies of the two groups is the factor that influences the activity of the bees and affects the growth of the brood area.

Based on the proven results, the technology of differential thermal regulation of bee activity can generally be considered as an operationally efficient technology. The technical design of the proposed technology is structurally simple and has demonstrated high technical and technological reliability under operating conditions.

## REFERENCES

- Aizen, M.A., Garibaldi, L.A., Cunningham, S.A. & Klein, A.M. 2009. How much does agriculture depend on pollinators? Lessons from long-term trends in crop production. *Annals of Botany* **103**, 1579–1588.
- Eckert, C.D., Winston, M.L. & Ydenberg, R.C. 1994. The relationship between population size, amount of brood, and individual foraging behaviour in the honey bee, *Apis mellifera* L. *Oecologia* **97**(2), 248–255.



- Gallai, N., Salles, J-M., Settele, J. & Vaissière, B.E. 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics* **68**, 810–821.
- Gordo, O. & Sanz, G. 2006. Temporal trends in phenology of the honey bee *Apis mellifera* (L.) and the small white *Pieris rapae* (L.) in the Iberian Peninsula (1952–2004). *Ecological Entomology* **31**(3), 261–268.
- Herbert, E.W. Jr. & Simanuki, H. 1978. Effect of the size of outdoor flight cages on brood rearing and food consumption by honey bees. *Journal of Apicultural Research* **17**(3), 114–117.
- Jaffé, R.R., Dietemann, V.V., Allsopp, M.H.M., Costa, C.C, Crewe, R.M.R., Dall'olio, R.R., De la Rúa, P., El-Niweiri, M.A.A., Fries, I., Kezic, N., Meusel, M.S., Paxton, R.J., Shaibi, T., Stolle, E. & Moritz, R.F.A. 2010. Estimating the density of honeybee colonies across their natural range to fill the gap in pollinator decline censuses. *Conservation Biology* **24**, 583–593.
- Jones, J.C., Myerscough, M.R., Graham, S. & Oldroyd, B.P. 2004. Honey bee nest thermoregulation: Diversity promotes stability. *Science* **305**, 402–404.
- Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A. & Kremen, C. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences* **274**, 303–313.
- Kronenberg, F. & Heller, H.C. 1982. Colonial thermoregulation in honey bees (*Apis mellifera*). *Journal of Comparative Physiology* **148**(1), 65–76.
- Matilla, H.R. & Otis, G.W. 2006. The effects of pollen availability during larval development on the behaviour and physiology of spring-reared honey bee workers. *Apidologie* **37**(5), 533–546.
- Seeley, T.D. 1985. Survival of honeybees in cold climates: the critical timing of colony growth and reproduction. *Ecological Entomology* **120**, 826–888.
- Severson, D.W. & Erickson, E.H. Jr. 1990. Quantification of cluster size and low ambient temperature relationships in the honey bee. *Apidologie* **21**(2), 135–142.
- Stabentheiner, A., Kovac, H. & Brodschneider, R. 2010. Honeybee colony thermoregulation – regulatory mechanisms and contribution of individuals in dependence on age, location and thermal stress. *PLoS ONE*. **5**(1):e8967.
- Todd, F.E. & Reed, C.B. 1970. Brood measurement as a valid index to the value of honey bees as pollinators. *Journal of Economic Entomology* **63**(1), 148–149.
- Williams, I.H. 1994. The dependence of crop production within the European Union on pollination by honey bees, *Agricultural Zoology Reviews* **6**, 229–257.

## **Evaluation of the mechanized harvest of grapes with regards to harvest losses and economical aspects**

J. Jobbágy, K. Krištof\*, A. Schmidt, M. Križan and O. Urbanovičová

Slovak University of Agriculture in Nitra, Faculty of Engineering, Department of Machines and Production Biosystems, Tr.A. Hlinku 2, SK949 76 Nitra, Slovakia

\*Correspondence: koloman.kristof@uniag.sk

**Abstract.** The aim of the study was to evaluate the mechanized grape harvest conducted by the trailed harvester. As a criterion was introduced the performance, harvest losses of grapes and economic efficiency from the perspective of its introduction. The calculations consist of total annual and partial unit costs also with regards to indirect costs which are formed by the grape losses during the harvest. Evaluation was conducted on three varieties of grapes. The losses of grapes, total annual and partial unit costs were calculated for selected varieties of grapes and also for whole production area. The mean grape losses for whole production area were determined at 470 kg ha<sup>-1</sup>, while the greatest portion of these losses (9.7%) was observed in case of Neronet variety. Among other analysed aspects were the efficiency of mechanized harvest introduction by definition of labour costs and the ransom price of grapes in relation with minimum required area. A substantial part of total costs for area 100 ha was formed by direct costs up to 15.24% greater than the indirect costs. The efficiency of machinery introduction into the harvest process was observed at minimum area of 16.92 ha in case of rental mechanized harvest of grapes. In case of mechanized harvest conducted by previously bought trailed grape harvester the value of minimal efficiency was determined at 27.42 ha year<sup>-1</sup>. As a result then serves an effective utilization of mechanized grape harvest in selected company but it is not limited to it and can be applied on any other scenario.

**Key words:** grapes, harvest, yields, harvest losses, economic benefits.

### **INTRODUCTION**

Currently, in conditions of Slovakia there are almost 300 various vine production and viticulture organisations (in different form of private production economies) which grown vine grapes and produce vines. According to registration for year 2017, the total area utilized for vine grapes production was calculated at area 17,598 ha (8,873 ha of directly produced vineyards and 11,159 ha managed for vine production and similar purposes), whereas this total area includes also not managed areas (UKSUP, 2016).

Mechanized vine grape harvest is spreading over Slovakia at slow motion in comparison with ratio of its utilization in other European countries (e.g. Italy or France). From the published statistics it is clear that mechanized vine grape harvest is fully utilized at level from 20 to 30% of total area used as vineyards in case of north Italy (Corazzina, 2010). In contrast, in case of Slovakia, mechanized vine grape harvest is established only at 10% of total area utilized to production of vine grapes. The issue of

establishment and utilization of mechanization in viticulture is limited due to several specific conditions. As critical criterions for its full establishment was emphasised by several conditions. Mainly, the selection of appropriate location of fields, it's landscaping in particular, since as a main issue which needs to be addressed are following. Subsequently, the employment of support constructions produced as a narrow aluminium pillars along with unified cropped varieties in single rows. In addition, on the fields which are characterised by good level of transverse slope along with employment of the appropriate landscaping techniques allows a non-problematic movement of machinery and therefore it allows the reduction of damaged to cropped vine grapes (Johnson et al., 2003; Jobbágy & Findura, 2013).

The main aim for introduction of mechanized vine grape harvest is to lower the need of manual labour and reduction of annual costs however initial costs needed for introduction of mechanized harvest are quite high (Bates & Morris, 2009; Jobbágy & Findura, 2013; Pezzi & Martelli, 2015). On the other hand, introduction of mechanized vine grape harvest cannot lead to decrease of harvested product quality (Morris, 2007; Pezzi, 2013). As it was stated, spacing of the support pillars should be in range from 4.5 to 8.0 m in accordance to the weight of vine grape crop row and to appropriate leaf area. In addition, the height of used support pillars should not exceed the height 1.8 m due to utilization of mechanization which is limited by this value. Moreover, the support pillars should not be produced from any wooden materials as they usually are (e.g. spruce wood, bamboo). Mainly, due to the caused vibration can easily lead to its damage or destruction and therefore the vine grape harvester mechanisms can be damaged or it may lead to complication of postharvest treatment of harvested products. As another limiting factor is considered the use of the same variety of vine grapes while it allows of the continual harvest of whole row at a single passage of machinery. It is also important to replace withered or other way damaged individual plants by the same variety (Fic, 1973; Žufánek & Zemánek, 1992; Zemánek & Burg, 2003; Walg, 2007; Keller, 2010).

In Europe (e.g. Italy), the trend is in increasing of mechanization of harvest labour in all phases of vine grape production including of its harvest. According to available sources, in latest ten years, the number of machinery used in this field was doubled. Statistics showed (UNIMA, 2013) that in case of Italy operates more than 2,600 vine grape harvesters which mostly (86 to 88%) utilize the horizontal harvest mechanism (horizontal impactors) while majority of this machinery is designed as trailed types (up to 85%). Moreover, 170 to 190 of new self-propelled vine grape harvesters are introduced into viticulture production which are subsequently also utilized by a various adapters designed for other field operation in viticulture production, e.g. pest controls, green and other operations (Pezzi & Balducci, 2012).

In conditions of Slovakia, as well as in other countries, the area for production of vine grapes was increased rapidly at different levels. Majority if this area is, in case of harvest, treated under mechanized harvest which is in year 2017 implicated in more than 10 companies of various types. By introduction of new technologies and machinery, the aim is to decrease final costs on manual labour along with preservation of limit values required for harvest losses and final quality of harvested product.

Due to the faster introduction of grape harvest by grape harvesters the aim of the study was to evaluate the mechanized grape harvest conducted by the trailed harvester. As a criterions was introduced the performance, harvest losses of grapes and economic efficiency from the perspective of its introduction.

## MATERIALS AND METHODS

### Field conditions

Field measurements were conducted on selected vine grape production company JM Vinárstvo Doľany, Ltd., Slovakia. Vineyards of selected company are situated at southern slopes of Small Carpathians on the area of 115 hectares. In selected company the mechanized vine grape harvest were tested on 3 different varieties of vine grapes, namely: Ruland blue (Pinot noir), Neronet and Veltliner green (Table 1). Varieties were tested in near locations to the company with average altitude 251 above sea level (48°25'03.5"N, 17°22'59.4"E).

Average yields of Ruland Blue variety range from 6 to 10 t ha<sup>-1</sup> (Kraus et al., 2004). The vineyard of this variety was established continuously in years 1997 till 2011 on the area of 3 ha, 13,900 individuals (4,630 individuals per hectare), specifically.

The average yields of Neronet variety range from 7 to 12 t ha<sup>-1</sup> (Kraus et al., 2004). The vineyard of this variety was established in years 1997 till 2011 on the area of 4 ha, 18,500 individuals (4,630 individuals per hectare).

Average yields of Veltliner Green variety range from 12 t ha<sup>-1</sup> (at medium height of support) up to 16 t ha<sup>-1</sup> (at high height of support) (Kraus, 2004). The vineyard of this variety was established in years 1997 till 2011 on the area of 16.1 ha, 74,500 individuals (4,630 individuals per hectare).

**Table 1.** Characteristics of vineyards

Cultivar	Spacing	Average Harvest, t ha <sup>-1</sup>	Surface, ha	Number of rootstock, pcs ha <sup>-1</sup>	Trellis system	Field slope, %	Plant Age, years
Ruland Blue	2.4 × 0.9	6.23	3	4,630	Double Guyot	4	15
Neronet	2.4 × 0.9	7.30	4	4,630	Spur cordon	3	13
Veltliner Green	2.4 × 0.9	9.70	16.1	4,630	Double Guyot	2	14

### Grape Harvester

For mechanized vine grape harvest of individual varieties was used the same type of trailed vine grape harvester ERO LS Traction (ERO-Gerätebau, GmbH, Niederkumbd, Germany). The ERO LS Traction (Table 2) grape harvester has a special hydrostatic transmission (the Load Sensing system) that actuates the wheels depending on the pulling force measured at the drawbar. The detachment system is an oscillatory shaker with bow rods on either side of the frame (maximum of ten per side). The grapes are collected by a series of overlapping spring-loaded plates, which carry the grapes on two lateral conveyer belts that unload into two lateral hoppers (3,000 L). The cleaning system is composed of two lateral suction fans at the end of the catching surface and one at the discharge into the hoppers.

Selected vine grape harvester was aggregated with four-wheel drive tractor Lamborghini RF 75 (Lamborghini Trattori, Italy). Such combination allows harvest on slopes at maximum 35% with maximum working speed 6 km h<sup>-1</sup> (Table 3).

**Table 2.** Technical parameters of grape harvester ERO LS Traction

Parameter	Value
Height of hopper during emptying, mm	2,600
Dimensions: Length/Width/Height, mm	3,300/2,550/2,650
Minimal space between rows, mm	1,350
Capacity of hopper, m <sup>3</sup>	1
Maximum side slope, %	27
Maximum slope, %	35
Minimal power input from tractor, kW	37
Number of striping rods, pcs	2×5

**Table 3.** Technical parameters of tractor Lamborghini RF 75

Parameter	Value
Cylinders/Displacement, cm <sup>3</sup>	4,000
Max. homologated power, kW	54.5
Nominal engine speed, rpm	2,200
Max. torque, Nm	257
Fuel tank capacity, L	55
Torque backup, %	15
Dimensions: Length/Width/Height, mm	3,440/1,460/2,090

### Performance of vine grape harvester

The height of the bunch of grapes ranged from 0.8 to 1.35 m, from 0.5 to 1.1 m and from 0.7 to 1.2 m above the ground in case of variety Ruland Blue, Neronet and Veltliner Green, respectively. The harvest mechanism of vine grape harvester consists of 5 wattles in the both sides of it. Therefore, it is able to harvest the whole range of grapes. Working speed, oscillation frequency and amplitude of wattles were set according to previous working experience and the results of preliminary harvest measurements. Post-harvest treatment and separation of harvested vine grapes was conducted by two ventilators at the bottom and single ventilator at the top of the harvest mechanism. The upper ventilator is connected with shredders and finger conveyors. The hopper for harvested vine grapes is equipped by screw separators which are responsible for emptying of hopper from the back of vine grape harvester. The effective field capacity of vine grape harvester  $C_a$  (ASABE, 2006) was evaluated according to measurements of effective working time during the harvest of all varieties (Eq. (1)).

$$C_a = 0.1 \cdot s \cdot w \cdot E_f, \text{ (ha h}^{-1}\text{)} \quad (1)$$

where  $C_a$  – area capacity (ha h<sup>-1</sup>);  $s$  – field speed (km h<sup>-1</sup>);  $w$  – distance between rows (m);  $E_f$  – field efficiency, considering the time required for turning and manoeuvring at the ends of the field and for hopper unloading (Srivastava et al., 2006; Pezzi & Martelli, 2015).

In case of all varieties of vine grapes were tested by measurements of effective working time, time needed for turning of machinery at the edges of rows and emptying of hopper. Evaluation consists of considering the mean values and standard error ( $\pm SE$ ).

### Field experiments

Evaluation of field experiments of vine grape harvester work quality followed a specific number of steps for all of the tested varieties. From each varieties were selected

a specific sections of lines represented by length 10 m. These sections were located in the middle part of rows. These procedures allows avoiding of influence of the results by working conditions of vine grape harvester, working speed and setting of the harvesting height and variety. The results then reflect the evaluation of losses which consist of the losses of grapes left on shrubs and losses caused by the slumping to the ground below vine grape harvester. The methodology of field measurements according to recorded losses of vine grape was done according to standard ISO (1980). The losses of vine grapes left on shrubs was measured by weighting of grape berries without the rest of tassel, leafs and stem on selected part of rows in length of 10 m. For measurement of grape losses by the slumping to ground was done by utilization of foil which was spread on the surface in length of 11 m from both sides of measured row in width 1.2 m. Subsequently, both parts of foils were then connected to avoiding of the slumping outside of measured parts. After the vine grape harvester passage, collected leafs, tassels and stems were removed and left grape berries were weighted. Statistical evaluation was conducted by statistical software Statistica 10 (StatSoft, Inc., Tulsa, USA). For evaluation of the results and analysis of statistically significant differences between mean values was used one-way analysis of variance (ANOVA). The results are presented as means  $\pm$  SE. The  $p$  value ( $p < 0.05$ ) was used to determine significant differences.

### Costs on vine grape harvest

Evaluation of costs on vine grape harvest was calculated with reflection of fixed and variable costs on vine grape harvester and those caused by the harvest (non-direct costs). While the study was focuses on evaluation of work quality of trailed vine grape harvester, the final costs needs to reflect also the cost of tractor which was used for aggregation. Therefore, fixed costs on tractor then consists of costs on amortization, interests, insurances and garaging (ASABE, 2005, 2006; Ďudák, 2016; Pristavka et al., 2017). Table 3 show the input parameters for determination of specific cost items which are valid in conditions of Slovakia.

*Total direct costs on vine grape harvest*

$$rN_{mC} = rN_{mT} + rN_{mZ} + rN_o + rN_e + rN_{zp}, \text{ (€ year}^{-1}\text{)} \quad (2)$$

where  $rN_{mT}$  – direct costs on tractor (€ year<sup>-1</sup>);  $rN_{mZ}$  – direct costs on vine grape harvester (€ year<sup>-1</sup>);  $rN_e$  – annual costs on energy (€ year<sup>-1</sup>);  $rN_o$  – annual costs on repairs of machinery (€ year<sup>-1</sup>);  $rN_{zp}$  – annual costs on manual labour (€ year<sup>-1</sup>).

*Constant (fixed) costs*

$$rN_k = rN_a + rN_{zu} + rN_{cd} + rN_p + rN_g, \text{ (€ year}^{-1}\text{)} \quad (3)$$

where  $rN_a$  – annual costs on amortization (€ year<sup>-1</sup>);  $rN_{zu}$  – annual costs on capitalization of equity and loan interest (€ year<sup>-1</sup>);  $rN_{cd}$  – annual costs on road tax (€ year<sup>-1</sup>);  $rN_p$  – annual costs on mandatory insurance (€ year<sup>-1</sup>);  $rN_{np}$  – annual costs on voluntary insurance (€ year<sup>-1</sup>);  $rN_g$  – annual costs on garaging (€ year<sup>-1</sup>).

*Annual costs on amortization*

$$rN_a = \frac{C_s \cdot a}{100}, \text{ (€ year}^{-1}\text{)} \quad (4)$$

where  $C_s$  – purchase (input) cost of machinery (€ year<sup>-1</sup>);  $a$  – depreciation rate, it is depreciation percentage for the selected period of use and depreciation strategy.

*Annual costs on capitalization of equity and loan interest*

$${}_rN_{zu} = \frac{(C_s + C_z) \cdot z}{200}, (\text{€ year}^{-1}) \quad (5)$$

where  $C_s$  – purchase (input) cost of machinery (€ year<sup>-1</sup>);  $z$  – interest rates on deposits (%);  $C_z$  – residual price of machinery (€ year<sup>-1</sup>).

*Annual costs on insurances and taxes*

$${}_rN_s = (L + 1) \cdot (B + 1) \cdot {}_rN_{gm2}, (\text{€ year}^{-1}) \quad (6)$$

where  $L$  – length of machinery (m);  $B$  – width of machinery (m);  ${}_rN_{gm2}$  – costs on 1 m<sup>2</sup> of area for garaging per year (€ m<sup>-2</sup> year<sup>-1</sup>).

Variable annual costs then consist of costs on repairs of machinery, energy and manual labour.

*Annual costs on repairs of machinery*

$${}_rN_o = RF_1 \cdot C_z \cdot (1 + i)^n \cdot \left(\frac{h}{1000}\right)^{RF_2}, (\text{€ year}^{-1}) \quad (7)$$

where  $RF_1$  and  $RF_2$  – coefficients of machinery repairs (wide range dependent on type and reliability of machinery) defined by ASABE Standards);  $C_s$  – purchase (input) cost of machinery (€ year<sup>-1</sup>);  $i$  – average inflation;  $n$  – age of machinery (years).

*Annual costs on energy*

$${}_rN_e = Q \cdot C_e \cdot {}_rW \cdot 1.1, (\text{€ year}^{-1}) \quad (8)$$

where  $Q$  – consumption of energy (L ha<sup>-1</sup>);  $C_e$  – cost of energy (€ L<sup>-1</sup>);  ${}_rW$  – annual utilization of machinery (ha year<sup>-1</sup>).

Annual utilization of vine grape harvester (machinery) was observed at value 300 hours. In selected company was harvested by this machinery about 100 ha from total area. Daily working shift was from 7 to 8 hours during the whole vine grape harvest season (35 days). Costs on garaging of machinery was calculate as 6 € m<sup>-2</sup>. The depreciation period time for vine grape harvester and tractor is usually set to 4 years. In our case it means that the costs on depreciation rate in observed year was not considered due to the age of machinery. For determination of annual costs on capitalization of equity and loan interest was used average value for interests 5.0%. Variable annual costs (repairs of machinery, energy and manual labour) were then determined according to standards (ASABE, 2011) and following Ďud'ák, 2016. Average fuel consumption was monitored during the all measurements and then converted on consumption for specific area. Average consumption of oil per hour was calculated considering its price 4.10 € L<sup>-1</sup>. Salary of vine grape harvester operator was considered 3.10 € without deductions. The price of fuel (diesel) was in the year of study at level 1.34 € L<sup>-1</sup>.

*Annual costs on manual labour*

$${}_rN_{zpm} = {}_hN_{zp} \cdot 1.352 \cdot \frac{{}_rW}{C_a}, (\text{€ year}^{-1}) \quad (9)$$

where  ${}_hN_{zp}$  – salary of operator per hour (€ h<sup>-1</sup>);  ${}_rW$  – annual utilization of machinery (ha year<sup>-1</sup>);  $C_a$  – effective field capacity (ha h<sup>-1</sup>).

Direct cost per unit  $jN_{mc}$  in conjunction with the period and strategy of depreciation (utilization) then can be calculated by below equation.

$${}_rN_{jC} = \frac{{}_rN_{mc}}{{}_rW}, (\text{€ ha}^{-1}) \quad (10)$$

where  ${}_rN_{mc}$  – total direct annual costs (€ year<sup>-1</sup>);  ${}_rW$  – annual utilization of machinery (ha year<sup>-1</sup>).

For the purpose of efficiency evaluation of vine grape harvester utilization was considered three types of parameters. Firstly, the obtained results during the measurements of different varieties of vine grapes. Secondly, by the costs which were generated by organization where the harvest was utilized. Thirdly, by the costs of services generated by other companies in form of outsourcing (Table 4). For this kind of evaluation it requires consideration of different potential areas of vineyards and estimation of maximum life period of vine grape harvester at 15 years. Economical evaluation was then expanded also with evaluation of minimum area treated as vineyards in subsequent differentiation of costs on manual labour which ranged from 2 to 14 € h<sup>-1</sup> not considering VAT.

**Table 4.** Machinery cost parameters

Parameter	Units	Trailed vine grape harvester	Tractor
Purchase price	Eur	60,000	45,000
Estimated life	h	3,000	10,000
Annual use	h	300	600
Remaining value*	%	25	25
Depreciation	Year	-	-
Insurance and housing	€	50.49	85,13
Dimensions of machinery	m × m	3.3 × 2.55	3.44 × 1.46
Repair factor	RF <sub>1</sub>	0.11	0.02
	RF <sub>2</sub>	1.80	1.35

\* based on purchase price of machinery.

Total costs were then considered also in case of utilization of manual vine grape harvest within the scope of the same vineyard areas while the selected company do not use mechanized harvest only at all of their vineyards. Total cost on manual labour (salary of workers with insurances) were the considered as 3 € h<sup>-1</sup>. However, in case of manual harvest, the losses from harvest were considered at zero level while this type of harvest is characterised by minimal losses (Johann et al., 2010). At the basis of our research the time period was used in accordance of harvest needed for vineyard area 102 h ha<sup>-1</sup>.

## RESULTS AND DISCUSSION

### Harvester working capacity

From the obtained results on vine grape harvester working capacity, total yields and subsequently recorded losses from harvest the significant differences were observed. Total yields were significantly dependent on variety of vine grape up to value 10 t ha<sup>-1</sup>. In case of variety Ruland Blue it was 6.23 ± 0.34 t ha<sup>-1</sup> (sugar content 23.2 NM), Neronet



7.30 ± 0.46 t ha<sup>-1</sup> (sugar content 24°NM) and Veltliner Green 9.7 ± 0.50 t ha<sup>-1</sup> (sugar content 21.5°NM) presented in form *mean ± SE*.

In evaluation of performance parameters the significant differences were observed in all harvested varieties as well. Working speed of harvester and frequency of wattle oscillations were dependent on thickening of the plant and the time of grape harvest (degree of maturation). The time needed for turning of machinery and emptying of harvester hopper were partially affected by specifically used machinery (type of machinery – self-propelled or trailed). However, at the first place, it is highly dependent on experience and skills of machinery operator and land conditions of vineyards. On the other hand, when the water content of vine grapes are at lower levels (degree of maturation and previous precipitations), the grape berries were better situated in grape tassels. Therefore the frequency of oscillations needs to be increased to the greater values. It means that the every single variety of vine grape and specific year weather conditions are also main driving factors which affect the operability and utilization of machinery and its working settings. Similar results and conclusions can be found in other studies (Bavaresco et al., 2008; Pezzi & Caprara, 2009; Caprara & Pezzi, 2011; Pezzi, 2011; Clingeffer, 2013; Novák & Burg, 2013; Pezzi & Martelli, 2015).

In the evaluation of 3 varieties of vine grapes it was observed that the harvest with the lowest need of oscillation frequency were for variety Neronet. During the observation of times needed for turning of machinery and emptying of harvester hopper there were found out very low differences where the lowest values of these times were observed for variety Ruland Blue. In evaluation of effective field capacity of vine grape harvester for different varieties there were observed only slight differences while the maximum was observed at level 6.4% in comparison of variety Neronet with Veltliner green. In evaluation of material field capacity in relation with yields of selected varieties it can be concluded that the highest yields was observed in case of variety Veltliner Green (about 36.11% higher in comparison to Ruland Blue). In contrast, evaluation of manual harvest by the same person and all of the varieties in conjunctions with density of the plant and size of the grape tassels were observed interested values which are shown in Table 5. From these results it can be concluded that utilization of the manual harvest in comparison with utilization of mechanization requires employment of dozens of workers to be concurrent to mechanized harvest while field capacity of vine grape harvester is immeasurably higher.

**Table 5.** Operating characteristics of vine grape harvesters (*mean ± SE*)

Characteristic	Units	Variety		
		cv. Ruland Blue	cv. Neronet	cv. Veltliner Green
Field speed	km h <sup>-1</sup>	2.2 ± 0.1	2.1 ± 0.1	1.9 ± 0.1
Turning time	s	82 ± 1.4	90 ± 1.8	103 ± 2.1
Unloading time	s	113 ± 1.4	112 ± 1.7	115 ± 1.8
Frequency of oscillation	beats min <sup>-1</sup>	550	525	580
Field efficiency, E <sub>f</sub>	-	0.61 ± 0.00	0.65 ± 0.00	0.69 ± 0.00
Effective field capacity, C <sub>a</sub>	ha h <sup>-1</sup>	0.32 ± 0.00	0.33 ± 0.00	0.31 ± 0.00
Material field capacity	t h <sup>-1</sup>	1.98 ± 0.14	2.45 ± 0.16	3.11 ± 0.22
Field Capacity (manual harvest)	10 <sup>-3</sup> ha h <sup>-1</sup>	2.12	2.22	2.01

Evaluation of effective field capacity was the main aim of the study conducted by Pezzi & Martelli (2015) with focus on cultivar variety cv. Trebbiano. In the study it was reported at value  $0.34 \text{ ha h}^{-1}$ . It was concluded that the differences may be obvious, however, utilization of smooth and flat vineyards in combination with used tractor Tractor CNH T5060 aggregated with the same vine grape harvester ERO LS Traction was the main difference driving parameter (Pezzi & Martelli, 2015). In comparison with our results, the lower values about 5.88% in average for all of the tested varieties of vine grapes harvested were observed. In other study, conducted by Zemánek & Burg (2005), was used the same trailed vine grape harvester ERO LS Traction aggregated with tractor Zetor 7311 and effective field capacity was reported at value  $0.24 \text{ ha h}^{-1}$  (harvested variety was Lemberger). It represents about 29.4% lower values in comparison with our results. These differences may be caused by lower manoeuvrability of used machine aggregation combined with specifications of harvested variety.

### **Harvest Losses**

In the frame of research activities there were observed total losses of harvested vine grapes caused by vine grape harvester and was divided into two groups. Firstly to the losses due to slumping through the harvest mechanism on the ground and observed at level of 3% from the total harvested volume. Secondly, to the losses due to omissions of harvest where grapes were left on grape plants and observed at level 7%. From the results it is possible to conclude that variety Neronet has a greater ability to stick on the grape plants and has a greater resistance against grape tassels to be harvested by vine grape harvester mechanisms. Therefore, increased attention should be paid to careful settings of harvester mechanisms as well as utilization of greater values of oscillation frequencies should be adopted. On the other hand, it can be concluded that the total harvest losses were not exceeded 10%. These values are acceptable for mechanized harvest of vine grapes. The greatest observed harvest losses were observed specifically in variety Neronet (9.72%). In case manual harvest of vine grapes it was hardly to observe any losses while precision of labour workers were in case of experimental measurement naturally increased. Therefore, losses by letting grape tassels on grape plants was at absolute minimum and losses by slumping was neutralized by picking up all grape tassels even in case of their falling on to the ground.

The observed vine grape variety, Ruland Blue is variety designed mainly for high height of support and its yields range from 7 to  $12 \text{ t ha}^{-1}$  (Hubáček & Míša, 1996; Pospíšilová, 2005). In our study, the variety was grown at medium height of support and the average yields were observed as  $6.23 \text{ t ha}^{-1}$  with observed total losses about  $152.64 \text{ kg ha}^{-1}$ . The differences among the individual measurements of losses due to letting of vine grapes on grape plants demonstrated greater deviations. It was mainly caused by the areas where the grapes were clamped on the grape plants. However, harvest mechanism of vine grape harvester does not provide any lowest limits for its settings according to lowest height. Therefore, adjustment of harvest mechanism should be recommended and carefully conducted in case of every single harvested variety.

As another tested variety was used Veltliner Green. The maturation of grapes for this variety is characterized as very late. The variety is designed to be grown at higher and medium height of support and it is characterised by high yields where the value of  $12 \text{ t ha}^{-1}$  of grapes are not unusual (Kraus et al., 2004). These values are easy achievable in high height support. However, in our case it was grown at medium height support

which is suitable for grape plants itself however not so suitable for utilization of mechanized harvest. Therefore, yields of this variety was observed only at  $9.70 \text{ t ha}^{-1}$ , and subsequently, average losses of vine grapes was measured at level  $548.05 \text{ kg ha}^{-1}$ . In comparison with previous variety (Ruland Blue) were the losses increased by 3.2%. According to fact that the harvest itself was performed during the appropriate term the losses were still quite high. In this case it means that those two factors appear to be among most important affecting variables. The first negative factor was the situation of grape tassels on grape plants and its proximity to supporting pillars. An omission in harvest of these vine grapes or its parts was therefore caused by the phenomenon that the oscillation frequencies of harvest mechanism were near the pillars reduced. This reduction was caused by prevention of damage caused to the support system and the harvest mechanism itself. As a second factor which was responsible for increase in losses were slight damage done on support system. Specifically, the upper mast was relaxed by harvest mechanism which is not unusual in case of mechanized harvest however in case of such a good yielding variety it means the great decrease of grape height position. This phenomenon negatively affected the harvest and results by increase of harvest losses due to slumping through harvest mechanism.

Observed variety Neronet is characterised by dense foliage along with earlier term of maturation. It is suitable to be grown in combination with medium and high height of support system however in case of harvest in later maturation the berries are easily dispatched from grape tassels to the ground (Webb et al., 2011). Tested variety in our study has the average yield at level  $7.30 \text{ t ha}^{-1}$  while average harvest losses were observed as  $709.52 \text{ kg ha}^{-1}$ . This variety has showed the greater percentage of harvest losses which was caused by three main factors. As the first negative factor can be mentioned the very late harvest time which was conducted in second half of October due to weather conditions. These conditions do not allow utilization of the earlier dates for the harvest. As the second negative factor has to be mentioned was non-ideal distribution of vine grapes at grape plants and their proximity to support pillars. As the third factor were observed the same phenomenon as in previous harvested variety where the main mast was relaxed. Therefore the weight of single grape tassels caused its easier falling and it's slumping through the harvest mechanism.

The issues and problems connected with quality of harvested products which is caused by mechanized vine grape harvest are linked with mechanical damage caused on berries. It is manifested mainly by the release of the mast. Delay in harvest time period and post harvest treatment, in some cases also by the increased temperatures, is responsible for direct increase of harvest losses by his release of must (Caprara & Pezzi, 2011).

The issue of evaluation of harvest losses in case of vine grapes are also addressed by other researchers. For example, Zemánek & Burg (2003) has reported, in case of grape variety Saint Laurent and utilization of the same trailed vine grape harvester ERO LS – Traction, the total losses at level 7.90%. These losses were divided into two groups. Losses defined as non-harvested was recorded as 2.6% and losses caused by slumping of the grapes through the harvest mechanism into the ground were 5.3%. In case of variety Lemberger the losses by slumping through the harvest mechanism was observed as 3.9% and losses by non-harvesting of grapes was 1.8% which means that total losses were calculated at level 5.7%. As contrast there was used another variant of vine grapes and different machinery, namely self-propelled vine grape harvester NEW HOLLAND

– BRAUD SB 58. In this case the total losses were calculated as 5.5% from which 0.6% were characterised as losses caused by slumping of the grapes through harvester mechanism and the rest (4.9%) as non-harvested losses (Zemánek & Burg, 2003).

Mechanised grape harvest and its effect on harvest losses were addressed also in conditions of Italy by Pezzi & Martelli (2005). The same vine grape harvester was utilised (ERO LS Traction) however aggregated with tractor CNH T5060. As observation variety were used Trebbiano with recorded total harvest losses of 6.4%, which consist of undetected grape (4.2%) and grapes on the ground of 2.2% (Pezzi & Martelli, 2005).

According to some other researchers, mechanized harvest of grapes is firstly dependent on technical parameters of grape harvester and secondly on physiological properties of harvested vine grapes (variety). It was concluded that irregular distribution of single grape tassels on individual grape plants, maturity of grape berries and density of foliage has a greater influence on subsequently recorded harvest losses than the type of supporting system or operating mode of vine grape harvester. It was also reported that supporting pillars has a great effect on quality of harvest along with negative increase in harvest losses (Novák & Burg, 2013).

**Table 6.** Harvest losses, % of production, cv. Ruland Blue, Neronet and Veltliner Green (*mean ± SE*)

Cultivar (Variety)	Undetached Grape	Grapes on the ground	Total
Ruland Blue	0.48 ± 0.05	1.97 ± 0.11	2.45 ± 0.15
Neronet	6.74 ± 0.87	2.98 ± 0.10	9.72 ± 0.97
Veltliner Green	3.68 ± 0.93	1.97 ± 0.15	5.65 ± 0.96

Moreover, analysis of the results in our study shows the dependence of harvest losses on tested variety of vine grape. It was observed that the highest values of harvest losses was recorded for variety Neronet with decreasing trend in case of Ruland Blue and Veltliner Green (Table 6).

### Harvesting costs

In calculation of cost units was total utilization of machinery assembly (tractor and vine grape harvester) set at level 300 ha where the utilization of those machinery in other type of use, e.g. providing services, was not considered. Total annual costs on whole vineyards area (100 ha) by employment of mechanized harvest was calculated as 7,611.11 €, therefore total cost per hour are 25.37 € h<sup>-1</sup>. In evaluation of harvest costs defined for different varieties the value of harvest costs were significantly higher. In case of Ruland Blue variety it was 315.27 € h<sup>-1</sup>, 246.32 € h<sup>-1</sup> and 65.67 € h<sup>-1</sup> in case of Neronet and Veltliner Green, respectively. From the results is can be concluded that by increasing of the area treated by mechanized vine grape harvest is able to decrease the costs on harvest per hour unit even to level where the costs meets the basic values for utilization of harvest provided in form of services. (ca 200 € h<sup>-1</sup>). Given that, in conditions of Slovakia, there are not so many vine grape harvesters available on the market. Moreover the price of those services is not given by any platform or agency. The prices of this kind of services was obtained from the companies which posses vine grapes harvesters in their machinery park and they were calculated as 200 € h<sup>-1</sup> in case of trailed harvester and 400 € ha<sup>-1</sup> in case of self-propelled harvester. Table 7 shows the total annual costs

which are divided onto fixed (costs on amortization, equity interest, road tax, insurances and costs for garaging) and variable (cost of repairs of machinery, energy and manual labour) costs. All of the cost units were showed for single varieties as well as for total area of vineyards where the mechanized harvest were employed (Table 8). Costs on amortization were considered as zero due to utilization of machinery with a higher age and road tax on this type of machinery is not given in case of Slovakia. A great share of costs was then represented by costs on equity interests from the total fixed costs (up to 95%). In evaluation of variable costs the greatest share was formed by costs on fuel (diesel) in dependence on grape varieties and lower vineyard areas however in case of considering the whole vineyard area treated by mechanized vine grape harvest (100 ha) it was formed by costs on machinery repairs (Table 7).

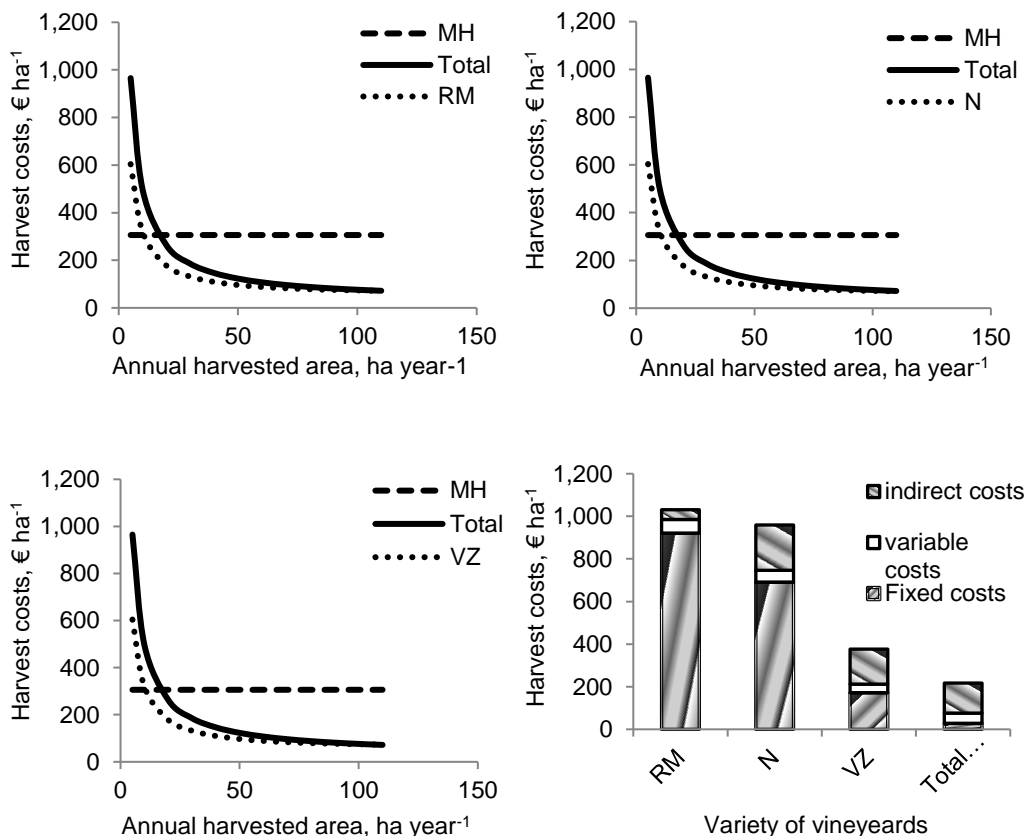
**Table 7.** Total (fixed and variable) annual costs on mechanized harvest of vine grapes

Items costs	Units	Vineyards, varieties			Total
		Ruland Blue	Neronet	Veltliner Green	
Area	ha	3	4	16.10	100
Costs of amortization, $rN_a$	€ year <sup>-1</sup>	0.00	0.00	0.00	0.00
Costs of interest on capital, $rN_z$	€ year <sup>-1</sup>	2,625.00	2,625.00	2,625.00	2,625.00
Costs of vehicle tax, $rN_{cd}$	€ year <sup>-1</sup>	0.00	0.00	0.00	0.00
Costs of insurance for damage, $rN_p$	€ year <sup>-1</sup>	55.00	55.00	55.00	55.00
Costs of optional insurance, $rN_{np}$	€ year <sup>-1</sup>	0.00	0.00	0.00	0.00
Costs of garaging, $rN_g$	€ year <sup>-1</sup>	80.62	80.62	80.62	80.62
Total fixed costs, $rN_k$	€ year <sup>-1</sup>	2,760.62	2,760.62	2,760.62	2,760.62
Costs of repairs and maintenance, $rN_o$	€ year <sup>-1</sup>	4.58	6.88	73.81	1,816.84
Costs of fuel, $rN_e$	€ year <sup>-1</sup>	151.14	167.36	363.55	1,723.90
Costs of live labour, $rN_{zp}$	€ year <sup>-1</sup>	39.29	50.80	217.67	1,309.75
Total variable costs, $rN_v$	€ year <sup>-1</sup>	195.01	225.04	655.02	4,850.49
Total annual costs, $rN_{mc}$	€ year <sup>-1</sup>	2,955.63	2,985.66	3,415.65	7,611.11

**Table 8.** Total (fixed and variable) unit costs on mechanized harvest of vine grapes

Items costs	Units	Vineyards, varieties			Total
		Ruland Blue	Neronet	Veltliner Green	
Area	ha	3	4	16.10	100
Costs of amortization, $jN_a$	€ ha <sup>-1</sup>	0.00	0.00	0.00	0.00
Costs of interest on capital, $jN_z$	€ ha <sup>-1</sup>	875.00	656.25	163.04	26.25
Costs of vehicle tax, $jN_{cd}$	€ ha <sup>-1</sup>	0.00	0.00	0.00	0.00
Costs of insurance for damage, $jN_p$	€ ha <sup>-1</sup>	18.33	13.75	3.42	0.55
Costs of optional insurance, $jN_{np}$	€ ha <sup>-1</sup>	0.00	0.00	0.00	0.00
Costs of garaging, $jN_g$	€ ha <sup>-1</sup>	26.87	20.16	5.01	0.81
Total fixed costs, $jN_k$	€ ha <sup>-1</sup>	920.21	690.16	171.47	27.61
Costs of repairs and maintenance, $jN_o$	€ ha <sup>-1</sup>	1.53	1.72	4.58	18.17
Costs of fuel, $jN_e$	€ ha <sup>-1</sup>	50.38	41.84	22.58	17.24
Costs of live labour, $jN_{zp}$	€ ha <sup>-1</sup>	13.10	12.70	13.52	13.10
Total variable costs, $jN_v$	€ ha <sup>-1</sup>	65.00	56.26	40.68	48.50
Total annual costs, $jN_{mc}$	€ ha <sup>-1</sup>	985.21	746.42	212.15	76.11

Graphical evaluation of the results of total unit costs on mechanized harvest in relationship with different levels of annual utilization of machinery shows the decreasing trend (Fig. 1). These relations present the results of individual varieties of grapes (Ruland Blue, Neronet and Veltliner Green), for all vineyards which was treated by mechanized harvest in contrast with costs in case of utilization of manual harvest. As another indicator of economic benefits were selected definition of the turning points where the mechanized harvest becomes cheaper than manual harvest from the point of costs view. It was calculated at 16.91 ha year<sup>-1</sup> in average for total area of vineyards 100 ha.



**Figure 1.** Harvesting costs with harvested area, individual varieties and all vineyards (MH – manual harvesting; Total – all vineyards, mechanized harvest; RM – Ruland Blue; N – Neronet; VZ – Veltliner Green).

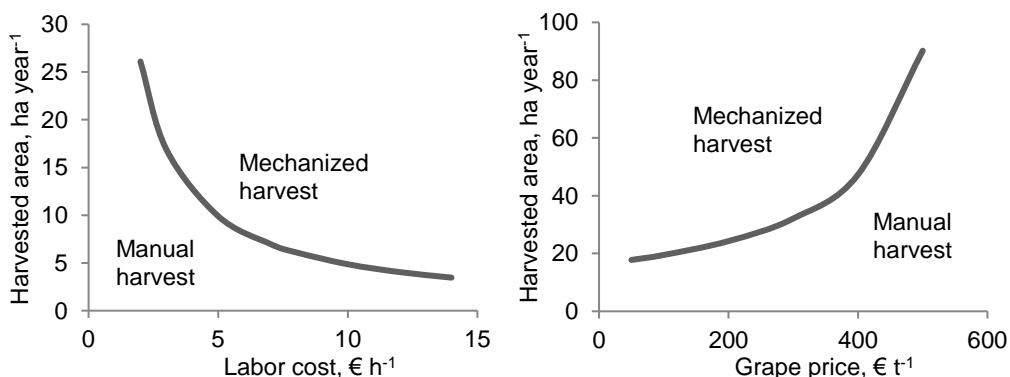
Indirect costs, caused by the measured total losses on yields should be then imputed into direct costs. In determination of indirect costs were considered average purchase price of vine grapes (market value) in time of harvest 0.30 € kg<sup>-1</sup> with VAT. In our study, the total harvest losses were observed at maximum of 9.72% (in case of Neronet variety). Economical evaluation then reveal that the relevant results with regards on total losses from yields are at level 45.79 € ha<sup>-1</sup> (Ruland Blue variety), 212.86 € ha<sup>-1</sup> (Neronet variety) and 164.42 € ha<sup>-1</sup> (Veltliner Green variety). Graphical evaluation of the results of indirect costs divided onto fixed and variable costs and showed at Fig. 1. The direct

costs of mechanized vine grape harvest on the total area of vineyards of 100 ha were characterised by the higher effect of indirect costs (in average) in comparison with direct costs about 15.24%, specifically.

In evaluation of total costs on vine grape harvest were the results highly dependent on individual country conditions where in many cases there are various changes in input units for calculation of fixed but as well as variable components (e.g. years of depreciation, costs on insurances, road taxes, cost on garaging, costs on machinery repairs, costs of fuel and costs on manual labour). However, the highest important role in calculation of costs are forming by direct costs (price of machinery or machines assembly) and indirect costs which are caused by harvest losses on yields (Demaldè & Spezia, 2006; Tudisca et. al, 2013; Pezzi & Martelli, 2015).

### Break-even analysis

The possibilities of exploitation of mechanized vine grape harvest in contrast with utilization of manual harvest are showed in Fig. 2 (left). In case of increasing of costs on manual labour and by calculation of unit costs on mechanized harvest and manual harvest results is decreasing trend of area of vineyards which needs to be treated. The performance in manual harvest were observed at value  $9.8 \times 10^{-3} \text{ ha h}^{-1}$ , while the average price of manual labour in our conditions can be calculated on level of  $306 \text{ € ha}^{-1}$ . The efficiency of investment into a new machinery (vine grape harvester) are then represented by minimal annual machinery utilization at level of  $16.92 \text{ ha year}^{-1}$ . If the calculation includes also indirect costs on mechanized harvest (in case of grape price  $0.3 \text{ € kg}^{-1}$ ) the minimum area of vineyards which need to be treated by mechanized harvest will be increased at level  $34.5 \text{ ha year}^{-1}$ . According to indicated prizes of mechanized harvest (rent of machinery) and services for purchase of own vine grape harvester with economic benefits it needs to be treated  $27.42 \text{ ha year}^{-1}$  of vineyards. In case of areas (vineyards), which are below those mentioned above the mechanized harvest and its utilization, is more beneficial to be obtained in form of services (Tisseyre et al., 2007).



**Figure 2.** Break–even analysis under different combinations of harvested area and labour cost and grape price (left), break-even areas under different combinations of harvested area and grape price (right).

The evaluation of machinery utilization and exploitation by the changes in grape prizes in relationship with change in direct and indirect unit costs showed the significant differences. By increasing of grape price the minimum area grows exponentially (Fig. 2, right). In case of average vineyard areas (for all varieties) the increase in prize of grapes results in more often utilization of manual harvest where are considered harvest losses by slumping to the ground and non-harvested on grape plants as zero.

The cost-effectiveness of mechanized harvest compared to manual harvest is obtained on small areas (7 to 14 ha) in both situations. Analysis of the cost-effectiveness between mechanized and manual harvest cannot exclude other aspects that are not easily quantifiable. Among the favours of manual harvest is the more intact product, which leads to fewer problems in the successive phases of delivery to the winery and winemaking companies. An aspect against manual harvest in the region is a shortage of labour, which causes problems in the organization of field works and delivery of the grapes (Letaief et al., 2008; Pezzi, 2011; Pezzi & Martelli, 2015).

The mechanized harvest can be effectively employed at minimum area of 16.92 ha according to our results. However if indirect costs are included into calculation and considering the prize of grapes (market prize) at level of 300 € t<sup>-1</sup> the minimum area which needs to be treated by mechanized harvest is increased to 34.5 ha year<sup>-1</sup>. By comparison of obtained results which defines the costs on rent of machinery in form of services the minimum treated area which will be needed for purchasing of own machinery will be at minimum of 27.42 ha year<sup>-1</sup>.

## CONCLUSIONS

The aim of the study was to evaluate the quality of mechanized vine grape harvest expressed firstly by total losses caused by harvest itself and secondly by total costs on mechanized harvest involving three varieties of vine grapes. Although, the manual harvest is defined by absolute minimum values of harvest the losses caused by mechanized harvest was observed in average 470.07 kg ha<sup>-1</sup> for the whole treated area of vineyards. Higher losses for variety Neronet was affected by non-ideal distribution of grape tassels with close proximity to support pillars and also by the improper hanging of individual grape stems which can be also affected by lower quality of used hanging material. In economical evaluation of mechanized harvest of vine grapes it was showed that cost-effective way of mechanized harvest is achievable however increased harvest losses has to be considered as it was described in our study. Many companies and individuals in the field of viticulture starting to employ mechanized vine grape harvest however it is usually supported by consideration and utilization of greater areas of vineyards as it was defined in this study (16.92 ha year<sup>-1</sup>). The evaluation and following specification of the turning point revealed that cost-effective utilization of mechanized harvest is achievable in comparison with manual harvest in different levels of costs on manual labour on one side and market prize of vine grapes on second side. As the greatest benefit of mechanized grape harvest is the time period needed for harvest itself but also regarding to decrease of work quality and increase in costs of hourly rate. However this phenomenon's combined leads to decrease of unit costs in both direct and indirect type. From the perspective of Slovakia and overview of commonly utilized machinery in viticulture can be concluded that greatest portion of mechanized harvest is done by trailed types of vine grape harvesters however in case of small vineyard holders (area



from 5–10 ha) it is mostly application of manual harvest. The consideration and decisions for selection of appropriate harvest technology was provided at the basis of proposed hypothesis of various labour costs and prizes of vine grapes. Those were determined as driving factors for economical evaluation. In the study was also pointed out that there is effect of indirect costs, harvest losses and other factors highly affect the affectivity of mechanized grape harvest however those can be decreased by specific changes in machinery settings.

ACKNOWLEDGEMENTS. This work was supported by AgroBioTech Research Centre built in accordance with the project Building ‘AgroBioTech’ Research Centre ITMS 26220220180; and by the Ministry of Education of the Slovak Republic, Project VEGA 1/0155/18.

## REFERENCES

- ASABE. 2005. S495.1. Uniform terminology for agricultural machinery management. St. Joseph, Mich.: ASABE.
- ASABE. 2006. EP496.3. Agricultural machinery management. St. Joseph, Mich.: ASABE.
- ASABE. 2011. D497.7. Agricultural machinery management data. St. Joseph, Mich.: ASABE.
- Bates, T & Morris, J. 2009. Mechanical Cane Pruning and Crop Adjustment Decreases Labor Costs and Maintains Fruit Quality in New York ‘Concord’ Grape Production. *HortTechnology* **19**(2), 247–253.
- Bavaresco, L., Gatti, M., Pezzutto, S., Fregoni, M. & Mattivi, F. 2008. Effect of leaf removal on grape yield, berry composition, and stilbene concentration. *American Journal of Enology and Viticulture* **59**(3), 292–298.
- Caprara, C. & Pezzi, F. 2011. Measuring the stresses transmitted during mechanical grape Harvesting. *Biosystems Engineering* **110**(2), 97–105.
- Clingeffer, P.R. 2013. Mechanization in Australian vineyards. *Acta Horticulturae* **978**, 169–177.
- Corazzina, E. 2010. Patto tra produttori e terzisti per la vendemmia meccanica. *L’Informatore Agrario* **21**, 41–43 (in Italy).
- Demalde, R. & Spezia, G. 2006. Quando conviene acquistare una vendemmiatrice. *L’Informatore Agrario* **22**, 57–60 (in Italy).
- Ďud’ák, J. 2016. *Management of machinery operation*. 1<sup>st</sup> edition. Slovak university of agriculture in Nitra, Nitra, 216 pp. (in Slovak).
- Fic, V. 1973. *Mechanization in Vineyards*. 1<sup>st</sup> edition. Príroda, Bratislava, 190 pp. (in Slovak)
- Hubáček, V. & Miša, D. 1996. *The year of vinarian*. 1<sup>st</sup> edition. Květ, Praha, 55 pp. (in Slovak)
- ISO (1980). ISO 5704: Equipment for vine cultivation and wine making: Grape-harvesting machinery: Test methods. Geneva, Switzerland: International Organization for Standardization.
- Jobbágy, J. & Findura, P. 2013. *Mechanization of viticulture*. 1<sup>st</sup> edition. Slovak university of agriculture in Nitra, Nitra, 165 pp. (in Slovak).
- Johnson, L.F., Roczen, D.E., Youkhana, S.K., Nemani, R.R. & Bosch, D.F. 2003. Mapping vineyard leaf area with multispectral satellite imagery. *Computers and Electronics in Agriculture* **38**(1), 33–44.
- Johann, J.A., Silva, M.C., Uribe-Opazo, M.A. & Dalposso, G.H. 2010. Variabilidade espacial da rentabilidade, perdas ncolheita e produtividade do feijoeiro. *Engenharia Agricola* **30**(4), 700–714.
- Keller, M. 2010. Managing grapevines to optimise fruit development in a challenging environment: A climate change primer for viticulturists. *Australian Journal of Grape and Wine Research* **16**(1), 56–69.

- Kraus, V., Hubáček, V. & Ackermann, P. 2004. *Handle of vinarian*. 1<sup>st</sup> edition. Brázda, Praha, 280 pp. (in Czech).
- Letaief, H., Rolle, L. & Gerbi, V. 2008. Mechanical behavior of winegrapes under compression tests. *American Journal of Enology and Viticulture* **59**(3), 323–329.
- Morris, J.R. 2007. Development and commercialization of a complete vineyard mechanization system. *HortTechnology* **17**(4), 411–420.
- Novák, P. & Burg, P. 2013. Evaluation Of Harvest Losses Within A Full Mechanised Grape Harvest. *Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis* **61**(3), 751–756.
- Pezzi, F. 2011. Solutions for critical points of mechanical harvesting and post-harvesting treatment. *L'Informatore Agrario* **26**, 55–58. (in Italy).
- Pezzi, F. 2013. Ridurre i costi senza penalizzare la qualità del prodotto. *Corriere Vinicolo* **39**, 13–15 (in Italy).
- Pezzi, F. & Balducci, G. 2012. Vendemmia meccanica una scelta possibile. *Corriere Vinicolo* **26**, 19–21 (in Italy).
- Pezzi, F. & Caprara, C. 2009. Mechanical grape harvesting: Investigation of the transmission of vibrations. *Biosystems Engineering* **103**(3), 281–286.
- Pezzi, F. & Martelli, R. 2015. Technical and economic evaluation of mechanical grape harvesting in flat and hill vine yards. *Trans of ASABE* **58**(2), 297–303.
- Pospíšilová, D. 2005. *Ampelography*. 1<sup>st</sup> edition. Research and Breeding station of vinery and vineyards, Modra, 368 pp. (in Slovak).
- Přistavka, M., Křištof, K. & Findura, P. 2017. Reliability monitoring of grain harvester. *Agronomy Research* **15**(3), 817–829.
- Srivastava, A.K., Goering, C.E., Rohrbach, R.P. & Buckmaster, D.R. 2006. *Engineering Principles of Agricultural Machines*. 2<sup>nd</sup> edition. ASABE, St Joseph, Michigan, 268 pp.
- Tisseyre, B., Ojeda, H. & Taylor, J. 2007. New technologies and methodologies for site-specific viticulture. *Journal International des Sciences de la Vigne et du Vin* **41**(2), 63–76.
- Tudisca, S., Trapani, A.M.D., Sgroi, F. & Testa, R. 2013. The cost advantage of Sicilian wine farms. *American Journal of Applied Sciences* **10**(12), 1529–1536.
- UKSUP. 2016. <http://www.uksup.sk/ovv-vinohradnický-register/> Accessed 7.12.2017.
- UNIMA. 2013. Tariffe 2013. Rome, Italy: Unione Nazionale.
- Walz, O. 2007. *Taschenbuch der Weinbautechnik*. 2<sup>nd</sup> edition. Fachverlag Dr. Fraund, Mainz, 198 pp. (in German).
- Webb, L.B., Whetton, P.H. & Barlow, E.W.R. 2011. Observed trends in winegrape maturity in Australia. *Global Change Biology* **17**(8), 2707–2719.
- Zemánek, P. & Burg, P. 2003. Special mechanization: machinery for viticulture. 1<sup>st</sup> edition. Mendel university in Brno, Brno, 93 pp. (in Czech).
- Zemánek, P. & Burg, P. 2005. Evaluation of mechanical harvesting in viticulture. *Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis* **53**(4), 191–194.
- Žufánek, J & Zemánek, P. 1992. Mechanization: harvesting machinery for vegetables, fruits and grapes. 1<sup>st</sup> edition. Mendel university in Brno, Brno, 115 pp. (in Czech).

## **The excretion of Ca, Mg, Zn and Cu via excreta of laying hens fed low phosphorus diets and phytase**

M.A. Kamberi, S. Muji\*, A. Kryeziu\*\*, R. Kastrati and N. Mestani

University of Prishtina, ‘Hasan Prishtina’, Faculty of Agriculture and Veterinary, Department of Biotechnology in Zootechnics, Tahir Zajmi 34, XK10000 Prishtinë, Republic of Kosovo

\*Correspondence: skender.muji@uni-pr.edu

\*\*This author contributed equally to this work

**Abstract.** An 8-week experiment was conducted to study the effect of adding phytase (Natuphos® 5000 BASF) to low and normal available phosphorus diets of laying hens on the excreta content and excretion of Ca, Mg, Zn and Cu. A total of 144 Hisex Brown laying hens that were 22 weeks old at the start of the experiment were randomly assigned to four dietary treatments. Treatments included three replicates (12 hens each) or 36 hens per treatment in total. Four corn-soybean meal-based diets were formulated to contain two levels of available phosphorus (AP; 0.12 and 0.46%) and two phytase levels (0 and 600 FTU kg<sup>-1</sup>). The results showed that there was no significant effect of added phytase on excreta Ca and Mg content ( $P > 0.05$ ), but there was a significant effect of the dietary treatment on the content of Zn ( $P = 0.0075$ ) and Cu ( $P = 0.0002$ ). In terms of the excretion of these minerals, the dietary treatment had no effect on Ca and Zn excretion and a borderline effect ( $P = 0.0522$ ) on Mg excretion measured as the amount of the mineral excreted per egg mass produced is observed. The results however showed a very strong effect of all three factors (available phosphorus, phytase and their interaction) on Cu excretion. The results indicate that adding 600 FTU to the corn-soybean meal laying hen diet with 0.12% or 0.46% AP beneficially affects the content and the excretion of Ca, Mg, Zn and Cu. Therefore, we can conclude that a laying hen diet containing 0.12% available phosphorus and 600 FTU during the first production cycle may not only satisfactorily support hens’ performance but will also beneficially affect the environment.

**Key words:** excretion, manure, minerals, pollution.

### **INTRODUCTION**

From the environmentalists’ point of view, animal production is one of the industries, faced with complaints about the possible negative implications on the environment. In this context, intensive animal production is a potential source of global pollution through waste, liquids, gases and odours. It has been estimated that 250,000 tons of manure P are produced annually, which contributes to water pollution (Kazempour & Jahanian, 2017). Waste products from livestock systems can directly affect water resources and the atmosphere in the whole process of food production (Owen, 1994). Phosphorus is an expensive element in poultry feed; however, it is regularly given to poultry to cover their needs owing to the inefficient use of phytate P

in plant ingredients used to feed them. Phytate P is the major natural storage form of phosphorus in animal feed (cereal grains, legumes, and oilseeds) (Sumengen et al., 2012). There is a danger of pollution of land from the excreta of animals owing to the addition of minerals such as copper and zinc, which are used in animal feed to improve their performance growth (Pallauf & Rimbach, 1997). Minerals are able to rapidly bind to phytic acid and form a phytate–mineral complex that can be resistant to hydrolysis by phytase of animal, vegetable or microbial origin (Maenz et al., 1999). Decreasing phytate levels may improve the bioavailability of various minerals, such as iron, manganese and zinc. Another tool to overcome such problems and also contribute efforts to improve the nutritive worth of feedstuffs (Slominski, 2011) is the use of exogenous enzymes to monogastric diets (Asmare, 2014) among which phytase was proven to additionally benefit the release of many nutrients other than P, which makes possible the reduction of the amount of high value ingredients in the complete feed (Silversides & Hruby, 2011). Common levels of phytase used in laying hen diets vary from were 300 FTU and 3% calcium in hens' diets improves the digestibility of P and significantly reduces the excretion of P. The addition of Ca increases the digestibility and reduces the excretion of calcium and N (Schwarz, 1994; Mc Knight, 1996; Vargas-Rodriguez et al., 2015). These authors pointed out the role of phytase in improving the relative retention time of Ca in male chickens as a consequence of Ca release from Ca-phytate complexes. Similarly, the results of many studies showed that the addition of phytase in diets with different levels of Ca was effective in maintaining the growth parameters in chickens and improving the uptake of macro and micro minerals. They found that the best retention of Ca was observed when low–Ca diet (0.6%) was supplemented with phytase which is well recognized tool to degrade phytate in animal feed (Dersjant-Li et al., 2015). In his research on the impact of the addition of phytase in broiler corn–soybean meal diets, Nelson (1994) showed significant improvement in retention of Zn and Cu. The author assumed that such a high relative Zn retention improvement (62.3%), relative to a phosphorus–deficient diet and without the addition of phytase, may result in higher digestibility of zinc complexes within phytate-mineral complexes. The concentration of Zn in the bones of chickens fed a diet with a high content of Zn can serve as a useful criterion for assessing the bioavailability of zinc from inorganic sources (Sandoval et al., 1997). Aoyagi & Baker (1995) reported that, despite expectations, the addition of 600 FTU microbial phytase per kg of feed had no positive effect on the efficiency of Cu uptake from soy or cotton pellets. They explained this as a possible influence of phytase leading to the increased utilisation of zinc, which can have an antagonistic effect on the efficiency of Cu absorption. Swiatkiewicz et al. (2001) ran an experiment to investigate the utilisation of Zn from organic and inorganic sources in chicks from 4 to 28 days of age. They found that the utilisation of Zn from organic sources (Zn complexed with amino acids) was better in the diet without phytase.

Sebastian et al. (1996) reported the ineffectiveness of phytase in improving the retention of Mg, Zn, Fe and Mn, but did not have a suitable explanation for it. They note only that their results correspond with the results of Roberson & Edwards (1994), who found that the addition of phytase had no effect on the retention of Zn in broilers. Liu et al. (2015) mention that standard (500 FTU kg<sup>-1</sup>) and elevated (1,000 FTU kg<sup>-1</sup>) phytase inclusions in diets with reduced nutrient specifications have the capacity to enhance the performance of broilers and compensate for these reductions. Viveros et al. (2002) reported that chickens fed a low phytic phosphorus diet exhibited lower retention of Mg

and Zn by 26.9% and 88.6%, respectively, in the third week and 24.5% and 91.2% in the sixth week of feeding as compared to normal diets. The possible explanation for this is that the wide Ca to P ratio in low-phosphorus diets causes increased pH in the intestine and reduces the digestibility of the mineral fractions. Therefore, the addition of phytase increases the retention of Zn and Mg, despite their adequate levels in feed. They believe that the increased retention of Zn may be a result of its higher availability from mineral-phytate complexes.

The use of large quantities of animal excreta as fertiliser for many years may undoubtedly result in high concentrations of nitrogen, phosphorus and potassium in soil and water (Webb & Archer, 1994). Calcium is the most important mineral in the diet of all living organisms. The amount of endogenous calcium excreted via the excreta largely depends on the efficiency of absorption of this mineral. Increasing the Ca content in the diet from 3 to 4% may lead to an increase in the total non-phytic P in hens' faeces. Laying hens fed diets containing 4% Ca and 0.64% P from 50 to 72 weeks of age had 28.4% more P excreted in the form of phytate (Scheideler & Sell, 1987). There are several papers reporting beneficial effects of different levels of phytase in performance of laying hens. Englmaierova et al. (2015) found that supplementation with 350 FTU microbial 3-phytase per kg in a low-P diet not only improved the digestibility of minerals but also led to a change in the microflora of the digestive tract. In a meta-analytical study done by Ahmadi and Rodehutsord (2012) analysing 14 experiments phytase levels from 150–500 FTU per kg were used in diets containing between 0.1–0.45% non-phytate phosphorus levels. Kim et al. (2017) compared the super dosing effect of phytase (10,000; 20,000 and 30,000 FTU) added to negative control diet with 0.26% non-phytate phosphorus (NPP) diet with positive control diet containing 0.38% NPP. They found that 20,000 FTU were effective only in hen-day egg production (%) but no effect was observed in all other productive and egg quality parameters.

Since the addition of microbial phytase to plant derived feed ingredients fed to poultry improved growth performance and the retention of minerals such as P, Ca, Zn and Cu (Singh, 2008), the objective of the experiment reported herein was to specifically focus on the effect of the addition of phytase on excreta content and excretion of Ca, Mg, Zn and Cu from laying hens fed low phosphorus diet.

## **MATERIALS AND METHODS**

### **Animals and diets**

The experiment reported here lasted 8 weeks and covered the first egg production cycle (22–30 weeks). The trial included 144 Hisex Brown laying hens aged 22 weeks at the beginning of the experiment. Prior to experiment, laying hens were prepared during two weeks. During this period, uniform groups in body weight ( $P = 0.6972$ ) and egg production ( $P = 0.7747$ ) were formed. During the pre-experimental period, the hens were fed standard mixtures for this category of laying hens formulated according to NRC (1994). Individual treatments consisted of 36 hens (three replicates with 12 hens). Diets were based on corn and soybean meal, which are raw materials known to contain low levels of available phosphorus.

Two basal diets are formulated (mixtures A and B) to contain all the necessary nutrients in accordance with the recommendations of NRC (1994), except total and available phosphorus (Table 1). Two main ingredients used to formulate diets were corn and soybean meal which are known to contain not only small amounts of phosphorus, but also in the form of phytate. Kiarie et al. (2015) concluded that supplemental phytase was effective in improving the nutritional value of corn- and soy-based diet formulated to be suboptimal in terms of available Ca and P. therefore, in a diet A, no Dicalcium phosphate was used to ensure low phosphorus content. The whole amount of feed produced is divided into two parts. First half was used as treatment 1, while second part of the same mixture (treatment 2) was afterward supplemented with 12 grams of phytase per 100 kg feed similar to that used by Silversides & Hruby (2009) to ensure 600 FTU kg<sup>-1</sup>. Applying the same procedure, the Diet B was used to prepare treatments 3 and 4. Therefore, four dietary treatments with two levels of Available phosphorus (0.12% and 0.46%) and two levels of Natuphos 5000 phytase (0 and 600 FTU<sup>1</sup> kg<sup>-1</sup>) were used in this experiment. The enzyme used in this experiment was a product of BASF Corporation–Canada with a guaranteed activity of 5,000 FTU kg<sup>-1</sup> and was derived from the fermentation of *Aspergillus niger*. Analysis of Ca, Mg, Zn and Cu in the feed and faeces was done using atomic absorption spectroscopy (Blanusa & Breski, 1981).

**Table 1.** The composition and nutritional value of experimental diets

	Diet A (%)		Diet B (%)	
	TREATMENTS			
Raw materials	0.12% AP no Phytase	0.12% AP + 600 FTU <sup>2</sup>	0.46% AP no Phytase	0.46% AP + 600 FTU
Ground yellow Corn	61.53	61.53	59.25	59.25
Soybean meal, 44% SP	26.64	26.64	27.08	27.08
Sunflower oil	2.00	2.00	3.00	3.00
Limestone	8.45	8.45	7.37	7.37
Dicalcium phosphate	0.00	0.00	1.92	1.92
Kitchen salt	0.30	0.30	0.3	0.3
Mineral Vitamin premix	1.00	1.00	1.00	1.00
DL–Methionine	0.08	0.08	0.09	0.09
Natuphos <sup>R</sup> Phytase	–	0.012	–	0.012
<b>TOTAL</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>
Calculated nutrient content of diets				
ME; MJ kg <sup>-1</sup>	12.17		12.25	
Crude protein, %	17.00		17.00	
Ca, %	3.30		3.30	
Total P, %	0.35		0.70	
Available P, %	0.12		0.46	
Lysine, %	0.88		0.88	
Met + Cys, %	0.64		0.64	
Mg, %	0.12		0.12	
Zn, mg	85.21		85.13	
Cu, mg	15.31		15.36	

<sup>1</sup> FTU stands for Phytase Unit and is defined as the amount of enzyme needed to release one micromole of inorganic phosphorus per minute from an excess of sodium phytate at 37 °C (98.6°F) at a pH of 5.5.

## Statistical methods

For statistical processing of the data, the GLM procedure of SAS (1985) was used. One way Analysis of Variance was used to find whether a significant probability value ( $P < 0.05$ ) exists. Duncan's New Multiple-Range Test was used as a post hoc test to compare individual means and determine to what extent they differ.

## RESULTS AND DISCUSSION

There are many confirmatory studies on the efficacy of phytase enzyme for maintaining optimum economical poultry production and laying hen performance (Shet et al., 2017), egg quality (Kim et al., 2017), nitrogen and phosphorus excretion (Kamberi et al., 2017), suggesting that this enzyme may be considered and eco-friendly feed additive to enhance the nutritive quality of phytate (Park et al. 2012). However, publications on the effect of phytase used with low phosphorus diets on Ca, Mg, Cu and Zn content and their excretion as the amount per egg mass produced are scarce.

### Content and Total Calcium Excretion

Results of egg mass produced and the content of minerals in excreta of minerals included in this study are presented in Table 2.

**Table 2.** Results of egg mass produced and mineral content, % (Mean  $\pm$  SEM<sup>1</sup>)

Treatments	Egg mass, kg per hen	Mineral content in dry faeces			
		Ca, %	Mg, %	Zn, mg kg <sup>-1</sup>	Cu, mg kg <sup>-1</sup>
0.12 AP/0FTU <sup>3</sup>	1.84 $\pm$ 0.169 <sup>b</sup>	7.90 $\pm$ 0.31	2.54 $\pm$ 0.15	387.92 $\pm$ 15.92 <sup>a</sup>	38.11 $\pm$ 0.74 <sup>a</sup>
0.12 AP/600FTU	2.84 $\pm$ 0.057 <sup>a</sup>	7.12 $\pm$ 0.26	2.06 $\pm$ 0.07	338.54 $\pm$ 8.90 <sup>b</sup>	28.64 $\pm$ 1.31 <sup>b</sup>
0.46 AP/0FTU	2.89 $\pm$ 0.064 <sup>a</sup>	7.58 $\pm$ 0.85	2.36 $\pm$ 0.17	402.08 $\pm$ 8.33 <sup>a</sup>	37.70 $\pm$ 0.78 <sup>a</sup>
0.46 AP/600FTU	2.67 $\pm$ 0.098 <sup>a</sup>	7.36 $\pm$ 0.26	2.08 $\pm$ 0.09	353.54 $\pm$ 3.61 <sup>b</sup>	29.19 $\pm$ 1.28 <sup>b</sup>
<i>Pr &gt; F</i>	0.0003	0.7128	0.0699	0.0075	0.0002
Main affect					
Available Phosphorus, (%)					
0.12	2.34 $\pm$ 0.238 <sup>b</sup>	7.51 $\pm$ 0.25	2.30 $\pm$ 0.13	363.23 $\pm$ 13.72	33.37 $\pm$ 2.22
0.46	2.88 $\pm$ 0.053 <sup>a</sup>	7.47 $\pm$ 0.41	2.22 $\pm$ 0.11	377.81 $\pm$ 11.59	33.45 $\pm$ 2.02
Added phytase (FTU kg <sup>-1</sup> )					
0	2.37 $\pm$ 0.249 <sup>b</sup>	7.74 $\pm$ 0.41	2.45 $\pm$ 0.11 <sup>a</sup>	395.00 $\pm$ 8.63 <sup>a</sup>	37.90 $\pm$ 0.49 <sup>a</sup>
600	2.85 $\pm$ 0.051 <sup>a</sup>	7.24 $\pm$ 0.17	2.07 $\pm$ 0.05 <sup>b</sup>	346.04 $\pm$ 5.45 <sup>b</sup>	28.91 $\pm$ 0.83 <sup>b</sup>
Analysis of variance					
	<i>Pr &gt; F</i>	<i>Pr &gt; F</i>	<i>Pr &gt; F</i>	<i>Pr &gt; F</i>	<i>Pr &gt; F</i>
AP	0.0010	0.9418	0.5371	0.1899	0.9462
Phytase	0.0019	0.3347	0.0155	0.0013	0.0001
AP and Phytase	0.0013	0.4601	0.5735	0.9684	0.0699

Values within the same column with different superscript differ significantly ( $P < 0.05$ ); <sup>1</sup>SEM – Standard Error of Mean; <sup>2</sup>AP – Available Phosphorus; <sup>3</sup>FTU Phytase Unit.

As shown in Table 2, hens fed low available phosphorus with the addition of phytase (treatment 2) managed to maintain comparable egg production with hens fed normal AP.

The average values of calcium in dry excreta of laying hens fed the different treatments did not show statistically significant differences between treatments with different levels of AP, so it can be concluded that phytase did not influence the content of this element in dry hen excreta. However, considering the results of the total amount of excreted calcium (g) in relation to the egg mass (kg) produced, it can be clearly seen that the use of phytase had an impact in reducing the amount of excreted calcium.

Experimental treatments with different levels of available phosphorus and phytase had a statistically significant effect on the total calcium excretion via faeces only in the last stage of the experiment when the hens fed with the low-AP treatment excreted the largest amounts of calcium per kg of egg mass produced (57.19 g).

Hens fed treatment two (low AP + phytase) excreted 21.99 g of Ca for each kg of eggs while hens receiving treatments 3 and 4 excreted about the same amount of calcium (18.99 and 18.75 g kg<sup>-1</sup> of eggs). The average values for excretion of this element found during this experiment suggest that the minimum amount excreted was seen in hens receiving treatment 2 (35.87 g kg<sup>-1</sup> of egg mass) compared to all other treatments (57.32, 36.05 and 40.27 g kg<sup>-1</sup> of egg mass for treatments 1, 3 and 4, respectively).

Studies of Chung et al. (2012) showed that apparent retention of Ca was improved when dietary phytase was supplemented to low-phosphorus maize-soyabean meal diet for broiler. However Rabie et al. (2015) found no effect of either non-phytate level or phytase on Ca retention in Dokki-4 Laying Hens fed diets containing 0.4%, 0.3%, and 0.2% NPP without or with the addition of 0.05% microbial phytase.

The content of Ca in the excreta of laying hens increases linearly with the increase of dietary Ca (Pelicia et al., 2009).

According to Lei & Stahl (2000), animals require less Ca when phytase is added as a result of increased Ca retention, which can also lead to increased phosphorus utilisation as a direct effect of increased Ca utilisation. It should be noted that, according to Parr (1996) in experiments with laying hens, the use of phytase requires the adjustment of the Ca amount owing to its higher utilisation after phytate degradation. This adjustment is equivalent to the reduction of 0.3 g of Ca for layers that consume 100 grams of feed per day.

### **Content and Total Excretion of Magnesium through Excreta**

After just 14 days of feeding hens different available phosphorus and phytase diets, an impact on the content of magnesium in dry excreta was observed, which was also seen in the third phase. The highest content of magnesium in the excreta (3.08%) was found in hens fed treatments 3 and 1 (2.97%). These treatments contained different amounts of AP but no phytase was added. The excreta of laying hens fed treatment 4 contained 2.55% Mg, while hens fed treatment 2 had the lowest Mg content of 1.91% in the excreta. Despite differences in the excreta of Mg content between treatments during this experiment (seen as main effect), there was no significant effect of different treatments on Mg content in the excreta of hens fed different AP level but there was a significant effect of phytase seen as the means effect.

The results of this research show that there is no significant effect of various levels of available phosphorus on the content of Mg in dry excreta. However, hens fed a diet deficient in AP after phytase addition excreted, on average, significantly less magnesium in relation to the egg output. The amount of excreted magnesium was the highest from hens fed treatment 1 during all phases of the experiment. For the other treatments, except



for the lower values, the content of magnesium in the excreta tended to be lower as the experiment progressed. The levels of excreted Mg from treatments 2, 3 and 4 were significantly lower in the third and fourth stage. Magnesium excretion from the laying hens fed treatment 1 was 16.77 g and 19.37 g kg<sup>-1</sup> egg mass produced during the third and fourth stage, respectively. Although hens that received treatment 2 were offered the same dietary AP level, they excreted significantly less Mg: 10.42 vs. 18.30 g kg<sup>-1</sup> egg mass compared with those that received treatment 1. This can only be attributed to the addition of phytase. There was no difference in Mg excretion between treatments 3 and 4 with sufficient AP levels, despite the addition of phytase.

No recent publication on the effect of phytase on Mg excretion was found, however, in a study done by Rabie et al. (2015), who investigated the effects of different NPP levels and phytase, no effect of dietary level of phosphorus or the addition of phytase was observed in terms of Mg retention. This may suggest no effect on excretion also.

### **Content and Excretion of Zinc through Excreta**

In this study, the content of zinc in the excreta (%) was analysed and the total excretion of this mineral, expressed in mg per kg of egg mass, is calculated. The results of this study indicate that there was no significant difference in the content of zinc in dry excreta regardless of different dietary treatments. However, the Zn content was observed to decrease with the duration of the experiment. However, there were differences in the average values during the whole experiment, particularly in the excreta of treatments with the addition of phytase (treatments 2 and 4). Phytase addition resulted in less zinc in the dry faeces compared with the treatments without phytase (treatments 1 and 3). The effects of Available phosphorus and phytase were significant only in the fourth phase of the experiment where hens given 0.12% AP excreted significantly less Zn (295.83 mg kg<sup>-1</sup> egg mass) compared with treatment with sufficient AP where excretion of 362.50 mg kg<sup>-1</sup> of egg mass was observed. The amount of excreted zinc was about 22% lower.

From the data, it can be seen that the highest amount of excreted Zn in milligrams per kilogram of produced eggs was obtained with treatment 1 during all phases of the experiment. Laying hens fed treatment 2 (low in available phosphorus with the addition of phytase) excreted less Zn over the entire experiment (32.7%, 6.5% and 4.88%) compared with treatments 1, 3 and 4, respectively.

It is already proven that dietary supplementation of Zn (especially in organic form) increases its bioavailability resulting in lower excretion (Yenice et al., 2015). Our results are generally consistent with the results of other authors stating that the addition of phytase in diets with low available P, increases its utilisation in swine and poultry owing to the high concentration of phytic acid. Lei & Stahl (2000) and Pallauf et al. (1994) found that the addition of phytase in diets based on wheat, peas and barley (rich in phytic acid) improved utilisation of phytate phosphorus and also increased the utilisation of Zn and Mg in piglets. Through in vitro experiments, Pallauf & Rimbach (1997) concluded that adding phytase at 500 FTU kg<sup>-1</sup> of diet led to an increase in utilisation of Zn by 31% compared to the control group (5%). Zacharias et al. (2002) stated that the liberation of zinc from the phytate complex may have an antagonist effect on copper utilisation though Ao et al. (2009) found that the antagonism between Zn and Cu occurred only if inorganic forms of these 2 minerals were included in a chick diet. Research done by Mohanna & Nys (1999) also showed that the addition of phytase in food reduced the

antagonistic effects of phytate on zinc bioavailability. Adding 800 FTU microbial phytase per kg of feed in a corn–soybean meal–based diet for broilers allow a limited reduction of the content of Zn in the diet (14 mg kg<sup>-1</sup>).

**Table 3.** The effect of AP and phytase levels on excretion, (Mean ± SEM<sup>1</sup>)

Treatments AP <sup>2</sup> and Phytase	Mineral excretion, g kg <sup>-1</sup> egg mass			
	Ca, g	Mg, g	Zn, mg	Cu, mg
0.12 AP/0FTU	57.32 ± 11.29	18.30 ± 2.93 <sup>a</sup>	271.88 ± 36.64	45.99 ± 4.73 <sup>a</sup>
0.12 AP/600FTU	35.87 ± 2.96	10.42 ± 0.15 <sup>b</sup>	182.87 ± 13.83	19.79 ± 1.82 <sup>b</sup>
0.46 AP/0FTU	36.05 ± 5.97	11.70 ± 0.68 <sup>b</sup>	195.48 ± 12.26	25.74 ± 0.72 <sup>b</sup>
0.46 AP/600FTU	40.27 ± 3.58	11.70 ± 1.92 <sup>b</sup>	192.25 ± 18.42	21.84 ± 1.27 <sup>b</sup>
Pr > F	0.1611	0.0522	0.0760	0.0004
<b>Main affect</b>				
Available Phosphorus, (%)				
0.12	46.59 ± 7.09	14.36 ± 2.20	227.38 ± 26.51	32.89 ± 6.28 <sup>a</sup>
0.46	38.16 ± 3.25	11.70 ± 0.91	193.86 ± 9.92	23.79 ± 1.09 <sup>b</sup>
Added phytase, (FTU kg <sup>-1</sup> )				
0	46.68 ± 7.43	15.00 ± 1.99 <sup>a</sup>	233.68 ± 24.30	35.86 ± 5.01 <sup>a</sup>
600	38.07 ± 2.30	11.06 ± 0.91 <sup>b</sup>	187.56 ± 10.51	20.812 ± 1.09 <sup>b</sup>
<b>Analysis of variance</b>				
	<i>Pr &gt; F</i>	<i>Pr &gt; F</i>	<i>Pr &gt; F</i>	<i>Pr &gt; F</i>
AP	0.2497	0.1748	0.1745	0.0087
Phytase	0.2404	0.0581	0.0744	0.0005
AP x Phytase	0.0956	0.0584	0.0929	0.0029

Values within the same column with different superscript differ significantly (P < 0.05); <sup>1</sup>SEM – Standard Error of Mean; <sup>2</sup>AP – Available Phosphorus, <sup>3</sup>FTU – Phytase Unit.

### Content and Excretion of Copper through Excreta

The results show that lower copper content was found in the excreta of hens at all stages of the experiment when diets were supplemented with 600 FTU. The highest concentration of Cu in dry excreta was found in hens of treatment 1 (fed low AP) and treatment 3 (normal AP) were 38.11 mg Cu kg<sup>-1</sup> and 37.70 mg Cu kg<sup>-1</sup> of dry excreta is observed. This content is significantly different with treatments where phytase is added indicating no effect of AP level. The addition of phytase significantly decreased Cu content of dry excreta although the dietary Cu levels were the same for all treatments. In a study with broilers, Skrivan et al. (2006) found that Cu concentration of the excreta is strongly related with its content in a diet and will linearly increase with dietary Cu supplementation. In a diets containing dietary Cu concentration in a range from 9.2 to 243.7 mg Cu kg<sup>-1</sup> DM, they observed an increase of Cu in excreta from 25.3 to 396.8 mg kg<sup>-1</sup> DM.

Copper excretion (mg kg<sup>-1</sup> egg mass) in the excreta of hens fed diets deficient in AP with added phytase (treatment 2) was significantly lower (24.85% on average) than for hens receiving treatment with the same content of phosphorus without phytase and 24.03% lower than in treatment with the appropriate amount of phosphorous (treatment 3). In particular, it is quite obvious that the lower phosphorus content resulted in higher excretion of Cu after calculating the egg mass produced. Differences in the amount of excreted copper started to be statistically significant after the third stage of

the experiment (27.64, 10.11, 16.48 and 9.35 mg kg<sup>-1</sup> egg mass) and also continued during the fourth phase (108.17, 30.83, 44.34 and 35.87 mg kg<sup>-1</sup>egg mass) for respective treatments. There was also a significant difference in the average values of Cu excretion (45.99, 19.79, 25.74 and 21.84 mg kg<sup>-1</sup>egg mass) for treatments 1, 2, 3 and 4, respectively. Seen as the main effect, phytase is found to significantly ( $P = 0.0005$ ) affect the amount of copper excreted. There is significant effect of available phosphorus, phytase and AP  $\times$  phytase interaction ( $P > 0.05$ ). Hens fed low phosphorus diet and those without the addition of phytase excreted more Cu (9.1 and 15.05 mg kg<sup>-1</sup>egg mass respectively). Chung et al. (2013) found better retention of Cu from low phosphorus diets of broilers after phytase supplementation. The rate of mineral excretion is related to the level and the form of trace mineral supplementation (Yenice et al., 2015). Same authors reported lower Cu excretion particularly if the mineral is given in organic form as a result of better bioavailability. However, the amount of excreted Cu was linear with its dietary level.

## CONCLUSIONS

The addition of 600 FTU to a corn–soybean meal laying hen diet containing either 0.12% or 0.46% AP, in general, was beneficial in terms of the content and excretion of Ca, Mg, Zn and Cu. The effect was more pronounced for Cu content and excretion, especially when phytase was added to the low–available phosphorus diet, with a reduction observed for all minerals. Thus, it can be concluded that a laying hen diet containing 0.12% AP and 600 FTU during the first production cycle may not only satisfactorily support hens' performance but will also beneficially affect the environment.

**ACKNOWLEDGEMENTS.** The experiment reported here was carried out at the premises of the Faculty of Agriculture and Veterinary of the University of Pristina and the authors acknowledge that this institution also provided the financial support to cover all running costs of the experiment.

## REFERENCES

- Ao, T., Pierce, J.L., Power, R., Pescatore, A.J., Cantor, A.H., Dawson, K.A. & Ford, M.J. 2009. Effects of feeding different forms of zinc and copper on the performance and tissue mineral content of chicks. *Poult. Sci.* **88**(10), 2171–2175.
- Aoyagi, S. & Baker, D.H. 1995. Effect of microbial phytase and 1, 25–dihydroxycholecalciferol on dietary copper utilization in chicks. *Poult. Sci.* **74**, 121–126.
- Asmare, B. 2014. Effect of common feed enzymes on nutrient utilization of monogastric animals. *Int. J. Biotechnol. Mol. Biol. Res.* **5**, 27–34.
- Blanuša, M. & Breški, D.J. 1981. Comparison of dry and wet ashing procedures for cadmium and iron determination in biological material by atomic-absorption spectrophotometers, *Talanta*, **28**(9), 681–684.
- Chung, T.K., Rutherford, S.M., Thomas, D.V. & Moughan, P.J. 2013. Effect of two microbial phytases on mineral availability and retention and bone mineral density in low-phosphorus diets for broilers. *British Poultry Science.* **54**, 362–373.

- Dersjant-Li, Y., Awati, A., Schulze, H. & Partridge, G. Partridge, 2015. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. *J Sci Food Agric.* **95**(5), 878–896.
- Englmaierova, M., Skrivan, M., Skrivanova, E., Bubancova, I., Cermák, L. & Vlcková, J. 2015. Effects of a low-phosphorus diet and exogenous phytase on performance, egg quality, and bacterial colonization and digestibility of minerals in the digestive tract of laying hens. *Czech J. Anim. Sci.* **60**(12), 542–549.
- Kamberi, M., Muji, S., Kryeziu, A., Kastrati, R. & Mestani, N. 2017. Ecological implications of phytase added to normal and deficient available phosphorus diets of laying hens measured by N and P excretion. *Bulg. J. Agric. Sci.* **23**, 632–638.
- Kazempour, F. & Jahanian, R. 2017. Effects of different organic acids on performance, ileal microflora, and phosphorus utilization in laying hens fed diet deficient in non-phytate phosphorus. *Anim. Feed Sci. Technol.* **223**, 110–118.
- Kiarie, E., Woyengo, T. & Nyachoti, C.M. 2015. Efficacy of new 6-phytase from *buttiauxella* spp. on growth performance and nutrient retention in broiler chickens fed corn soybean meal-based diets. *Asian Australas. J. Anim. Sci.* **28**, 1479–1487.
- Kim, J.H., Pitargue, F.M., Jung, H., Han, G.P., Choi, H.S. & Kil, D.Y. 2017. Effect of superdosing phytase on productive performance and egg quality in laying hens. *Asian-Australas J Anim Sci.* **30**, 994–998.
- Lei, X. G. & Stahl, C.H. 2000. Nutritional benefits of phytase and dietary determinants of its efficacy. *Journal Appl. Animal Res.* **17**(1), 97–12.
- Liu, S.Y., Bold, R.M., Plumstead, P.W. & Selle, P.H. 2015. Effects of 500 and 1000 FTU/kg phytase supplementation of maize-based diets with two tiers of nutrient specifications on performance of broiler chickens. *Anim. Feed Sci. Technol.* **207**, 159–167.
- Maenz, D.D., Carmen. M., Schan, E., Newrik, W.R. & Classen, H.L. 1999. The effect of minerals and mineral chelators on the formation of phytase-resistant and phytase-susceptible forms of phytic acid in solution and in a slurry of canola meal. *Anim. Feed Sci. Technol.* **81**, 177–192.
- Mc Knight, W.F., 1996. Phytase technical specifications and properties. *BASF Technical Symposium*, Ithaca New York, 1–13.
- Mohanna, C. & Nys, Y. 1999. Changes in zinc and manganese availability in broiler chicks induced by vegetal and microbial phytase. *Anim. Feed Sci. Technol.* **77**, 241–253.
- National Research Council (NRC). 1994. Nutrient Requirements of Poultry, 9th Edition. National Academy Press, Washington DC.
- Nelson, E.W. 1994. Phytase: A new enzyme in poultry nutrition and management. *BASF technical symposium* page, 48–61.
- Owen, J.B. 1994. Pollution in Livestock production system – An overview. In I. Ap Dewi ... [et al.]. *Pollution in Livestock Production Systems*, Wallingford, CAB International, 1–15.
- Pallauf, J. & Rimbach, G. 1997. Nutritional significance of phytic acid and phytase. *Arch. Animal Nutrition.* **50**, 301–319.
- Pallauf, J., Rimbach, G., Pippig, S., Schindler, B., Hohler, D. & Most, E. Most. 1994. Dietary effect of phytogenic phytase and an addition of microbial phytase to a diet based on field beans, wheat, peas and barley on the utilization of phosphorus, calcium, magnesium, zinc and protein in piglets. *Z. Ernährungswiss* **33**, 128–135.
- Park, I., Leea, J. & Cho, J. 2012. Degradation of phytate pentamagnesium salt by bacillus sp. T4 phytase as a potential eco-friendly feed additive. *Asian-Aust. J. Anim. Sci.* **25**, 1466–1472.
- Parr, J. 1996. Practical feed formulation and return on investment with natyphos phytase for layer. *BASF Canada* pp. 589–595.
- Pelicia, K., Garcia, E., Móri, C., Faitarone, A.B.G., Silva, A.P., Molino, A.B., Vercese, F. & Berto, D.A. 2009. Calcium levels and limestone particle size in the diet of commercial layers at the end of the first production cycle. *Braz J Poultry Sci.* **11**(2), 87–94.

- Rabie, M.H., Ismail, F.S.A., Hassan, R.A. & Abo Ahmed, E.A.H. 2015. Performance, egg quality and mineral utilization of dokki-4 laying hens fed diets containing three levels of nonphytate phosphorus in absence or presence of microbial phytase, citric acid or both. *Asian J. Anim. Vet. Adv.* **10**, 918–929.
- Roberson, K.D. & Edwards, H.M. 1994. Effects of 1.25 dihydroxycholecalciferol and phytase on zinc utilization in broiler chicks. *Poult.ry Sci.* **73**, 1312–1326.
- Sandoval, M., Henry, P.R., Ammerman, C.B., Miles, R.D. & Littell, R.C., 1997. Relative bioavailability of supplemental inorganic zinc for chicks. *J. Anim. Sci.* **75**, 3195–3205.
- SAS, Institute. 1985. SAS user guide: statistics. Version 5 edition. SAS Institute, Inc., Cary NC.
- Scheideler, S.E. & Sell, J.L. 1987. Utilization of phytate phosphorous on laying hens as influenced by dietary phosphorous and calcium. *Nutr. Reports Int.* **35**(5), 1073–1081.
- Schwarz, G. 1994. Phytase supplementation and waste management. BASF Technical symposium Arkansas, pp. 21–44.
- Sebastian, S., Touchburn, S.P., Chavez, E.R. & Lague, P.C. 1996. The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper and zinc in broiler chickens fed corn-soybean diets. *Poult.ry sci.* **75**(6), 729–736.
- Shet, D., Ghosh, J., Ajith, S. Awachat Arumbackam, V.B. & Elangovan, V. 2017. Efficacy of dietary phytase supplementation on laying performance and expression of osteopontin and calbindin genes in eggshell gland. *Animal Nutrition*. (In Press, Corrected Proof) <https://doi.org/10.1016/j.aninu.2017.10.004>
- Silversides, F.G. & Hruby, M. 2009. Feed formulation using phytase in laying hen diets. *J. Appl. Poult. Res.* **18**, 15–22.
- Singh, P.K. 2008. Significance of phytic acid and supplemental phytase in chicken nutrition: a review. *World's Poultry Science Journal* **64**, 553–580.
- Skriwan, M., Skriwanová, V. & Marounek, M. 2006. Effect of various copper supplements to feed of laying hens on cu content in eggs, liver, excreta, soil, and herbage. *Arch Environ Contam Toxicol.* **50**, 280–283.
- Slominski, B.A. 2011. Recent advances in research on enzymes for poultry diets. *Poult. Sci.* **90**, 2013–2023.
- Sumengen, M., Dinçer, S. & Kaya, A. 2012. Phytase production from *Lactobacillus brevis*. *Turk J of Biology.* **36**, 533–541.
- Swiatkiewicz, S., Koreleski, J. & Zhong, D.Q. 2001. The bioavailability of zinc from inorganic and organic sources in broiler chickens as affected by addition of phytase. *J. Anim. Feed Sci.* **10**(2), 317–328.
- Vargas-Rodriguez, L.M., Herrera, J.G., Morales, E.J., Arcos-Garcia, J.L., Lopez-Pozos, R. & Ruelas, G. 2015. Effect of citric acid, phytase and calcium in diets of laying hens on productive performance, digestibility and mineral excretion. *Inter. J. of Poultry science* **14**(4), 222–228.
- Viveros, A., Brenes, A. Arija, I. & Centeno, C. 2002. Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chick fed different levels of phosphorus. *Poultry sci.* **81**(8), 1172–1183.
- Webb, J. & Archer, J.R. 1994. Pollution of soils and watercourses by wastes from livestock production systems. In I. Ap Dewi ... [et al.]. CAB international. *Pollution in Livestock Production Systems*, Wallingford, CAB International, pp. 189–203.
- Yenice, E., Mızrak, C., Gültekin, M., Atik, Z. & Tunca, M. 2015. Effects of organic and inorganic forms of manganese, zinc, copper, and chromium on bioavailability of these minerals and calcium in late-phase laying hens. *Biol Trace Elem Res.* **167**(2), 300–307.
- Zacharias, B., Ott, H. & Drochner, W. 2002. The influence of phytase and cooper supplementation of barley–soybean meal diets on copper status in growing pigs. *Proc. soc. nutr. physiol.* **11**, pp.117.

## Research on the mineral composition of cultivated and wild blueberries and cranberries

A. Karlsons\*, A. Osvalde, G. Čekstere and J. Pormale

University of Latvia, Institute of Biology, Laboratory of Plant Mineral Nutrition, Miera street 3, Salaspils, LV-2169 Salaspils municipality, Latvia

\*Correspondence: andis.karlsons@lu.lv

**Abstract.** European cranberry (*Vaccinium oxycoccos* L.) and European bilberry (*Vaccinium myrtillus* L.) are among the most popular wild-harvested fruits in Latvia, traditionally used in folk-medicine and food. The commercial cultivation of American cranberry (*Vaccinium macrocarpon* Ait.) and highbush blueberry (*Vaccinium corymbosum* L.) was successfully started during last 20 years. With a berry production increase due to considerable hectareage of plantings and growing consumer interest in health-improving foods cultivated blueberries and cranberries have found a place in a daily intake as an excellent source of phenolic and nutritive compounds, vitamins and minerals. As the chemical composition of *Vaccinium spp.* has an important implication on human health, detailed information on the nutritional content of berries are of special importance. The aim of this study was to compare the contents of twelve biologically essential elements (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B) in berries of four *Vaccinium* species: cultivated and wild blueberries (*Vaccinium corymbosum* and *Vaccinium myrtillus*) and cranberries (*Vaccinium macrocarpon* and *Vaccinium oxycoccus*). Together 136 (leaf and berry) samples were collected from 7 main cranberry and blueberry producing sites and 17 native woodland areas and bogs in Latvia. A comparison of wild and cultivated species showed similar concentrations for the macroelements K, Ca and S in cranberry and N, P in blueberry fruits. While statistically significant differences were found for N, P and Mg in case of cranberries and Ca, K, Mg and S for blueberries. The research revealed statistically significant differences of most micronutrients in cultivated and wild berries. Plant leaf and fruit analysis revealed the organ-specific distribution of mineral elements in all species studied. In most of the cases, leaf analysis supported concentration differences in fruits.

**Key words:** *Vaccinium myrtillus*, *Vaccinium corymbosum*, *Vaccinium oxycoccus*, *Vaccinium macrocarpon*, mineral composition of fruits.

### INTRODUCTION

American cranberry (*Vaccinium macrocarpon* A.) and highbush blueberry (*Vaccinium corymbosum* L.) are perennial flowering plants from the family *Ericaceae* of the genus *Vaccinium*, commercial cranberry and blueberry varieties, in general, are indigenous to eastern and central North America including the eastern territories of Canada (Trehane, 2004). Commercial production of *Vaccinium* species in the United States of America has existed since the latter part of the eighteenth century (Eck, 1990). While European bilberry (*Vaccinium murtillus* L.) and European cranberry (*Vaccinium*

*oxycoccus* L.) is one of the most popular wild-harvested fruits in many North countries, traditionally used as healthy food as well as in folk medicine. Headaches, fever, eye problems, diarrhea and other problems have all apparently been eased or cured by various *vacciniums*. Unfortunately, there are wide fluctuations in yield from year to year depending on several factors. The yield of wild berries are often reduced by insufficient water in summer, winter damage and competition from other plant species (Karlsons & Osvalde, 2017).

Many species of *Vaccinium* have a long history of being used for medical purposes. Recent advances in nutrition science have shown that diet has a potential effect on human health and development, dietary guidance is persistent in recommending greater consumption of fruit and vegetables to promote health (Blumberg et al., 2016; Istek & Gurbuz, 2017). Increased consumption of fruits and vegetables can replace foods high in saturated fats, sugar and salt and thus improve the intake of most micronutrients and dietary fiber (Ekholm et al., 2007). Cranberry and blueberry fruits are rich sources of bioactive compounds, such as phenolics, organic acids, anthocyanins, proanthocyanidins, flavonol glycosides, vitamin C, carbohydrates as well as dietary fiber and minerals (Reed, 2002; Nile & Park, 2014; Liu et al., 2015; Michalska & Lysiak, 2015; Szajdak & Inisheva, 2016).

Bioactive compounds from blueberries and cranberries are widely reported to demonstrate a number of health advantages including: inhibition of development and progression of cancer and cardiovascular diseases, antimicrobial activities and prevention of urinary tract infections, tooth and gum disease, stomach ulcers, obesity, diabetes, aging, reduction of cholesterol and biofilm formation (Dugoua et al., 2008; Mckay & Blumberg, 2008; Hwang et al., 2014; Koupy et al., 2015; Shi et al., 2017; Drózdź et al., 2018).

*Vaccinium* berries are also valued for their fresh taste as well as their potential for being processed. Today, an increasing demand for healthy ingredients by the food industry and changed consumer consciousness provide great opportunities for further progress of cranberry and blueberry production. The cultivation of American cranberries and blueberries in Latvia is comparatively recent – while the first experimental plantations were established in the middle of 1980's, commercial cultivation of these berries started in last 20 years (Osvalde & Karlsons, 2010). In general, about 98% of global production of cranberries and 85% of blueberries comes from the United States of America and Canada alone (FAOSTAT). However, with more than 125 ha of commercial plantings, Latvia is the sixth major cranberry producing country (10 top countries in cranberry production, 2018). In 2018, there was an estimated 280 ha of highbush blueberries planted in Latvia with increasing annual hectareage.

While the content of anthocyanins and other phenolic compounds, as well as the role of different species of *Vacciniums* in health promotion is quite often determined and compared, the concentration of essential mineral elements, which are also the important components of fruits, was rarely reported (Drózdź et al., 2018). It should be noted, that many external factors as growth environment (soil, geographical conditions), cultivation and fertilization practices are widely diverse in different cranberry and blueberry production and wild harvesting countries and could contribute to the mineral composition of fruits.

A survey was carried out to compare the contents of twelve biologically essential elements (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B) in berries and leaves of four *Vaccinium* species: cultivated and wild blueberries and cranberries. Plant tissue (leaf) and berry analysis were used to evaluate the content of analyzed nutrients.

## MATERIALS AND METHODS

Together 136 (leaf and berry) samples of four *Vaccinium* species: cultivated and wild blueberries – Highbush blueberry, (*Vaccinium corymbosum*) and European bilberry, (*Vaccinium myrtillus*) and cranberries (American cranberry, (*Vaccinium macrocarpon*) and European cranberry, (*Vaccinium oxycoccus*) were collected from 7 main cranberry and blueberry producing sites (Gaujienas, Talsu, Krāslavas, Babītes, Beverīnas, Smiltenes and Varakļānu district) and 17 native woodland areas and bogs (Jelgavas, Saldus, Mārupes, Ķekavas, Olaines, Līvānu, Salaspils, Tukuma, Jūrmalas, Jaunpiebalgas, Valkas, Jaunpils district) in Latvia.

Berry and leaf materials were collected at each site as a composite sample from locations representative of the planting or woodland area in berry harvest time (July – August for blueberries, September for cranberries. For each composite sample approximately 500 g of berries and 200 g of leaves were collected.

The leaf and berry material was oven-dried at 60 °C to a constant weight and finely ground using a laboratory mill. Then the samples were dry-ashed in concentrated HNO<sub>3</sub> vapors and re-dissolved in HCl solution (HCl – distilled water mixture 3:100) (Rinkis et al., 1987). Concentrations of 12 biogenous elements (N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo, B) were determined in all berry and leaf samples. The levels of Ca, Mg, Fe, Cu, Zn, and Mn were estimated by atomic absorption spectrophotometer (Perkin Elmer AAnalyst 700, acetylene-air flame) (Page et al., 1982) those of N, P, Mo, B by colorimetry, S by turbidimetry, and K by a flame photometer (Jenway PFP7, air propane-butane flame). Mineral concentrations in plant tissue for macronutrients were expressed as mass percent (%), and for micronutrients as mg kg<sup>-1</sup> of dry weight, while macro- and microelements in berries were expressed as mg 100 g<sup>-1</sup> of fresh weight. The contribution of 100 g of fresh berries to the Recommended Dietary Allowance (RDA) for adults per day was calculated. All chemical analyses were done in the Laboratory of plant mineral nutrition of the Institute of Biology, University of Latvia. The levels of statistical significance were determined with MS Excel 2016. Standard errors (SE) were calculated in order to reflect the mean results of chemical analysis of leaves and berries. T-test ‘Two-Sample Assuming Unequal Variances’ (p < 0.05) was used to compare the mean element concentrations of fruits and leaves of four *Vaccinium* species. To assess differences between the chemical compositions of wild and cultivated cranberry and blueberry leaves and berries, the principal component analysis (PCA) was done using PC-ORD Version 6 (McCune & Mefford, 1999).

## RESULTS AND DISCUSSION

The relationship between food and health becomes increasingly significant as consumers now demand healthy, tasty and natural foods that have been grown in uncontaminated environments. Mean macro- and micronutrient concentrations in berry and leaf samples, as well as concentration range, are shown in Table 1 to Table 3. In



general, our research revealed statistically significant differences for majority analyzed mineral elements between studied species in fruits and leaves.

**Table 1.** Mineral element concentrations in *Vaccinium spp.* leaf samples

Element	<i>V. oxycoccus</i>	<i>V. macrocarpon</i>	<i>V. myrtillus</i>	<i>V. corymbosum</i>
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Macroelements (% dry weight)				
N	0.81 ± 0.048a <sup>1</sup>	0.65 ± 0.017a	1.08 ± 0.051a	1.64 ± 0.064b
P	0.07 ± 0.005a	0.12 ± 0.011b	0.12 ± 0.006a	0.13 ± 0.005a
K	0.35 ± 0.014a	0.54 ± 0.029b	0.77 ± 0.031a	0.43 ± 0.041b
Ca	0.75 ± 0.039a	0.89 ± 0.055a	0.84 ± 0.046a	0.74 ± 0.049a
Mg	0.12 ± 0.01a	0.22 ± 0.01b	0.32 ± 0.029a	0.28 ± 0.016a
S	0.09 ± 0.004a	0.10 ± 0.007a	0.15 ± 0.005a	0.14 ± 0.019a
Microelements (mg kg <sup>-1</sup> dry weight)				
Fe	95.18 ± 21.0a	71.31 ± 7.71b	81.87 ± 6.76a	102.45 ± 4.23b
Mn	1,531.76 ± 158.9a	298.00 ± 71.2b	814.40 ± 146.42a	288.36 ± 26.82b
Zn	36.94 ± 1.7a	26.44 ± 1.2b	18.60 ± 0.96a	9.59 ± 0.19b
Cu	4.88 ± 0.42a	9.06 ± 1.6b	4.97 ± 0.24a	3.97 ± 1.50a
Mo	0.28 ± 0.02a	0.41 ± 0.05b	0.23 ± 0.01a	0.36 ± 0.08b
B	23.94 ± 1.34a	39.13 ± 2.8b	22.73 ± 1.21a	23.73 ± 2.11a

<sup>1</sup>Means with different letters separately for cranberries (*V. oxycoccus* and *V. macrocarpon*) and for blueberries (*V. myrtillus* and *V. corymbosum*) in a row were significantly different (t-Test, p < 0.05).

**Table 2.** Mineral element concentration mg·100 g<sup>-1</sup> in wild and cultivated cranberry fresh fruit

Element	<i>V. oxycoccus</i>		<i>V. macrocarpon</i>	
	Range	Mean ± SE	Range	Mean ± SE
Macroelements (mg 100 g <sup>-1</sup> fresh weight)				
N	14.9–81.8	54.9 ± 6.1a <sup>1</sup>	9.6–62.4	42.1 ± 3.7b
P	5.0–8.7	6.12 ± 0.26a	6.0–10.8	8.59 ± 0.32b
K	50.8–89.3	67.19 ± 2.86a	52.8–88.8	72.51 ± 2.06a
Ca	8.7–17.4	12.74 ± 0.63a	7.2–13.2	10.19 ± 0.50a
Mg	7.4–9.4	8.09 ± 0.16a	4.8–8.4	6.61 ± 0.22b
S	5.0–17.4	8.14 ± 1.10a	4.8–13.2	7.85 ± 0.52a
Microelements (mg·100 g <sup>-1</sup> fresh weight)				
Fe	0.12–1.02	0.31 ± 0.05a	0.22–3.88	0.72 ± 0.20b
Mn	0.97–4.19	2.59 ± 0.20a	0.058–0.51	0.19 ± 0.04b
Zn	0.11–0.18	0.15 ± 0.005a	0.04–1.18	0.16 ± 0.06a
Cu	0.04–0.08	0.06 ± 0.002a	0.03–0.07	0.049 ± 0.003a
Mo	0.002–0.006	0.003 ± 0.0003a	0.0002–0.007	0.004 ± 0.0004a
B	0.03–0.16	0.09 ± 0.009a	0.03–0.11	0.065 ± 0.005b

<sup>1</sup>Means with different letters in a row were significantly different (t-Test, p < 0.05).

In leaves, significant differences were stated for P, Mg, and all microelements in cranberries and N, K, Fe, Mn, Zn and Mo in blueberries. Thus supporting previous research on cranberries (Karlsons et al., 2009) and partly results gained by Pormale et al. (2010) on blueberries.

As a nitrogen is one of the controlling elements for American cranberry nutrition and adequate fertilization, in general, is used to maintain renewal growth, crop production, and flower bud development for the next crop (DeMoranville, 1997).

However, surprisingly similar (or even higher in wild cranberries) N concentrations were found between wild and cultivated cranberry leaf samples. It should be stressed that wild cranberry growing medium – sphagnum peat is especially N poor (Osvalde et al., 2010). It is evident, based on the present results, that wild cranberry has the ability to improve nitrogen uptake in suboptimal nutritional conditions.

**Table 3.** Mineral element concentration mg 100 g<sup>-1</sup> in bilberry and cultivated blueberry fresh fruit

Element	<i>V. myrtillus</i>		<i>V. corymbosum</i>	
	Range	Mean ± SE	Range	Mean ± SE
Macroelements (mg 100 g <sup>-1</sup> fresh weight)				
N	67.5–150.0	91.4 ± 6.9a <sup>1</sup>	74.4–103.1	93.0 ± 6.6a
P	12.2–36.4	19.3 ± 2.2a	6.8–20.3	16.5 ± 3.3a
K	60.0–180.5	110.8 ± 8.9a	66.2–98.0	81.6 ± 6.6b
Ca	12.3–51.6	21.8 ± 3.7a	6.6–15.2	9.1 ± 0.7b
Mg	6.3–28.5	12.45 ± 2.0a	4.5–10.1	6.10 ± 0.5b
S	12.0–30.3	16.7 ± 1.1a	10.1–25.4	20.7 ± 3.5b
Microelements (mg 100 g <sup>-1</sup> fresh weight)				
Fe	0.23–0.68	0.38 ± 0.04a	0.25–0.59	0.47 ± 0.02a
Mn	0.23–4.35	1.72 ± 0.3a	0.14–1.52	0.57 ± 0.11b
Zn	0.09–0.30	0.15 ± 0.02a	0.08–0.12	0.10 ± 0.003b
Cu	0.047–0.14	0.072 ± 0.008a	0.01–0.09	0.026 ± 0.01b
Mo	0.001–0.005	0.002 ± 0.0003a	0.003–0.012	0.008 ± 0.002b
B	0.08–0.12	0.095 ± 0.004a	0.07–0.15	0.095 ± 0.007a

<sup>1</sup>Means with different letters in a row were significantly different (t-Test, p < 0.05).

Our research revealed a considerably higher content of Mn in *V. oxycoccus* leaves (1,531.8 mg kg<sup>-1</sup>) in comparison to American cranberry leaves (298.0 mg kg<sup>-1</sup>). In like manner, Mn concentration in bilberries was 814.4 mg kg<sup>-1</sup> while in cultivated – 288.4 mg kg<sup>-1</sup>. Such phenomenon could be explained by the different pH of the growing substrate and genetic differences between species. As reported previously, fertilization practice usually elevates substrata pH in cranberry and blueberry plantations (Osvalde et al., 2010). While significantly lower pH consequentially promotes the availability of Mn in natural high bogs. Fruit Mn contents of all analyzed *Vaccinium* species supported these differences.

It should be noted, that numerous conditions, like plant variety, growing conditions, harvesting, maturity stage can affect the chemical composition of fruit. Besides, the methods of sample preparation and methodology of chemical analyses also influence the obtained results. Thereby it can be complicated to compare and interpret the results obtained by different researchers.

A comparison of wild and cultivated species showed similar concentrations for the macroelements K, Ca and S in cranberry and N, P in blueberry fruits. While statistically significant differences (p < 0.05) were found for N, P and Mg in case of cranberries and Ca, K, Mg and S for blueberries. The obtained data indicated that nitrogen and potassium were the major mineral constituents in all analyzed species. The richest source of Ca, Mg (on average, 21.8 and 12.5 mg 100 g<sup>-1</sup> FW) in this study was cultivated blueberry, while highest P (19.3 and 16.5 mg 100 g<sup>-1</sup> FW) contents were found in both blueberry species.

The highest mean concentration of Fe (0.72 mg 100 g<sup>-1</sup> fresh fruit) was found in the American cranberry, while the highest Mn and B (on average 2.59 and 0.09 mg 100 g<sup>-1</sup> fresh fruit, respectively) concentrations were found in the European cranberry.

Overall, there are scarce comparable data in the literature which show the detailed mineral content of wild cranberries and wild blueberries. In general, our observations on wild and cultivated blueberries mineral content support studies made by Drózdź et al. (2018) in Poland, Miljković et al. (2018) in Serbia and Pormale et al. (2010) in Latvia. Drózdź et al. (2018) showed that the average Cu concentrations in wild and cultivated berries were similar while in our research Cu content in *V. myrtillus* was more than three times higher (0.072 to compare with 0.026 mg 100 g<sup>-1</sup>) despite the fact that in leaves concentrations were similar. Comparable results of Cu concentration in *V. myrtillus* (0.60 mg 100 g<sup>-1</sup>) berries show research made by Stanoeva et al. (2017) in Macedonia. In the present research, relatively high level of Mn was found in the studied wild blueberries and cranberries (2.59 and 1.72 mg 100 g<sup>-1</sup>). Similar Mn levels were reported for bilberries consumed in Latvia (1.88 mg 100 g<sup>-1</sup> FW) (Skesters et al., 2014) and higher in berries from Finland – 2.56 mg 100 g<sup>-1</sup> (Ekholm et al., 2007). While the content of Mn in wild cranberry was similar in Finland (2.37 mg 100 g<sup>-1</sup>) and previously determined by Karlsons et al. (2009) in Latvia.

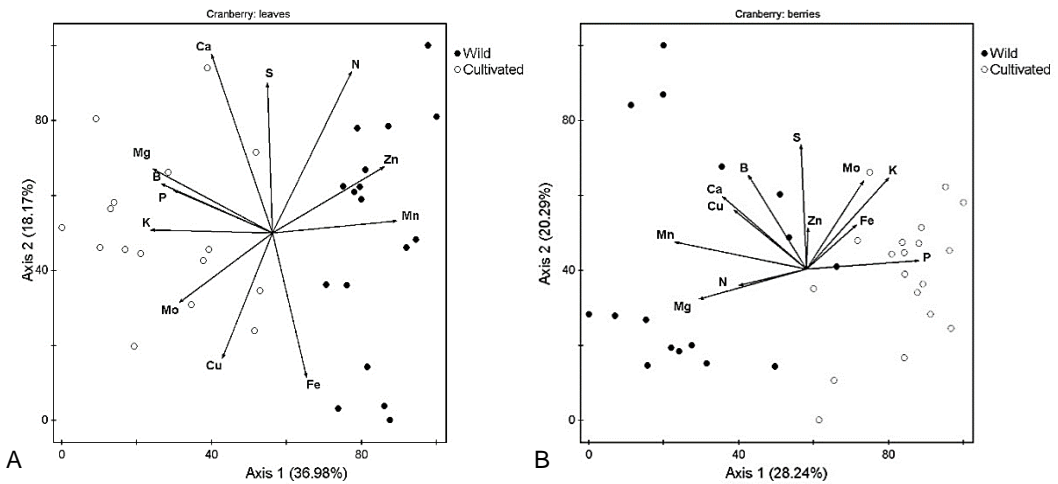
The nutritional value of studied fruits as a dietary source of minerals is related to the contribution it makes to the Recommended Dietary Allowance (RDA). Our research shows that berries of both wild species (*V. oxycoccos* and *V. myrtillus*) are excellent sources of Mn (112.5% and 74.6% from recommended daily dose, accordingly) in human nutrition (Table 4). Also, wild berries could be qualified as a valuable source of macroelement P and microelements Cu and B. While cultivated berries provide more Fe and Mo. It should be stressed that cultivated cranberries and blueberries had significantly higher concentrations of Mo in their leaves and fruits, apparently due to use of Mo containing fertilizers. Therefore 100 g of cultivated cranberry and blueberry fruits could provide 8.9–18.1% Mo of RDA.

**Table 4.** The contribution of 100 g of fresh berries to the Recommended Dietary Allowance (RDA) for adults per day

Element	RDA*, mg	% of RDA supplied by 100 g fresh berries			
		<i>V. oxycoccus</i>	<i>V. macrocarpon</i>	<i>V. myrtillus</i>	<i>V. corymbosum</i>
P	700	7.8	6.0	13.1	13.3
K	2,500	2.7	2.9	4.4	3.3
Ca	1,000	1.3	1.0	2.2	0.9
Mg	420	1.9	1.6	3.0	1.5
S	850	1.0	0.9	2.0	2.4
Fe	8	3.8	9.0	4.8	5.9
Mn	2.3	112.5	8.3	74.6	24.8
Zn	11	1.4	1.5	1.4	0.9
Cu	0.9	7.1	5.4	8.0	2.9
Mo	0.045	7.3	8.9	4.4	18.1
B	1.5	6.0	4.3	6.4	6.4

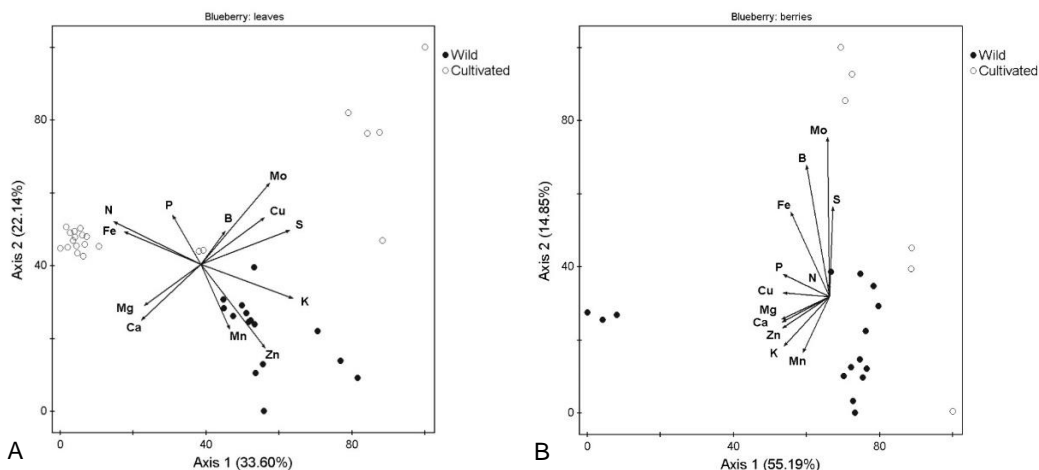
\* USDA National nutrient database for standard reference, (2017).

In general, the PCA of the chemical composition of cranberry leaves and berries demonstrated a good structure of the sampling points in the ordination space according to cranberry species. The first two components explained 55.15% and 48.53% of the total variance for leaves and berries, respectively (Fig. 1. A, B). The most important factors for the Axis 1 of the cranberry leaf PCA were Mn, K and Mg with eigenvector values ranging from 0.820 (Mn) to 0.793 (Mg), for the Axis 2 – Ca, N and S, with eigenvector values ranging from 0.720 (Ca) to 0.603 (S). The highest eigenvector value for the PCA of the berry results was also characteristics for Mn (0.889). In general, the samples of wild cranberry leaves were markedly located on the right side in the ordination space in the direction of N, Zn, Mn, and Fe, but the cultivated cranberries – mainly on the left side in the direction of K, Mg, Ca, B, Mo. The pronounced differences in the chemical composition were also clearly demonstrated for berries of wild and cultivated cranberries. The samples of wild berries were mainly located on the left side in the ordination space in the direction of such nutrients as Mn, Mg, N, and B, cultivated – on the right side in the direction of P and K.



**Figure 1.** Principal component analysis (PCA) of the chemical composition of wild and cultivated cranberry leaves (A) and berries (B) in Latvia.

The PCA of the chemical results of wild and cultivated blueberry leaves and berries also confirmed the differences in the nutrient concentrations between species. The first two components of the PCA explained 55.74% and 70.04% of the total variance for leaves and berries, respectively (Fig. 2. A, B). In both PCA, the leaf and berry samples of cultivated blueberries were located on the side of the positive of the Axis 2, while the individual sampling sites of bilberry on the opposite side in the ordination space. There were two nutrient groups which mainly affected site distribution for leaf samples of cultivated blueberries: N and Fe, as well as Mo, S, and Cu with eigenvector values from 0.678 (Fe) to 0.785 (S). Generally, these differences could be largely attributed to peculiarities of management practices in blueberry cultivation. The distribution of bilberry leaves in the ordination space was mainly determined by Mn, Zn, and K, with eigenvector values in the range between 0.579 (Mn) and 0.811 (K).



**Figure 2.** Principal component analysis (PCA) of the chemical composition of bilberry and cultivated blueberry leaves (A) and berries (B) in Latvia.

The results of the PCA for blueberry berries (Fig. 2. B) revealed that the most important elements for the 1<sup>st</sup> axis, which explained 55.19% of the total variance, were P, K, Ca, Mg, Zn and Cu with eigenvector values ranging from 0.930 to 0.979. The most important factors for the Axis 2 were Mo and B with eigenvector values 0.826 and 0.681, respectively. The PCA results showed a tendency that cultivated berries had higher concentrations of Mo, while the samples of bilberry berries were located in the direction of Mn. Although the most of the wild berry samples were located on the right side of the ordination space, three sites were on the opposite site, thus manifesting also a relatively wide variance of the wild berry chemical composition. Admittedly, the part of samples was located in the center of ordination space revealing certain similarity in the chemical composition of wild and cultivated blueberries.

## CONCLUSION

As a conclusion, our study reveals, that studied species differ in their elemental composition. In general, cultivated blueberry and cranberry fruits had a higher content of Fe, Mo while wild berry fruits showed higher levels of Ca, Mg and especially Mn. All berries studied could be qualified as a good source of microelements: excellent source of Mn and valuable source of Fe, Cu, Mo, and B in human nutrition.

## REFERENCES

- Blumberg, J.B., Basu, A., Krueger, C.G., Lila, M.A., Neto, C.C., Novotny, J.A., Reed, J.D., Rodriguez-Mateos, A. & Toner, C.D. 2016. Impact of cranberries on gut microbiota and cardiometabolic health: *Proceedings of the Cranberry Health Research Conference 2015. Advances in nutrition* **7**, 759–770.
- DeMoranville, C.J. 1997. Cranberry nutrition and fertilizers. In: H.A. Sandler (ed), *Cranberry production: a guide for Massachusetts*, Univ. of MA Extensions, pp. 81–85.
- Drózdź, P., Šežienė, V. & Pyrzynska, K. 2018. Mineral composition of wild and cultivated blueberries. *Biological Trace Element Research* **181**(1), 173–177.

- Dugoua, J. J., Seely, D., Perri, D., Mills, E. & Koren, G. 2008. Safety and efficacy of cranberry (*Vaccinium macrocarpon*) during pregnancy and lactation. *The Canadian Journal of Clinical Pharmacology* **15**, 80–86.
- Eck, P. 1990. *The American Cranberry*. Rutgers University Press, New Brunswick, NJ, pp. 420.
- Eckholm, P., Reinivuo, H., Mattila, P., Pakkala, H., Koponen, J., Happonen, A., Hellström, J. & Ovaskainen, M.-L. 2007. Changes in the mineral and trace element contents of cereals, fruits and vegetables in Finland. *Journal of Food Composition and Analysis* **20**(6), 487–495.
- Hwang, S.J., Yoon, W.B., Lee, O.H., Cha, S.J. & Kim, J.D. 2014. Radical scavenging- kinked antioxidant activities of extracts from black chokeberry and blueberry cultivated in Korea. *Food Chemistry* **146**, 71–77.
- Istek, N. & Gurbuz, O. 2017. Investigation of the impact of blueberries on metabolic factors influencing health. *Journal of Functional Foods* **38**(A), 298–307.
- Karlsons, A., Osvalde, A. & Nollendorfs, V. 2009. Research on mineral composition of American cranberry and wild cranberry fruits in Latvia. *Latvian Journal of Agronomy* **12**, 65–71.
- Karlsons, A., Osvalde, A. 2017. Nutrient status of the American cranberry in Latvia (2005–2016). *Agronomy Research* **15**, 196–204.
- Koupy, D., Kotolova, H. & Kucerova, J. 2015. Effectiveness of phytotherapy in supportive treatment of type 2 diabetes mellitus Bilberry (*Vaccinium myrtillus*). *Ceska A Slovenska Farmacie* **64**(1–2), 3–6.
- Liu, S.X., Yang, H.Y., Li, S.Y., Zhang, J.Y., Li, T., Zhu, B.Q. & Zhang, B.L. 2015. Polyphenolic compositions and chromatic characteristics of bog bilberry syrup wines. *Molecules* **20**(11), 19865–19877.
- McCune, B. & Mefford, M.J. 1999. PC-ORD. Multivariate analysis of ecological data, Version 4. MjM Software Design, Oregon.
- Mckay, D.L. & Blumberg, J.B. 2008. Cranberries (*Vaccinium macrocarpon*) and cardiovascular disease risk factors. *Nutrition Reviews* **65**, 490–502.
- Michalska, A. & Lysiak, G. 2015. Bioactive compounds of blueberries: Post-harvest factors influencing the nutritional value of products. *International Journal of Molecular Sciences* **16**(8), 18642–18663.
- Miljković, V.M., Nikoloč, G.S., Zvezdanović, J., Mihajlov-Krstev, T., Arsič, B.B. & Miljković, M.N. 2018. Phenolic profile, mineral content and antibacterial activity of the methanol extract of *Vaccinium myrtillus* L. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **46**(1), 122–127.
- Nile, S.H. & Park, S.W. 2014. Edible berries: bioactive components and their effect on human health. *Nutrition* **30**, 134–144.
- Osvalde, A., Karlsons, A., Pormale, J. & Nollendorfs, V. 2010. Renovation of extracted high bogs in Latvia: mineral nutrition and ecological aspects of American cranberry cultivation. Proceedings of the International soil science congress 'Management of Natural Resources to Sustain Soil Health and Quality', Samsun, Turkey, pp. 503–509.
- Osvalde, A. & Karlsons, A. 2010. Evaluation of the nutrient status of American cranberry in Latvia during 2001–2007. *Proceedings of the 6th international symposium on mineral nutrition on fruit crops. Acta Horticulturae* **868**, 213–218.
- Page, A.L., Miller, R.H., Keeney, D.R. (eds.). 1982. *Methods of soil analysis. Part 2. Chemical and microbiological properties*. Wisconsin.
- Pormale, J., Osvalde, A., Karlsons, A. 2010. Investigation on the essential mineral element contents of cultivated and wild blueberry fruits in Latvia. Proceedings of the International Conference „Environmentally friendly and safe Technologies for quality of fruit and vegetables”, COST Action 942, Faro, Portugal, pp. 108–112.
- Reed., J. 2002. Cranberry flavonoids, atherosclerosis and cardiovascular health. *Critical Reviews Food Science Nutrition* **42**(Suppl.3), 301–316.

- Rinkis, G.J., Ramane, H.K. & Kunickaya, T.A. 1987. Methods of soil and plant analysis. Zinatne, Riga, pp. 174 (in Russian).
- Shi, M., Loftus, H., McAinch, A.J. & Su, X.Q. 2017. Blueberry as a source of bioactive compounds for the treatment of obesity, type 2 diabetes and chronic inflammation. *Journal of Functional Foods* **30**, 16–29.
- Skesters, A., Kleiner, D., Blázovics, A., May, Z., Kurucz, D., Silova, A. & Szentmihályi, K. 2014. Mineral element content and antioxidant capacity of some Latvian berries. *European Chemical Bulletin* **3**, 98–101.
- Szajdak, L.W. & Inisheva, L.I. 2016. Cranberry: a plant growing on organic soils with a broad spectrum of pharmaceutical and medical use. In: Szajdak L. (eds). *Bioactive compounds in agricultural soils*. Springer, Cham. 273–286.
- Stanoeva, J.P., Stefova, M., Baceva Andonovska, K., Vankova, A. & Stafilov, T. 2017. Phenolics and mineral content in bilberry and bog bilberry from Macedonia. *International journal of food properties* **20**(1), 863–883.
- Trehane, J. 2004. Blueberries, cranberries and other Vaccines. Portland, London, 173–175.
- Crop statistics. <http://www.fao.org/faostat/en/#data/QC>. Accessed 09.01.2018.
- USDA National nutrient database for standard reference. (2017) [www.nal.usda.gov/](http://www.nal.usda.gov/). Accessed 30.01.2018.
- 10 top countries in cranberry production. <https://www.worldatlas.com/articles/10-top-countries-in-cranberry-production.html>. Accessed 09.01.2018.

## **Solution for remote real-time visual expertise of agricultural objects**

V. Komasilovs<sup>1</sup>, A. Zacepins<sup>1,\*</sup>, A. Kviesis<sup>1</sup>, A. Nasirahmadi<sup>2</sup> and B. Sturm<sup>2</sup>

<sup>1</sup>Latvia University of Life Sciences and Technologies, Faculty of Information Technologies, Department of Computer Systems, Liela iela 2, LV-3001 Jelgava, Latvia

<sup>2</sup>University of Kassel, Process and Systems Engineering in Agriculture Group, Department of Agricultural and Biosystems Engineering, Nordbahnhofstrasse 1a, D-37213, Witzenhausen, Germany.

\*Correspondence: [aleksejs.zacepins@llu.lv](mailto:aleksejs.zacepins@llu.lv)

**Abstract.** In recent years automated image and video analyses of plants and animals have become important techniques in Precision Agriculture for the detection of anomalies in development. Unlikely, machine learning (i.e., artificial neural networks, support vector machine, and other relevant techniques) are not always able to support decision making. Nevertheless, experts can use these techniques for developing more precise solutions and analysis approaches. It is labour-intensive and time-consuming for the experts to continuously visit the production sites to make direct on-site observations. Therefore, videos from the site need to be made available for remote viewing and analysis. In some cases it is also essential to monitor different parts of objects in agriculture and animal farming (e.g., bottom of the plants, stomach of the animal, etc.) which are difficult to access in standard recording procedures. One possible solution for the farmer is the use of a portable camera with real-streaming option rather than a stationary camera.

The aim of this paper is the proposition of a solution for real-time video streaming of agricultural objects (plants and/or animals) for remote expert evaluation and diagnosis. The proposed system is based on a Raspberry Pi 3, which is used to transfer the video from the attached camera to the YouTube streaming service. Users will be able to watch the video stream from the YouTube service on any device that has a web browser. Several cameras (USB, and Raspberry Pi camera) and video resolutions (from 480p till 1,080p) are compared and analysed, to find the best option, taking into account video quality, frame rates, and latency. Energy consumption of the whole system is evaluated and for the chosen solution it is 645 mA.

**Key words:** precision agriculture, video streaming, visual observations.

### **INTRODUCTION**

Precision agriculture and its sub-branches, such as Precision Livestock farming and Precision Beekeeping (Zacepins et al., 2015) is based on detailed information on the status of agricultural objects using various technologies (Terry 2006; Wrest 2009; Abassi et al. 2014). Agricultural objects can be an individual plant, a set of plants or animals. Crop and animal protection, plant watering and fertilization processes need frequent updates in data, which afterwards is analysed and a decision is proposed to the farmer.



In current years automated image and video analyses of plants or animals for detection of anomalies in their development have become an important and emerging techniques in Precision Agriculture (Vranken & Berckmans 2017). The technology (hardware, camera and periphery) is relatively cheap and non-invasive, which facilitate the collection of more frequent data over longer time periods. The use of cameras and automated image processing techniques, makes it possible to obtain information on the behaviour of animals, analyse the data and detect possible deviations from expected values (Kashiha et al., 2013). The technology of monitoring animals by image processing is not new and already have been widely applied by many scientists and practitioners (Aydin et al., 2010). For example, broilers can be monitored by image analysis to estimate daily body weight changes (De Wet et al., 2003), to assess the chicken activity (Aydin et al., 2010), to investigate possibility of detecting leg disorders in broiler chickens (Kristensen & Cornou, 2011). Also image processing is widely used for pig monitoring (Tillett et al., 1997; Shao et al., 1998; Nasirahmadi et al., 2016; Nasirahmadi et al., 2017) and for cow wellness monitoring (Cangar et al., 2008).

It should be mentioned that image processing could be used not only for animal monitoring, but also for the plants. Image processing is a promising tool for non-destructive analysis of biological objects, and has been widely used in botanical research and practical agriculture (Ibaraki & Dutta Gupta 2010; Yadav et al., 2010; Dutta Gupta et al., 2012; Vesali et al., 2015; Mahlein 2016; Bodner et al., 2017; Vesali et al., 2017).

Unfortunately, modern information and communication technologies (ICT), like machine learning, satellite navigation, sensor network, grid computing, ubiquitous computing and other relevant technologies supporting the domain for improved monitoring and decision making capabilities (Shaikh, 2009) are not always able to support adequate and fast decision making. Nevertheless, experts can be more precise in analysing scenarios and supporting decisions, particularly when analysing images or videos. In commercial livestock houses as well as greenhouses, image analysis for behaviour/growth classification becomes more complex. Lighting, camera characteristics, background and the test subjects' traits all influence the ability of the system to detect or recognise the subject and determine its features accurately.

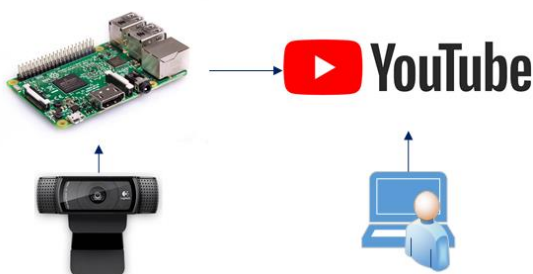
It is not always economically feasible for experts to visit geographically remote production sites to make on-site observations, so there is a need for making videos from the site available for remote viewing and analysis. Furthermore, it is necessary to provide videos from different angles and perspectives (such as bottom of the plants leaves, abdomen of the animal, etc.). Therefore, usage of automated recording is not always possible. Additionally, experts require on-demand details on the observed object. Thus, bi-directional real-time communication between the farmer and the expert is mandatory, as pre-recorded videos are not always suitable for precise diagnosis.

The aim of this paper is to propose a solution for real-time video streaming of agricultural objects (plants and/or animals) for remote expert evaluation and diagnosis. The proposed system is based on a Raspberry Pi 3, which is used to transfer the video from an attached camera to the YouTube streaming service. The system can be used by farm workers to transmit real-time video for remote expert analysis.

## DEVELOPED SOLUTION BASED ON RASPBERRY PI

The proposed system is based on a Raspberry Pi 3, which is a replacement for the Raspberry Pi 2 Model B, released in February 2016. The Raspberry Pi is a credit card sized single board computer developed by the Raspberry Pi Foundation, United Kingdom. The board is a miniature PC, packs extreme computing power and is capable to develop sophisticated projects. The Raspberry Pi is well suited to perform a multitude of computing tasks and for interfacing various types of devices via GPIO (Nayyar & Puri 2015). The key specification of the Raspberry Pi 3 is: Quad Core 1.2GHz Broadcom BCM2837 64bit CPU, 1GB RAM, BCM43438 wireless LAN and Bluetooth Low Energy (BLE) on board, 4 USB 2 ports, CSI (camera serial interface) camera port for connecting a Raspberry Pi camera. Due to its small dimensions, low weight and power consumption it is suitable for the development of wearable devices and solutions.

The proposed system architecture is shown in Fig. 1. The external web camera is connected to the Raspberry Pi USB port, and is used to capture high definition video (resolution 1,080p). The system automatically delivers the video stream to YouTube live service using the wireless transmitting method. A Raspberry Pi is connected to external Wi-Fi router. The intended users (experts) are able to watch the video stream from the YouTube service on any supported device.



**Figure 1.** Schematic view of the developed system architecture.

In this study the YouTube service was used for real-time video streaming as it can be considered as major video stream service nowadays. YouTube was started as a project with the aim to remove the technical barriers to share videos online. YouTube continues working on further optimization of their video streaming service.

YouTube is not the only service to choose from. For live video streaming, there are several services from different providers. Netflix open connect (<https://openconnect.netflix.com/>) - Netflix works together with NGINX, this project wants to improve the load balancing for video streaming over the whole network. Another service is WOWZA (<http://www.wowza.com/>), which provides video streaming for companies and universities.

Main advantage of the YouTube service is, that infrastructure solution is already in place: for the described project there is no need for a special solution for video record archiving, which is also valuable for agricultural experts.

To stream real-time video on YouTube it is necessary to use RTMP (real time messaging protocol), which was originally developed for streaming data between a video

player and a server. RTMP is encapsulated in HTTP to traverse firewalls. RTMP has more video player options compared to normal HTTP, thus giving the user a better experience. A disadvantage compared to normal HTTP is that it is sensitive for data spikes. These data spikes can result in an overload of the buffer, which in turn can lead to a stop in the video playing.

One more requirement of the YouTube service is that streamed videos should be encoded in the H.264 format. Not every web camera provides raw access to this format by hardware encoding, so there might be a need to transcode the camera output to H.264, and this process is consuming significant CPU resources.

To activate the option for real-time streaming it is necessary to sign up for the YouTube service. Then after log in and account confirmation, it is required to open the Creator Studio and choose the Live Streaming option. Two main parameters for streaming are: Server URL (for example, `rtmp://a.rtmp.youtube.com/live2`) and unique stream name/key (in format like `xxxx-xxxx-xxxx-xxxx`). When this information is known it is possible to start live streaming from a Raspberry Pi using the following Linux command:

```
ffmpeg -re -ar 44100 -ac 2 -acodec pcm_s16le -f s16le -i /dev/zero \
-input_format h264 -video_size 1,920x1,080 -i /dev/video0 \
-vcodec copy -acodec aac -ab 128k \
-f flv -movflags faststart \
rtmp://a.rtmp.youtube.com/live2/xxxx-xxxx-xxxx-xxxx
```

In this research FFmpeg is used which is a free software project that produces libraries and programs for handling multimedia data. It is possible to configure audio and video inputs, transcoding options and output parameters.

The system components with their approximate unit prices, which were available for the authors, are summarized (Table 1) below.

**Table 1.** System components with approximate unit price used in this study

Nr.	Name of the component	Quantity (pieces)	Price per piece (EUR)
1	Raspberry Pi 3 (including 8GB SD card)	1	44.00
2	Raspberry Pi 3 case	1	10.00
3	Logitech c920 web camera	1	84.00
4	Power Bank (Adata P12500D, capacity 12500mAh)	1	18.00
		<b>Total</b>	<b>156.00</b>

## COMPARISON OF DIFFERENT CAMERAS

The described solution was developed iteratively: the authors tested different web cameras and output modes. The results of the conducted experiments are summarised below.

The Raspberry Pi 3 was set up with the Linux operating system *Raspbian* (release: 9.1). No additional configurations were performed, to reduce resource consumption. During inactive periods (*idling*), the device consumed approximately 79 MB of RAM with a load average of: 0.23, 0.24, 0.2 (average values during 1, 5 and 15 minute periods, respectively) as provided by the *htop* utility. In such conditions the Raspberry Pi consumed approximately 280 mA. Testing was conducted in the laboratory with microclimate conditions with an ambient temperature of 22 °C and a relative humidity

of 30%. Wi-Fi connection speeds were as follows: download speed up to 43 MBps and upload speed up to 91 MBps. As the Raspberry Pi itself is a well exposed naked circuit board which cannot be used without any extra casing for agriculture related surveys the authors used standard a plastic casing with a radiator for CPU.

To test and compare resource and power consumption (crucial, when powering from battery pack) a separate operating system (DietPi) was installed. DietPi is a lightweight operating system for single board computers (<http://dietpi.com/#noAction>).

The core installation of DietPi was supplemented with the following software: ffmpeg, Dropbear SSH server. During inactive periods, approximately 38 MB of RAM was used with a load average of: 0.07, 0.07, 0.03. Tests proved that there was no significant difference regarding power consumption – it was constant (approx. 280 mA). To reduce the power consumption, the HDMI and activity LED of the Raspberry Pi 3 were disabled (as described by Geerling (2017)). This resulted in a current reduction by 30 mA.

The very first attempt was to use the Raspberry Pi camera module, which is capable to take HD videos as well as still photos, for video capturing. The Raspberry Pi camera has effective resolution of 5 Mega-Pixel and supports video recording at: 1080@30fps, 720p@60fps and Vga@90fps. It can be connected to the Raspberry Pi via a CSI port (Nayyar & Puri 2015).

In the second phase the authors used a Logitech HD PRO webcam C920 (<https://www.logitech.com/en-us/product/hd-pro-webcam-c920#specification-tabular>) as external USB web camera. The main features of importance for the research were: full HD video recording (up to 1,920 x 1,080 pixels) and H.264 video hardware compression. Camera output formats can be verified in the Linux system using the command below:

```
$ v4l2-ctl --list-formats -d /dev/video1
ioctl: VIDIOC_ENUM_FMT
Index      : 0
Type       : Video Capture
Pixel Format: 'YUYV'
Name       : YUYV 4:2:2

Index      : 1
Type       : Video Capture
Pixel Format: 'H264' (compressed)
Name       : H.264

Index      : 2
Type       : Video Capture
Pixel Format: 'MJPG' (compressed)
Name       : Motion-JPEG
```

Logitech C920 camera output is available in several formats: YUYV (raw), H.264 and MJPG. It is important, that the camera has built-in hardware compression to H.264 that is accepted by YouTube directly. Other formats should be transcoded to H.264 using additional software. It should be taken into account that the camera can have hardware encoding H.264 in the specification, but sometimes it is encapsulated in MJPG format and can be used only by specific software, like Skype and cannot be transmitted to YouTube directly (e.g. Logitech c925e web camera).

A comparison of different possible scenarios using the above-mentioned cameras and different video formats was conducted. Several system parameters were compared, such as: energy consumption, CPU load, amount of used RAM, consumed power by the

Raspberry Pi, latency to broadcaster on YouTube (from viewer’s perspective) and video quality.

Video quality was evaluated in a testing environment with room lighting conditions (day lights): a printed document with 10 text rows written in different font sizes, starting from 26 pt till 8 pt was placed in front of a camera with a distance of 50 cm. Image quality is estimated by the smallest text row, that is easy human readable on resulting video stream.

For real latency evaluation a real time clock was recorded, and it was compared with the time on the local PC. The current consumed by the Raspberry was measured using a KW203 USB Current and Voltage Detector, with a measurement range for voltage of: DC 3.2-10 V; and current: DC 0-3 A (accuracy 1 percent (about 2 digits)).

For the Raspberry Pi CPU and RAM loads the Linux system utility (`$ ps -C ffmpeg -o %cpu,%mem,cmd`) was used. The stream bitrate was reported by ffmpeg software itself.

Conducting various experiments the authors strived to find the possible maximal video resolution that can be streamed from Raspberry Pi 3 using software and hardware transcoding.

Due to the YouTube service requirement necessity for the existence of an audio channel in a video stream the authors faked the audio input by interpreting the /dev/zero device output as audio stream.

The results of the experiments are summarised below in Table 2.

**Table 2.** Comparison of different streaming scenarios

Resolution	Format	CPU, %	RAM, %	Power, mA	Lat, s	R.lat, s	Remarks	Quality	Score	Bitrate, kbps
Raspberry Pi camera hardware encoding										
640 x 480	H.264	15.1	1.4	335	1.6	14	Partial FOV, Pixel bins	1	Yellow	1,350
720 p	H.264	17.3	1.6	380	1.7	10	Pixel bins	2	Green	2,300
1,640 x 922	H.264	19.7	1.6	400	1.5	7	Full width	2	Yellow	3,900
1,080p	H.264	21.9	1.7	425	1.9	6	Partial FOV	3	Green	5,000
Logitech c920 software encoding										
640 x 400	yuv422	223.0	8.2	565	3.2	13	5 fps	1	Yellow	550
1,280 x 800	yuv422	338.0	25.1	620	7.5	57	1 fps	4-5	Red	1,500
1,280 x 800	yuv422	194.0	13.1	550	2.29	10	5fps, ultrafast	4-5	Green	31,290
1,280 x 800	yuv422	189.0	17.0	545	2.8	6	5fps, ultrafast,crf=12	5-6	Green	19,190
1,280 x 800	yuv422	161.0	17.1	515	3.65	12	5fps, ultrafast,crf=17	5-6	Green	11,100
Logitech c920 hardware encoding										
1,080 p	H.264	18.3	15.9	645	2.1	4	-	8-9	Green	3,030

During the experiments with the Raspberry Pi camera, the highest achieved quality score was 3, which is not acceptable for detailed observation of agricultural objects. The following command for the video streaming utilizing the Raspberry camera was used during the experiment:

```
raspivid -o - -t 0 -vf -hf -w 1920 -h 1,080 -fps 30 -md 1 -n -fli 50hz | \
ffmpeg -re -ar 44100 -ac 2 -acodec pcm_s16le -f s16le -ac 2 -i /dev/zero \
    -input_format h264 -i - \
    -vcodec copy \
    -acodec aac -ab 128k \
    -f flv -movflags faststart \
    rtmp://a.rtmp.youtube.com/live2/xxxx-xxxx-xxxx-xxxx
```

Different camera modes were selected by changing `-w`, `-h` and `-md` parameters of raspivid command.

It was observed, that increasing the video quality, there are small increase in CPU usage and in Raspberry power consumption. The amount of RAM stays the same. Real latency decreased from 14 s to 6 s, this can be explained by the fact, that in lower video quality there is pixel binning method applied, but in higher quality pixels are mapped directly to camera pixel sensors. In several high-resolution modes the camera produces partial FOV (field of view) images, which are not always suitable for agricultural object observations.

During the experiments with the external web camera and software video encoding, the highest achieved quality score was 5–6. The main limitation of software encoding is the enormous CPU usage, which limits the highest resolution to be used for video streaming.

```
ffmpeg \
    -re -ar 44100 -ac 2 -acodec pcm_s16le -f s16le -ac 2 -i /dev/zero \
    -video_size 1280x800 -pix_fmt yuv422p -r 1 -i /dev/video0 \
    -acodec aac -ab 128k \
    -vcodec libx264 -g 8 -profile:v high422 -level 4.0 -bf 2 -coder 1 \
    -f flv -movflags faststart \
    rtmp://a.rtmp.youtube.com/live2/xxxx-xxxx-xxxx-xxxx
```

Different software encoding configuration parameters were selected by changing `-profile`, `-level`, `-bf` and `-crf` parameters of ffmpeg command.

The best results for software encoding can be achieved with parameters producing video streams with high bitrates and lower image compression, which in turn uses less CPU power. In this case the data transmission channel becomes the limiter for better quality streaming. The authors tested bitrates up to 60 Mbit which did not achieve significant quality improvement.

During the experiments with the external web camera and H.264 hardware video encoding, the highest achieved quality score was 8–9 and it was considered to be the best solution for real time video streaming. The streaming bitrate was about 3,030 kbps, Raspberry CPU usage and RAM were also relatively low at 18.3% and 15.9% respectively. The average power consumption was 645 mA. The observed real latency was 4s, which is acceptable for the mentioned task.

Using the provided power bank with a capacity of 12,500 mAh the developed system would be able to operate more than 13 hours. The battery life or capacity can be calculated from the input current rating of the battery and the load current of the circuit. The calculation to find the capacity of the battery can be mathematically derived from the following formula (1):

$$BL = \frac{BC}{LC} \cdot 0.7, \quad (1)$$

where BL – Battery Life, BC – Battery Capacity, LC – Load Current, the factor of 0.7 makes allowances for external factors, which can affect battery life.

So in case of this study Battery Life = 13.57 Hours (2).

$$BL = \frac{12,500}{645} \cdot 0.7 \quad (2)$$

That system life is extended enough for remote visual expertise of needed agricultural objects.

Usually farms are located outside the urban areas, where Wi-Fi connection might not be available. Therefore, an additional test was carried out to identify if the system can operate using mobile internet connections. It was concluded that 3G technology is not suitable for real-time video streaming. However, using the 4G technology system performed well.

As Raspberry Pi is used with a plastic case in real environments, the CPU temperature during streaming was measured. As in practice the video is streamed for remote experts, one stream episode cannot not be longer than 30 minutes. The observed CPU temperature was starting from 25 °C in idle state, increased by 4 °C in the first ten minutes, then increased by 2 °C in next ten minutes. At the final time of 30 streaming minutes the CPU temperature was still 31 °C. Measurements of the CPU temperature conducted using a infrared thermometer Standard ST-8811.

## CONCLUSIONS

Continuous and real time monitoring of agricultural objects has become a common tool not only for research but also for practical agriculture and livestock farming.

Application of video observation systems has also become widely used in both precision agriculture and farming systems. Unfortunately, not every task can be solved by automated and fixed camera systems. There are cases, when it is necessary to record video manually by changing the position of the cameras.

Proposed system can be used in various scenarios, when remote expert consultation is needed.

Comparing different scenarios and camera configurations, the results showed that usage of an external web camera with built-in H.264 encoding is the best option, despite the price of such camera (up to 100 EUR).

Usage of video software encoding on a Raspberry Pi is challenging due to limited CPU power, which needs to be considered for larger scale monitoring.

In the future, it is planned to test the described proof of concept in real agricultural situation, with different environmental conditions (e.g., climate changes, lighting situations for camera).

**ACKNOWLEDGEMENTS.** Scientific research, publication and presentation are supported by the ERA-Net SusAn Project ‘Improving pig system performance through a whole system approach (PigSys – 2817ERA08D)’ funded by the Latvian State Education Development Agency, subordinated to the Ministry of Education and Science and by the German Federal Ministry of Agriculture and Nutrition, represented by the Federal Agency of Agriculture.

## REFERENCES

- Abbasi, A.Z., Islam, N. & Shaikh, Z.A. 2014. A review of wireless sensors and networks' applications in agriculture. *Computer Standards & Interfaces* **36**(2), 263–270.
- Aydin, A., Cangar, O., Ozcan, S.E., Bahr, C. & Berckmans, D. 2010. Application of a fully automatic analysis tool to assess the activity of broiler chickens with different gait scores. *Computers and Electronics in Agriculture* **73**(2), 194–199.
- Bodner, G., Alsalem, M., Nakhforoosh, A., Arnold, T. & Leitner, D. 2017. RGB and Spectral Root Imaging for Plant Phenotyping and Physiological Research: Experimental Setup and Imaging Protocols. *Journal of Visualized Experiments* **8**(126) doi: 10.3791/56251.
- Cangar, Ö., Leroy, T., Guarino, M., Vranken, E., Fallon, R., Lenehan, J., Mee, J. & Berckmans, D. 2008. Automatic real-time monitoring of locomotion and posture behaviour of pregnant cows prior to calving using online image analysis. *Computers and Electronics in Agriculture* **64**(1), 53–60.
- De Wet, L., Vranken, E., Chedad, A., Aerts, J.M., Ceunen, J. & Berckmans, D. 2003. Computer-assisted image analysis to quantify daily growth rates of broiler chickens. *British Poultry Science* **44**(4), 524–532.
- Dutta Gupta, S., Ibaraki, Y. & Pattanayak, A.K. 2012. Development of a digital image analysis method for real-time estimation of chlorophyll content in micropropagated potato plants. *Plant Biotechnology Reports* **7**(1), 91–97.
- Geerling, J. 2017. Raspberry Pi Zero - Conserve power and reduce draw to 80mA | Jeff Geerling. Available at: <http://www.jeffgeerling.com/blogs/jeff-geerling/raspberry-pi-zero-conserve-energy>.
- Ibaraki, Y. & Dutta Gupta, S. 2010. Image analysis for plants: Basic procedures and techniques. In *Plant Image Analysis. Fundamentals and Applications*. pp. 25–40.
- Kashiha, M., Pluk, A., Bahr, C., Vranken, E. & Berckmans, D. 2013. Development of an early warning system for a broiler house using computer vision. *Biosystems Engineering* **116**(1), 36–45.
- Kristensen, H.H. & Cornou, C. 2011. Automatic detection of deviations in activity levels in groups of broiler chickens – A pilot study. *Biosystems Engineering* **109**(4), 369–376.
- Mahlein, A.-K. 2016. Plant Disease Detection by Imaging Sensors – Parallels and Specific Demands for Precision Agriculture and Plant Phenotyping. *Plant Disease* **100**(2), 241–251. Available at: <http://apsjournals.apsnet.org/doi/10.1094/PDIS-03-15-0340-FE>.
- Nayyar, A. & Puri, V. 2015. Raspberry Pi- A Small, Powerful, Cost Effective and Efficient Form Factor Computer: A Review. *International Journal of Advanced Research in Computer Science and Software Engineering* **5**(12), 720–737.
- Nasirahmadi, A., Hensel, O., Edwards, S.A. & Sturm, B. 2016. Automatic detection of mounting behaviours among pigs using image analysis. *Computers and Electronics in Agriculture* **124**, 295–302.
- Nasirahmadi, A., Edwards, S.A. & Sturm, B. 2017. Implementation of machine vision for detecting behaviour of cattle and pigs. *Livestock Science* **202**, 25–38.
- Shaikh, Z.A., 2009. Smart agriculture. *Application of Modern High Performance Networks*, pp.120–129.
- Shao, J., Xin, H. & Harmon, J.D. 1998. Comparison of image feature extraction for classification of swine thermal comfort behavior. *Computers and Electronics in Agriculture* **19**(3), 223–232.
- Terry, B. 2006. *Precision Agriculture*, Thomson Delmar learning. New York: Clifton Park. 224 pp.



- Tillett, R.D., Onyango, C.M. & Marchant, J.A. 1997. Using model-based image processing to track animal movements. *Computers and Electronics in Agriculture* **17**(2), 249–261.
- Vesali, F., Omid, M., Kaleita, A. & Mobli, H. 2015. Development of an android app to estimate chlorophyll content of corn leaves based on contact imaging. *Computers and Electronics in Agriculture* **116**, 211–220.
- Vesali, F., Omid, M., Mobli, H. & Kaleita, A. 2017. Feasibility of using smart phones to estimate chlorophyll content in corn plants. *Photosynthetica* **55**(4), 603–610. Available at: <http://link.springer.com/10.1007/s11099-016-0677-9>.
- Vranken, E. & Berckmans, D. 2017. Precision livestock farming for pigs. *Animal Frontiers* **7**(1), 32pp.
- Wrest, P.H.C. 2009. Animal production and welfare. *Biosystems Engineering* **103**, 102–110.
- Yadav, S.P., Ibaraki, Y. & Gupta, S.D. 2010. Estimation of the chlorophyll content of micropropagated potato plants using RGB based image analysis. *Plant Cell, Tissue and Organ Culture* **100**(2), 183–188.
- Zacepins, A., Brusbardis, V., Meitalovs, J. & Stalidzans, E. 2015. Challenges in the development of Precision Beekeeping. *Biosystems Engineering* **130**, 60–71.

## **Energy potential of densified biomass from maize straw in form of pellets and briquettes**

M. Krížan, K. Krištof\*, M. Angelovič, J. Jobbágy and O. Urbanovičová

University of Agriculture in Nitra, Faculty of Engineering, Department of Machines and Production Biosystems, Tr. A. Hlinku 2, SK94976 Nitra, Slovakia

\*Correspondence: koloman.kristof@uniag.sk

**Abstract.** The aim of the study was the evaluation and comparison of energy potential of briquettes and pellets produced from the maize straw and woody biomass based on various diameters of pellets. By experimental measurements a calorific value and ash content was observed. Calorific value was measured by laboratory calorimeter IKA C 6000 (IKA® Works, Inc., USA) and laboratory combustion chamber Lindberg/Blue M (Thermo Fisher Scientific, Inc., USA). Individual calorific values and ash content was observed and subsequently confronted to obtain differences with replication. The analysis showed that calorific value of pellets with diameter 6 mm ranged from 16.99 MJ kg<sup>-1</sup> to 17.80 MJ kg<sup>-1</sup>. Calorific value of pellets with 8 mm diameter ranged from 16.63 MJ kg<sup>-1</sup> to 17.20 MJ kg<sup>-1</sup>. However, compared calorific value of briquettes ranged from 14.99 MJ kg<sup>-1</sup> to 15.66 MJ kg<sup>-1</sup>. Further analysis showed that ash content of samples varied as well and it's even affected by diameter of pellets. While ash content of pellets with diameter 6 mm was observed as 4.9% of total volume in case of pellets with 8 mm it was observed at value 5.5%. Briquettes produced from maize straw have ash content at value 5.4%. In contrary, ash content of woody biomass was significantly higher, 11% of volume, specifically. At the basis of observed parameters it can be concluded that maize straw densified in form of briquettes and pellets have a great energy potential which is comparable and competitive with currently used materials for production of briquettes and pellets.

**Key words:** biomass, maize straw, briquettes, pellets, calorific value, ash content.

### **INTRODUCTION**

From the perspective of the combustion of biomass its properties are essential where the main indicators of quality are values such as moisture content in biomass, chemical content of biomass, volatile matter content and calorific value (Maga & Piszalka, 2006). The usable biomass comes from a variety of plants and includes a wide range of chemicals and even though its energy content is in the most cases similar. As it was reported by Maga & Piszczalka (2006) and Pepich (2006) energy production from biomass has a great potential in the frame of agriculture and similar fields even in case of Slovakia (Table 1). Calorific value of dry biomass is typically in the range from 15 to 19 MJ kg<sup>-1</sup>. The heat produced from biomass materials can be obtained directly by combustion or indirectly, e.g. by cooling of the engines combusting the biogas or by electricity generation. In the case of direct way of energy production it means the combustion of plant or woody biomass. And in combination with more currently

advanced energy source devices defined by increased thermal efficiency it means less bio fuel requirements for the same amount of energy produced (Piszczalka & Jobbágy, 2011). The harvest of energy crops can be realized by different technologies. An annual willow or grass crops can be harvested by using machinery which is designed for harvest of the maize (Urbanovičová, 2011). Chopped biomass can be used for additional purposes such as production of the briquettes. The particle fractions are one of the most important features of chopped biomass (Lisowski et al., 2010). However, the harvest of crops has a high energy demands and therefore it is inevitable to optimize the whole harvest process (Lisowski et al., 2009).

**Table 1.** Energy potential of biomass in Slovakia (adopted from Maga & Piszczalka (2006) and Pepich (2006))

Type of biomass	Total amount	Energy potential, PJ
Biomass from agriculture for combustion	2,031 Mt	28.6
Woody biomass	1,810 Mt	16.9
Wood processing industry	1,410 Mt	18.1
Mouldings in the production of bio fuels	0.1 Mt	1.8
Purpose-grown biomass for energy production by combustion	100×10 <sup>-3</sup> ×ha	10.6
Total	-	76

PJ – Peta joules; Mt – Mega tons; ha – hectare.

Biomass is an ideal renewable energy with advantages of abundance resources and neutral in the greenhouse gas circulation (Krištof et al., 2011; Fei et al., 2014). Maize is one of the agricultural crops, which have wide use from the view of phytomass production and is considered as the third millennium crop. Alcohol, oil and biogas, also plastics, thermal insulation and other materials can be produced from maize, even electric energy by means of biogas cogeneration. Maize is primarily an economically profitable crop (Križan et al., 2017). Considering combustion the characteristics of biomass are important whereas the main indicators of quality are values of water content in biofuel, chemical composition of combustible fuel, content of volatile matter, biofuel heat value (Findura et al., 2006; Maga & Piszczalka, 2006; Jobbágy et al., 2011). Maize stalks have a heat value of 14.4 MJ kg<sup>-1</sup> at moisture level of 10%, at the volumetric weight of 100 kg m<sup>-3</sup> in packages. However, straw, Miscanthus, maize, and horse manure were reviewed in terms of fuel characteristics by Carvalho et al. (2008; 2013) with conclusion that all the fuels showed problems with ash lumping and slag formation.

At the same time it needs to be noted that treatment of biomass is required for its use improvement. Moreover, biomass material pressing at very high pressure is a working process, which we refer to as compaction in the final phase (Pepich, 2006). Traditional multi-operational maize straw harvesting is performed in the following steps, which are defined by primary method of the grain maize harvesting, it means what type of machine was used to harvest maize crop (Jandačka & Mikulík, 2008). Grain harvest is performed by conventional combine harvesters with adapter for grain maize harvesting with crushing maize stalks under combine-harvester. After that, maize straw crushing is performed. This is followed by maize straw and stubble grinding by means of hammer and knife mulching machine (Birrell, 2006; Collection of Laws, 2010).

The aim of the study was the evaluation and comparison of energy potential of briquettes and pellets produced from the maize straw and woody biomass based on various diameters of pellets. By experimental measurements a calorific value and ash content was observed.

## MATERIALS AND METHODS

Since the aim of the study was to compare pellets and briquettes produced from maize straw (residues) with regards to its physical and mechanical properties in relationship with differences in diameters. Therefore, by experimental measurements was observed calorific value and ash content as a main criterion. Various laboratory devices were employed in measurements, e.g. laboratory calorimeter control unit (Fig. 1) and laboratory combustion furnace.



**Figure 1.** Calorimeter IKA C 6000 control unit (IKA® Works, Inc., USA).

The experimental field was selected in accordance of sufficient experimental sampling grid needed with area of 120 ha (N 48.069135, E 17.959389). From selected area were collected samples in regular grid by manual harvest. Ten sampling points were selected with average area represented by 1 m<sup>2</sup>, from which the whole maize were collected at the end of maturity before field harvest. Subsequently, a maize cob were removed and only leaves and stalks were utilized in pellets and briquettes production while their represents the maize residues left on the field after harvest of maize. Obtained residue mixture of leaves and stalks were shredded and crushed in order to produced an appropriate particle size of this elements which can be utilized in pellets and briquettes production. This mixture were then densified in form of briquettes by employment of automatic briquetting press BrikStar MAGNUM (BRIKLIS – Slovakia, Inc., Slovakia) which operates in pressure range from 12 to 18 MPa. Produced briquettes were then analysed in order to determination of its calorific value by employment of calorimeter device IKA C 6000 (IKA® Works, Inc., USA). For each sample of produced briquettes were conducted ten replications. Subsequently, ash content of individual samples were determined by employment of laboratory combustion chamber Lindberg/Blue M (Thermo Fisher Scientific Inc., USA),

The same methodologies were followed in case of production and analysis of pellets produced from maize residues however two types of pressing matrices were utilized. Those matrices differ in diameter of its holes (6 mm and 8 mm) and pelletizing press was used (UDKL 120, TIANYUYOUDO, Shandong, China).

The production pellets and briquettes was conducted according following standards: DIN 51900, BS 1016 part 5 1977, ASTN D3286-91, ASTM D240-87, ASTM E711-87, ISO 1928-1976, ASTM D 1989-91 and BSI. In case of determination of the calorific value and ash content of produced briquettes were followed standards STN ISO 1928 – Calorific value of biological materials and STN ISO 1171 – Ash content of biological materials.

## RESULTS AND DISCUSSION

According to Frei (2013) lignin is a plant component with important implications for various agricultural disciplines. It confers rigidity to cell walls, and is therefore associated with tolerance to abiotic and biotic stresses and the mechanical stability of plants. In animal nutrition, lignin is considered an anti-nutritive component of forages as it cannot be readily fermented by rumen microbes. In terms of energy yield from biomass, the role of lignin depends on the conversion process. It contains more gross energy than other cell wall components and therefore confers enhanced heat value in thermo chemical processes such as direct combustion.

Our study as well as in those conducted by Kaliyan et al. (2009) and Kaliyan & Morey (2010) confirmed that highly dense, strong, and durable briquettes and pellets from corn stover and switch grass could be produced without adding chemical binders (i.e., additives) by activating (softening) the natural binders such as water-soluble carbohydrates, lignin, protein, starch, and fat in the biomass materials by providing moisture and temperature in the range of glass transition of the biomass materials. Moreover, the same study as well as those conducted by Urbanovičová et al. (2017) has proved that the roll press briquetting appears to be a promising low-cost, low-energy, high-capacity densification approach for commercial production of the biomass briquettes.

The energy potential analysis reveal that in case of produced pellets with diameter of 6 mm the average calorific value was obtained at level 17,381.6 kJ kg<sup>-1</sup> while in case of pellets with diameter of 8 mm was observed lower average value at level 17,041.7 kJ kg<sup>-1</sup>. From this point of view it can be concluded that the lower diameter of utilized holes matrices leads to increase of the pellets densities which was then transformed into the increased energy potential. In comparison with the average calorific value of produced briquettes the significantly lower value (15,402.8 kJ kg<sup>-1</sup>). This phenomenon can be explained by conclusion that in briquetting process do not secure comparable densification of utilized material while its energy potential was observed at level 15,402.8 kJ kg<sup>-1</sup> while in case of pellets were about 11.38 and 9.61% lower than in case of pellets with diameters of 6 and 8 mm (Table 2).

Wongsiriamnuay & Tippayawong (2015) has reported that the compact density increased with pressure and temperature to around 95–1,100% higher than the residue density. The relaxed density was stable at 60–80°C, but at 30 °C, it was found to decrease from 800–1,000 kg m<sup>-3</sup> to 660–700 kg m<sup>-3</sup>. The durability index was observed to improve with increasing pressure and temperature by 30–60% and 70–90%,

respectively. This corresponded well with the lignin glass transition temperatures being in the range of 60–80 °C at moderate pressure values between 150 MPa and 250 MPa. Pellet density was also found to increase with increasing compression pressure and temperature. Pellet density was three times higher than bulk density and similar to the particle density. Heating the feed materials during compression decreased the compaction pressure from 250 MPa to 150 MPa, resulting in the formation of pellets with a higher durability index and more stable relaxed density.

Moreover, Brunerová et al. (2017) concluded that utilization of Poppy waste material (by extension, all tested waste materials utilization) for briquette production can be recommended, however, with necessary improvements related to their mechanical parameters. It can be recommended using of extremely high briquetting pressure (> 60 MPa) or using of external additives, for example wood dust or chips (with high level of lignin), to improve mechanical properties of briquettes. Overall evaluation of all obtained results proved satisfactory level of chemical quality and high energy potential of all investigated materials but low level of their mechanical quality (Brunerová et al., 2017).

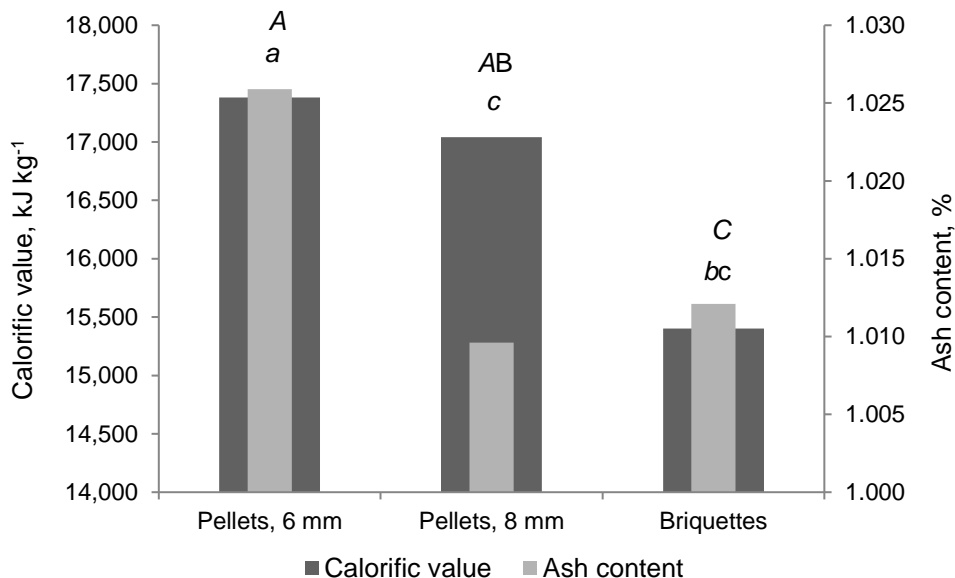
**Table 2.** Comparison of calorific values and ash content of selected diameters of pellets and briquettes

<i>n</i>	Calorific value, kJ kg <sup>-1</sup>			Ash content, %		
	Pellets, 6 mm	Pellets, 8 mm	Briquettes	Pellets, 6 mm	Pellets, 8 mm	Briquettes
1	17,303	17,092	15,531	1.026	1.007	1.012
2	17,221	16,638	15,516	1.024	1.010	1.017
3	17,502	16,889	15,307	1.030	1.003	1.013
4	16,991	17,110	15,446	1.025	1.012	1.009
5	17,150	17,087	15,293	1.027	1.006	1.006
6	17,207	17,200	15,662	1.023	1.007	1.012
7	17,449	17,128	14,994	1.027	1.008	1.016
8	17,783	16,993	15,334	1.025	1.015	1.011
9	17,801	17,105	15,405	1.028	1.019	1.014
10	17,409	17,175	15,540	1.024	1.009	1.011
Mean	17,381.6	17,041.7	15,402.8	1.026	1.010	1.012

In order to maintain a high operating comfort for end users in the residential heating sector, high ash content must be avoided. On the one hand because of the demand of emptying the ash box at periodical intervals, on the other hand because of the increasing danger of slag and deposit formation in the furnace as well as due to the rising dust emissions. The ash content of pellets could be higher, however, if the pellets are used in medium and large-scale applications due to the higher robustness as well as to the more sophisticated combustion and process control technology applied for such plants (Oberberger & Thek, 2004; Niedziółka et al., 2015).

The ash content analysis reveal that in the case of pellets with diameter 6 mm the average value of ash content was recorded at level of 1.026% while in case of pellets with diameter of 8 mm was observed lower average value at level 1.010%. In comparison with ash content of produced briquettes it was observed at value 1.012% which means the difference about 0.014% in case of pellets with diameter of 6 mm. Moreover, in comparison of the briquettes and pellets with diameter of 8 mm there were

observed significantly greater energy potential of pellets however in the case of ash content the difference were at minimum 0.002% (Fig. 2).



Where: Different letters means statistically significant differences (A,B,C for calorific values; a, b, c for ash content); *LSD* test,  $\alpha = 0.05$ .

**Figure 2.** Comparison of calorific values and ash content of selected diameters of pellets and briquettes (according statistics: *LSD* test,  $\alpha = 0.05$ ;  $n = 10$ ).

In comparison of pellets with the different diameters were energy potential recorded at minimum however in the case of ash content of individual pellets was observed difference about 0.016%. This phenomenon support the previous assumption that with decreasing diameter of pellets with no difference in the pressing condition it leads to increased and more efficient densification of pressed material and therefore to increased energy potential and increased ash content of selected pellets.

On the other hand the differences may be caused by the different composition of maize biomass used in densification process. According to Zhang et al. (2012) and Fei et al. (2014), corn residues (cobs, leaves and stalks) are abundantly available renewable materials that can be used as an energy source in gasification and combustion systems. Proper understanding of the physical properties of these materials is necessary for their use in thermo chemical conversion processes. It was observed that the leaves had an increasing trend of particle size distribution between the particle sizes 0.106 and 0.925 mm. The average particle sizes for the cobs, leaves and stalks were 0.56, 0.70 and 0.49 mm, respectively. The average bulk density was 282.38, 81.61 and 127.32 kg m<sup>-3</sup> for the corn cobs, leaves and stalks, respectively. The average porosity was 67.93, 86.06 and 58.51% for the corn cobs, leaves and stalks, respectively. A positive relationship between the average particle size and the porosity was observed for the corn residues. The differences in the physical properties among the corn residues (cobs, leaves and

stalks) observed in the study are due to variations in the compositions and structures of these materials (Zhang et al., 2012).

The study of Witters et al. (2012) examines the renewable energy production of crops used for phytoremediation. Cultivation of crops for energy purposes on such land offers the opportunity to come up with an approach that efficiently uses contaminated agricultural land and that can be beneficial for both farmer and society. Performing a Life Cycle Analysis (LCA), it was examined the energy and CO<sub>2</sub> abatement potential of willow (*Salix spp.*), silage maize (*Zea mays L.*), and rapeseed (*Brassica napus L.*) originating from contaminated land. Taking into account the marginal impact of the metals in the biomass on the energy conversion efficiency and on the potential use of the biomass and its rest products after conversion, digestion of silage maize with combustion of the contaminated digestate shows the best energetic and CO<sub>2</sub> abating perspectives. The replacement of cokes based electricity by willow is more efficient in CO<sub>2</sub> abatement than willow used in a Combined Heat and Power (CHP) unit, despite lower net energy production in the former option. Willow reaches the same energy production and same CO<sub>2</sub> abatement per hectare per year as silage maize when its biomass yield is respectively 13 and 8.7 Mg dm ha<sup>-1</sup> y<sup>-1</sup>.

However, Meyer-Aurich et al. (2012) conducted a study to observe the impact of uncertainties on greenhouse gas mitigation potential of biogas production from agricultural resources. Their analysis demonstrates the variability of the mitigation effect due to uncertainties with technical and environmental processes, which are difficult to control. Uncertainties due to fertilizer induced N<sub>2</sub>O emissions from the soil had the biggest impact on the mitigation effect of biogas use when the digestate is stored gas-tight. Otherwise, the uncertainty of emissions from the digestate dominates the variability of GHG emissions of the whole process. Moderate effects are caused by the biogas yield from feedstock, methane leakage, the electrical efficiency of the combined heat and power unit (CHP), and nitrate leaching. A minor impact can be expected from fertilizer volatilization and from the power consumption of the biogas plant.

In addition, Prade et al. (2012) in their comprehensive study focused on energy balances for biogas and solid bio fuel production from industrial hemp. This study evaluated and compared net energy yields (NEY) and energy output-to-input ratios (R<sub>O/I</sub>) for production of heat, power and vehicle fuel from industrial hemp. Four scenarios for hemp biomass were compared; (I) combined heat and power (CHP) from spring-harvested baled hemp, (II) heat from spring-harvested briquetted hemp, and (III) CHP and (IV) vehicle fuel from autumn-harvested chopped and ensiled hemp processed to biogas in an anaerobic digestion process. The results were compared with those of other energy crops. Calculations were based on conditions in the agricultural area along the Swedish west and south coast. There was little difference in total energy input up to storage, but large differences in the individual steps involved. Further processing to final energy product differed greatly. Total energy ratio was best for combustion scenarios (I) and (II) (R<sub>O/I</sub> of 6.8 and 5.1, respectively). The biogas scenarios (III) and (IV) both had low R<sub>O/I</sub> (2.7 and 2.6, respectively). They suffer from higher energy inputs and lower conversion efficiencies but give high quality products, i.e. electricity and vehicle fuel. It was concluded that the main competitors for hemp are maize and sugar beets for biogas production and the perennial crops willow, reed canary grass and miscanthus for solid biofuel production. Hemp is an above-average energy crop with a large potential for yield improvements (Prade et al., 2012).



## CONCLUSIONS

In this study was observed the energy potential and related ash content of sampled briquettes and pellets produced from maize residues (leaves and stalks, cobs not included) and following comparison of individual differences. The analysis of individual samples reveals that energy potential of pellets with diameter 6 mm ranged from 16.99 MJ kg<sup>-1</sup> to 17.80 MJ kg<sup>-1</sup>. In case of pellets with diameter 8 mm it was observed in range from 16.63 MJ kg<sup>-1</sup> to 17.20 MJ kg<sup>-1</sup>. The energy potential of compared briquettes produced from the same material mixture was significantly lower and ranged from 14.99 MJ kg<sup>-1</sup> to 15.66 MJ kg<sup>-1</sup>. Further analysis has shown that the differences were also in the recorded ash content of briquettes and pellets moreover the differences were observed also in case of pellets in relation with their different diameters. At the basis of this information it can be concluded that maize residues in form of briquettes and pellets has a great energy potential which is comparable with currently used materials for production of briquettes and pellets. Taken together, utilization of all present waste materials is highly recommended, as well as all kinds of waste materials (especially the biological ones) in attempt to keep the main key factors of proper waste management.

ACKNOWLEDGEMENTS. This work was supported by AgroBioTech Research Centre built in accordance with the project Building 'AgroBioTech' Research Centre ITMS 26220220180; and by the Ministry of Education of the Slovak Republic, Project VEGA 1/0155/18.

## REFERENCES

- Birrell, S. 2006. Biomass harvesting, transportation and logistics. Iowa state university. 2006, [www.bioeconomyconference.org/images/Birrell,%20Stuart.pdf](http://www.bioeconomyconference.org/images/Birrell,%20Stuart.pdf).
- Brunerová, A., Brožek, M. & Müller, M. 2017. Utilization of waste biomass from post-harvest lines in the form of briquettes for energy production. *Agronomy Research* **15**(2), 344–358.
- Carvalho, L., Lundgren, J. & Wopienka, E. 2008. Challenges in small-scale combustion of agricultural biomass fuels. *International Journal of Energy for a Clean Environment* **9**(1–3), 127–142.
- Carvalho, L., Wopienka, E., Pointner, C., Lundgren, J., Verma, V.K., Haslinger, W. & Schmidl, C. 2013. Performance of a pellet boiler fired with agricultural fuels. *Applied Energy* **104**, 286–296.
- Collection of Laws. 2010. *Emissions and technical requirements for stationary energy production facilities (no. 356/2010)*. Slovak ministry of Agriculture, Environment and Regional Development, Bratislava, pp. 2931–2945 (in Slovak).
- Fei, H., Shi, J.-M., Li, Y.-L. & Luo, K. 2014. Study on kinetics of straw stalk gasification in CO<sub>2</sub> with random pore model. *Applied Mechanics and Materials* **618**, 316–320.
- Frei, M. 2013. Lignin: Characterization of a multifaceted crop component. *The Scientific World Journal* **2013**, Article ID 436517, 25 pages.
- Findura, P., Maga, J., Jobbágy, J. & Ponjičan, O. 2006. The effect of sowing technology on production of biomass. *Acta technologica agriculture* **9**(3), 75–78 (in Slovak).
- Jandačka, J. & Mikulík, M. 2008. *Ecological aspects of biomass and fossil fuel combustion*. GEORG press, Žilina, 116 pp. (in Slovak).
- Jobbágy, J., Gabaj, D. & Árvay, J. 2011. Evaluation of selected agro-physical properties of a root vegetables. *Acta technologica agriculture* **14**(3), 61–66.

- Kaliyan, N. & Morey, R.V. 2010. Natural binders and solid bridge type binding mechanisms in briquettes and pellets made from corn stover and switchgrass. *Bioresource Technology* **101**(3), 1082–1090.
- Kaliyan, N., Morey, R.V., White, M.D. & Doering, A. 2009. Roll press briquetting and pelleting of corn stover and switchgrass. *Transactions of the ASABE* **52**(2), 543–555.
- Krištof, K., Blaško, M., Angelovič, M. & Chudá, Z. 2011. The use of maize stalks for energy purposes and emissions measurement during their combustion. *Mechanizace zemědělství* **61**, 178–185 (in Slovak).
- 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* **15**(2), 456–467.
- Lisowski, A., Klonowski, J. & Sypuła, M. 2010. Comminution properties of biomass in forage harvester and beater mill and its particle size characterization. *Agronomy Research* **8**(S II), 459–464.
- Lisowski, A., Nowakowski, T., Sypuła, M., Chołuj, D., Wiśniewski, G. & Urbanovičová, O. 2009. Suppleness of energetic plants to chopping. *Annals of Warsaw University of Life Sciences – SGGW. Agriculture*, **53**, 33–40.
- Maga, J. & Piszczalka, J. 2006. *Biomass as a source of renewable energy*. Slovak University of Agriculture in Nitra, Nitra, 104 pp. (in Slovak).
- Meyer-Aurich, A., Schattauer, A., Hellebrand, H.J., Klauss, H., Plöchl, M. & Berg, W. 2012. Impact of uncertainties on greenhouse gas mitigation potential of biogas production from agricultural resources. *Renewable Energy* **37**(1), 277–284.
- Niedziółka, I., Kachel-Jakubowska, M., Kraszkiewicz, A., Szpryngiel, M., Szymanek, M. & Zaklika, B. 2015. Assessment of quality and energy of solid biofuel production. *Bulgarian Journal of Agricultural Science* **21**(2), 461–466.
- Obernberger, I. & Thek, G. 2004. Physical characterisation and chemical composition of densified biomass fuels with regard to their combustion behaviour. *Biomass and Bioenergy* **27**(6), 653–669.
- Pepich, Š. 2006. Economical aspects of biomass utilization as an energy source at farm. *Agrobioenergia* **1**, 9–10 (in Slovak).
- Piszczalka, J. & Jobbágy, J. 2011. *Bioenergy: Green energy*. 1<sup>st</sup> edition. Slovak university of agriculture in Nitra, Nitra. 2011. 143 pp. (in Slovak)
- Prade, T., Svensson, S.-E. & Mattsson, J.E. 2012. Energy balances for biogas and solid biofuel production from industrial hemp. *Biomass and Bioenergy* **40**, 36–52.
- Urbanovičová, O., Krištof, K., Findura, P., Jobbágy, J. & Angelovič, M. 2017. Physical and mechanical properties of briquettes produced from energy plants. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* **65**(1), 219–224.
- Zhang, Y., Ghaly, A.E. & Li, B. Physical properties of corn residues. *American Journal of Biochemistry and Biotechnology* **8**(2), 44–53.
- Wongsiriamnuay, T. & Tippayawong, N. 2015. Effect of densification parameters on the properties of maize residue pellets. *Biosystems Engineering* **139**, 111–120.

## **The European performance indicators of broiler chickens as influenced by stocking density and sex**

A.J. Kryeziu, N. Mestani, Sh. Berisha and M.A. Kamberi\*

University of Prishtina, Faculty of Agriculture and Veterinary, Department of Biotechnology in Zootechny, “Bill Clinton” Boulevard, nn, XK10000, Prishtinë, Republic of Kosovo.

\*Correspondence: muhamet.kamberi@uni-pr.edu

**Abstract.** The aim of this study was to investigate the influence of different stocking densities on the growth performance of Ross 308 broiler chickens up to six weeks of age. A total of 216 one-day broiler chicks were randomly assigned to three treatment groups based on the stocking density: Low (LSD) = 14 chickens m<sup>-2</sup>, Medium (MSD) = 18 chickens per m<sup>2</sup> and High (HSD) = 22 chickens m<sup>-2</sup>, with four replications. Higher body weight gain (TWG) was observed for the low (2,043.89 g) and medium (2,008.03 g) compared to the high (1,901.51 g) density. The study revealed that chickens of the LSD treatment consumed significantly ( $P < 0.01$ ) more feed compared to the HSD chickens. High stocking density (22 m<sup>-2</sup>) tended to improve feed conversion ratio compared to medium (18 m<sup>-2</sup>) and low (14 m<sup>-2</sup>) stocking density, but the differences were not significant ( $P > 0.05$ ). From the results of this study it can be concluded that broiler chicks can be stocked up to 22 chickens m<sup>2</sup>, as far as required standards are assured.

**Key words:** broiler chickens, feed intake, growth, sex, stocking density.

### **INTRODUCTION**

Broiler contribution in meat production has increased markedly in the last few decades, and this is mainly due to improved genetic and management practices. Due to its low fat and high protein content, broiler meat is considered as a high quality food by consumers.

Broiler chicken can grow almost anywhere. They are small and therefore need less space compared to other animals. Housing of broiler chickens in large numbers and groups is a mean of reducing housing costs. A study by Package et al. (2015) demonstrated that a closed cage housing system enables the control of the microclimate inside the facilities, improves productivity, land and labour efficiency, and renders broiler production more environmentally friendly. The housing environment is extremely important and has an influence on broiler performance and welfare. Beyond stocking density, another factor that is very important is the quality of litter material, as meat type chickens spend most of their time lying on litter and their foot pads, hock and breast are in constant contact with the material on the floor (Terčič et al., 2015). Among the many factors that can affect broiler growth performance, stocking density and sex are very important (Abudabos et al., 2013b; Qaid et al., 2016). Stocking density can be

expressed as a number of birds per unit area or body mass per unit area. According to previous researchers (Estevez et al., 2007; Buijs et al., 2009), stocking density can be different in various countries and husbandry systems. It is one of the most important non-genetic factors in poultry breeding and has critical implications for the broiler industry, as higher returns can be obtained as the number of birds per unit area increases.

From the farmers' perspective, the housing of more birds per unit area may increase the income. However, if densities are over exceeded, economic profit may be lower due to the impairment of bird performance, health and welfare (Adeyemo et al., 2016). Welfare standards of chickens kept for meat production are strictly defined by the Council Directive (2007). This document describes the necessary welfare criteria depending on the total load of yield per unit area. A load of maximum of 33 kg m<sup>-2</sup> is set as a limit. This directive allows 39 kg m<sup>-2</sup> if stricter welfare standards are documented, or even 42 kg m<sup>-2</sup> if exceptionally high welfare standards are met over a prolonged period.

The maximum stocking density may be defined as the number of birds or weight per floor surface (Berg & Yngvesson, 2012) and could affect growth rate and body weight per floor area. High stocking density can reduce growth and final body weight at 42 days (Sekeroglu et al., 2009; Hassanein et al., 2011; Simitzis et al., 2012). In studies implemented by Uzum & Toplu (2013) and Das & Lacin (2014) effects of stocking density on the final body weight, feed intake and feed conversion ratio were also observed. Some researchers (Tsiouris et al., 2015) have also concluded that stocking density may affect the viscosity of intestinal content and the prevalence of necrotic enteritis in chicken. Sex is a factor that could also affect final body weight (Marcu et al., 2013a) with males being superior to females (Azahan et al., 2014; Beg et al., 2016) in growth parameters such as body weight. Being aware of the importance of the number of birds kept in specific surface area, and due to the fact that in Kosovo broilers are normally bred un-sexed, the goal of this study is to find the optimum stocking density and its effects on growth performance in broiler chickens of both sexes.

## MATERIALS AND METHODS

The present study was conducted at the experimental facilities of Agriculture and Veterinary Faculty of the University of Prishtina and lasted six weeks. This experiment was carried out using a total of 216 one day old commercial Ross 308 broiler chicks. The experiment was organised in two factorial design with stocking density and sex as independent variables. One day-old chicks obtained from a leading broiler commercial company, KonSoni, were first feather sexed and individually weighed to make uniform replicate groups ( $P > 0.05$ ) for each density treatment. Chicks were randomly assigned to 3 stocking density groups (treatments): High Stocking Density (HSD) = 22 chicks (11 female and 11 male), Medium Stocking Density (MSD) = 18 (9 female and 9 male) and Low Stocking Density (LSD) = 14 (7 female and 7 male) birds m<sup>-2</sup>. Each group was replicated four times. Broiler chickens were housed in four tier wired cages with 1 x 1 metre dimensions for each floor. The height between floors was 0.5 metres. Birds were kept under controlled environmental conditions from day one until day 42. All birds were offered free access to feed and water during the entire rearing period. Diets were formulated based on nutritional requirements according to recommendations by National Research Council (1994). The chickens were fed starter (first three weeks) and grower

diet until the end of trial (22–42 days). Birds of each group were individually weighed at 7, 14, 21, 28, 35 and 42 days of age. Total Weight Gain (TWG), Daily Weight Gain (DWG), Total Feed Intake (TFI), Daily Feed Intake (DFI) and Feed Conversion Ratio (FCR) were calculated weekly and at the end of the feeding period. Feed intake was calculated from the difference between the amount of feed added every week and feed residues of each group at the end of the phase. In the case of mortality, the body weight of the dead bird was recorded. After calculation of viability percentage and FCR, the European Production Efficiency Factor (EPEF) and European Broiler Index (EBI) were used to evaluate the growing performance of broilers as suggested by Van, (2003), Marcu et al. (2013b) and Aviagen, (2015). EPEF and EBI were calculated according to the following formula (Marcu et al., 2013b).

TWG = Body weight (g) at the end – Body weight (g) at start;

ADG (g/chick/d) = TWG/ days of growth period;

FCR (kg feed/kg gain) = Cumulative feed intake (kg) /Total weight gain (kg);

Viability, % = 100 – Mortality, %

$$\text{EPEF} = \frac{\text{Viability (\%)} \times \text{BW (kg)}}{\text{Age (d)} \times \text{FCR (kg feed/kg gain)}} \times 100$$

$$\text{EBI} = \frac{\text{Viability (\%)} \times \text{ADG (g/chick/d ay)}}{\text{FCR (kg feed/kg gain)} \times 10} \times 100$$

Statistical analysis: Raw data obtained from measurements was processed using the Microsoft Excel spreadsheet application. Collected data on growth performance parameters were subjected to analysis by statistical package JMP IN 7 (business unit of SAS). One way Analysis of Variance was used to compare the means of the data and alfa level of 0.05 were used as a borderline to define the significance. Tukey-Kramer HSD post hoc test was used to compare mean group differences.

## RESULTS AND DISCUSSION

Broiler chicken growth performance at two ages (1–35 and 1–42), housed in different stocking densities is presented in Tables 1 and 2. The different number of chickens per square metre has significantly ( $P < 0.05$ ) influenced total weight gain, daily weight gain and yield per unit area in both ages. Total (TFI) and daily feed intake (DFI) were significantly affected by stocking density only in 1–42 days of age (Table 2). All other performance indicators were not significantly affected by the number of birds per unit area irrespective of the age. However increasing stocking density decreased total weight gain (TWG).

This tendency is also observed by Farhadi et al. (2016), who reported lower weight gain on day 42 in broilers kept at 18 compared to that reared at the stocking density of 22 birds m<sup>2</sup>. TWG was reduced from 142.38 g to 106.52 g with increasing stocking density from 14 to 22 chickens m<sup>2</sup> at the age of 42 days. Similar observations were also made by Cengiz et al. (2015), who reported higher body weight gain in six week old Ross 308 broilers reared at LSD (10 birds m<sup>2</sup>) than those at HSD (20 birds m<sup>2</sup>). Ghosh et al. (2012) also concluded that the increase in bird stocking density significantly affects body weight gain. In fact, our results are inconsistent with those of Nogueira et al. (2013), who worked with 10, 14 and 18 birds m<sup>2</sup>. These authors found no significant effect of the stocking density in total weight gain. However, they reported higher body

weight gain of broilers at 14 and 18 birds m<sup>-2</sup> (257.11 g and 330.97 g respectively) compared with the results of the present study for same densities. Our results are also different from that of Uzum & Toplu (2013), who reported lower TWG of birds reared at densities of 18 birds m<sup>-2</sup>.

**Table 1.** Main effect of treatment on growth performance of broiler chicken (mean±SEM) (1–35 days)

Parameters	Treatment, birds m <sup>-2</sup>			P-value
	HSD	MSD	LSD	
TWG, g per bird	1,511.10 <sup>b</sup> ± 24.16	1,626.15 <sup>a</sup> ± 39.28	1,606.21 <sup>ab</sup> ± 28.44	0.0372
DWG, g per bird per day	43.17 <sup>b</sup> ± 0.69	46.46 <sup>a</sup> ± 1.12	45.85 <sup>ab</sup> ± 0.81	0.0372
TFI, g per bird	2,505.95 ± 46.19	2,666.63 ± 60.16	2,681.96 ± 54.80	0.0586
DFI, g per bird per day	71.59 ± 1.32	76.18 ± 1.72	76.62 ± 1.57	0.0586
FCR	1.66 ± 0.02	1.64 ± 0.03	1.67 ± 0.03	0.7312
Mortality, %	0.68 ± 0.33	0.55 ± 0.56	0.35 ± 0.36	0.8641
YUA, (kg m <sup>-2</sup> )	33.03 <sup>a</sup> ± 0.71	29.21 <sup>b</sup> ± 0.37	23.14 <sup>c</sup> ± 0.52	<.0001
EPEF	266.73 ± 5.61	290.40 ± 9.69	282.25 ± 7.36	0.1135
EBI	258.82 ± 5.46	282.21 ± 9.55	274.33 ± 7.28	0.1113

Note: HSD – High Stocking Density; MSD – Medium Stocking Density; LSD – Low Stocking Density; SEM – Standard error of mean; TWG – Total Weight Gain; DWG – Daily Weight Gain; TFI – Total Feed Intake; DFI – Daily Feed Intake; FCR – Feed Conversion Ratio; YUA – Yield per unit area; EPEF – European Production Efficiency Factor; EBI – European Broiler Index.

<sup>abc</sup> – means with different superscripts within the same row are significantly different at  $P \leq 0.05$ .

**Table 2.** Main effect of treatment on growth performance of broiler chicken (mean ± SEM) (1–42 d)

Parameters	Treatment, birds m <sup>-2</sup>			P-value
	HSD	MSD	LSD	
TWG, g per bird	1,901.51 <sup>b</sup> ± 33.23	2,008.03 <sup>ab</sup> ± 45.48	2,043.89 <sup>a</sup> ± 31.81	0.0355
DWG, g per bird per day	45.27 <sup>b</sup> ± 0.79	47.81 <sup>ab</sup> ± 1.08	48.66 <sup>a</sup> ± 0.76	0.0355
TFI, g per bird	3,337.77 <sup>b</sup> ± 85.54	3,589.13 <sup>a</sup> ± 65.20	3,731.52 <sup>a</sup> ± 70.12	0.0041
DFI, g per bird per day	79.47 <sup>b</sup> ± 2.04	85.46 <sup>a</sup> ± 1.55	88.85 <sup>a</sup> ± 1.67	0.0041
FCR	1.75 ± 0.02	1.79 ± 0.01	1.83 ± 0.02	0.1044
Mortality, %	0.57 ± 0.32	0.46 ± 0.32	0.30 ± 0.30	0.8285
YUA, (kg m <sup>-2</sup> )	41.30 <sup>a</sup> ± 0.66	35.87 <sup>b</sup> ± 0.44	29.27 <sup>c</sup> ± 0.52	<.0001
EPEF	261.68 ± 5.25	273.24 ± 7.66	273.06 ± 6.82	0.4083
EBI	255.47 ± 5.12	266.96 ± 7.58	266.99 ± 6.72	0.3981

Note: HSD – High Stocking Density; MSD – Medium Stocking Density; LSD – Low Stocking Density; SEM – Standard error of mean; TWG – Total Weight Gain; DWG – Daily Weight Gain; TFI – Total Feed Intake; DFI – Daily Feed Intake; FCR – Feed Conversion Ratio; YUA – Yield per unit area; EPEF – European Production Efficiency Factor; EBI – European Broiler Index.

<sup>abc</sup> – means with different superscripts within the same row are significantly different at  $P \leq 0.05$ .

The DWG of LSD and HSD groups were 48.66 and 45.27g per day, respectively (Table 2). This is not in accordance with the results of Adeyemo et al. (2016), who found no effect on daily weight gain. This is probably due to the much lower stocking densities in their research (10, 12 and 14 birds m<sup>-2</sup>) and longer duration of experiment (8 weeks). In our study, the LSD group had higher DWG (+8.95 g) compared with the results of Adeyemo et al. (2016), at the same stocking density. However, TWG reported by the same authors was 180.11 g higher than in our experiment. Other authors (Tong et al.,

2012) have also reported different results for daily body weight gain as an effect of different stocking densities (12.5, 17.5 and 22.5 birds m<sup>-2</sup>).

Stocking density has also significantly affected ( $P < 0.05$ ) feed intake (total and daily) of broilers at six weeks of age (Table 2). Higher total (TFI) and daily (DFI) feed intake were found in LSD and MSD compared to HSD group. Differences were also observed between MSD–HSD while no significant feed intake was observed between LSD–MSD treatment. Results show that chickens of the MSD group had higher TFI than those reported by Uzum & Toplu (2013) and Tong et al. (2012), who also reported different results for DFI. The total amount of feed intake (3,731.52 g per bird) reported in the present study for the LSD group is lower than reported by Beg et al. (2011), who observed intake of 4,307 g per broiler kept at the same stocking density (14 birds m<sup>-2</sup>). Differences in feed intake may be attributed to different feeding space available (Lemons & Moritz, 2016). Birds in our experiment were offered 4.55; 5.56 and 7.14 cm feeder space for HSD, MSD and LSD treatment respectively, which is higher than in commercial practice.

Results for feed conversion ratio (FCR) and mortality are also shown in Tables 1 and 2. FCR was not significantly influenced ( $P > 0.05$ ) by stocking density. However, FCR tended to be lower in the HSD compared to the other groups. A possible explanation is that birds of the LSD group had more available feeding and moving space, and although consumed more feed, they did not convert it effectively into tissues due to energy losses. This is in agreement with the results of Abudabos et al. (2013a) and Türkyilmaz (2008), who observed that stocking density had no significant effect on FCR. However, these results do not agree with those from trials of Ravindran et al. (2006), who found significant differences in FCR as a result of three different stocking densities (16, 20 and 24 birds m<sup>-2</sup>). Moreover, according to the findings of the present study, it seems that stocking density has no significant influence on mortality rates ( $P > 0.05$ ), since the applied stocking densities were not over the critical levels according to the Council Directive (2007), and there was no competition of chickens for space. Our findings are also in agreement with the results of Feddes et al. (2002), Guardia et al. (2011), Zuowei et al. (2011), Tong et al. (2012), Adeyemo et al. (2016) and Farhadi et al. (2016), which have shown that stocking density had no effect on mortality rates. Results of the yield per unit area (YUA, kg m<sup>-2</sup>) shown in Table 2 were significantly ( $P < 0.05$ ) different among density groups at the age of 42 days. As indicated, YUA increased by raising stocking density.

Feddes et al. (2002) also reported that YUA was affected by stocking density. They found a YUA of 46.9 kg m<sup>-2</sup> in higher stocking densities (23.8 bird m<sup>-2</sup>), which is significantly higher than that of 34.6, 28.6 and 22.9 kg m<sup>-2</sup> produced in the stocking densities of 17.9, 14.3 and 11.9 birds m<sup>-2</sup>, respectively.

The performance of broiler birds was also evaluated in terms of European Broiler Index (EBI) and European Production Efficiency Factor (EPEF), which includes daily weight gain and survival percentage. Higher values of these indicators indicate that the bird's body weight gain is uniform and the flock is in good health (Bhamare et al., 2016). No significant ( $P > 0.05$ ) differences among groups in EPEF and EBI values were observed. However these values were lower than those reported from Aviagen (2015).

The effect of sex on the performance of birds is shown in Tables 3 and 4. Results show non-significant differences ( $P > 0.05$ ) between male and female broilers at both ages in all measured and calculated parameters.

**Table 3.** Main effect of sex on growth performance of broiler chicken (mean±SEM) (1–35 days)

Parameters	Sex		<i>P</i> -value
	Male	Female	
TWG, g per bird	1,596.85 ± 27.10	1,565.46 ± 30.40	0.3980
DWG, g per bird per day	45.62 ± 0.77	44.72 ± 0.87	0.3980
TFI, g per bird	2,659.40 ± 42.73	2,576.96 ± 52.26	0.1930
DFI, g per bird per day	75.98 ± 1.22	73.63 ± 1.49	0.1930
FCR	1.66 ± 0.02	1.64 ± 0.02	0.4920
Mortality, %	0.54 ± 0.29	0.52 ± 0.39	0.9688
YUA, (kg m <sup>-2</sup> )	28.40 ± 1.73	28.52 ± 1.96	0.9628
EPEF	281.39 ± 7.70	278.20 ± 5.82	0.7301
EBI	273.10 ± 7.57	270.47 ± 5.78	0.7735

*Note:* HSD – High Stocking Density; MSD – Medium Stocking Density; LSD – Low Stocking Density; SEM – Standard error of mean; TWG – Total Weight Gain; DWG – Daily Weight Gain; TFI – Total Feed Intake; DFI – Daily Feed Intake; FCR – Feed Conversion Ratio; YUA – Yield per unit area; EPEF – European Production Efficiency Factor; EBI – European Broiler Index.

**Table 4.** Main effect of sex on growth performance of broiler chicken (mean±SEM) (1–42 days)

Parameters	Sex		<i>P</i> -value
	Male	Female	
TWG, g per bird	2012.90 ± 33.468	1956.05 ± 34.252	0.2478
DWG, g per bird per day	47.93 ± 0.796	46.57 ± 0.815	0.2478
TFI, g per bird	3,587.86 ± 65.393	3,517.66 ± 85.502	0.5205
DFI, g per bird per day	85.43 ± 1.557	83.75 ± 2.035	0.5205
FCR	1.78 ± 0.019	1.80 ± 0.020	0.6318
Mortality, %	0.45 ± 0.263	0.43 ± 0.248	0.9649
YUA, (kg m <sup>-2</sup> )	35.54 ± 2.121	35.42 ± 2.344	0.9692
EPEF	274.50 ± 6,080	264.15 ± 4.440	0.1828
EBI	268.04 ± 6.014	258.24 ± 4.406	0.2021

*Note:* HSD – High Stocking Density; MSD – Medium Stocking Density; LSD – Low Stocking Density; S<sub>em</sub> – Standard error of mean; TWG – Total Weight Gain; DWG – Daily Weight Gain; TFI – Total Feed Intake; DFI – Daily Feed Intake; FCR – Feed Conversion Ratio; YUA – Yield per unit area; EPEF – European Production Efficiency Factor; EBI – European Broiler Index.

However male broilers had slightly higher TWG and DWG (2.82 vs. 2.84%) compared to females. The total feed intake and daily feed intake was nearly similar in both sexes; however, female broilers consumed less feed (3,517.66 vs. 3,587.86 and 83.75 vs. 85.43) than males. Although feed intake was similar in both sexes ( $P > 0.05$ ), male chickens showed better FCR (1.78 vs. 1.80) than females. These findings are comparable to those of San et al. (2010) who reported that up to eight weeks of conventional rearing there is no significant benefit in separate sex growing of broilers. However our results do not agree with those of Ozturk, et al. (1998) who found that Ross PM3 female broilers grew slower than male ones ( $P < 0.05$ ). Same authors observed higher feed consumption of males at 35 days ( $P < 0.01$ ) and 42 days ( $P < 0.01$ ). Shim et al. (2012), also observed significant effect ( $P < 0.001$ ) of sex on body weight gain of broilers during grower (0–35 days) and finisher phase (0–48 days).

Mortality was not significantly affected by the sex of chickens ( $P > 0.05$ ) either at 1–35 (Table 3) or 1–42 days (Table 4) of feeding. A similar finding was reported by Beg et al., 2016 who found that mortality was not different in male, female or unsexed



chickens. Shim et al. (2012), reported no effect of sex on mortality during grower phase (0–35 d), but they found significant effect ( $P < 0.005$ ) on this parameter during finisher phase (0–48 days). As seen from the results (Table 4), the average yield per unit area (YUA) for both sexes was almost the same (35.54 and 35.42 g, respectively). Sex did not significantly affect EPEF and EBI values, although these parameters were lower in female chickens than in males.

## CONCLUSIONS

Based on the results of this experiment, it can be concluded that growth rate of broiler chickens decreased and the yield per unit area increased as a result of the increase in the stocking density.

The results suggest that the best YUA is obtained when 22 birds per m<sup>2</sup> are reared. Following recommendations of Council Directive (2007), this load requires high welfare standards which cannot be actually assured in common rearing conditions in Kosovo. In this case, slaughtering before the age of 42 days may be more appropriate. For example, if slaughtering is done at the age of 35 days, a calculated load will be 34.26 kg m<sup>-2</sup>, which is very close to minimum welfare standard set by Council Directive (2007).

Regarding the sex of the broilers, results show no significant effect on the measured parameters.

## REFERENCES

- Abudabos, A.M., Samara, E.M., Hussien, E.O., Al-Ghadi, M.Q. & Al-Atiyat, R. 2013a. Impacts of Stocking Density on the Performance and Welfare of Broiler Chickens. *Italian J. Anim. Sci.* **12**, 66–71.
- Abudabos, A.M., Samara, E.M., Hussien, E.O., Al-Atiyat, R. & Al-Haidary, A.A. 2013b. Influence of stocking density on welfare indices of broilers. *Italian J. Anim. Sci.* **12**, 213–218.
- Adeyemo, G.O., Fashola, O.O. & Ademulegun, T.I. 2016. Effect of Stocking Density on the Performance, Carcass Yield and Meat Composition of Broiler Chickens. *Brit. Biotechn. J.* **14**, 1–7.
- Aviagen. 2015. *Ross broiler pocket guide*. <http://eu.aviagen.com> (2015) (Retrieved August 20 2017)
- Azahan, E., Azma, E.A.A. & Noraziah, M. 2014. Effects of strain, sex and age on growth performance of Malaysian Kampong chickens. *Mal. J. Anim. Sci.* **17**, 27–33.
- Beg, M.A.H., Baqui, M.A., Sarker, N.R. & Hossain, M.M. 2011. Effect of Stocking Density and Feeding Regime on Performance of Broiler Chicken in Summer Season. *Intern. J. Poult. Sci.* **10**, 365–375.
- Beg, M.A.H., Islam, S.K.B.M., Aftabuzzaman, M. & Mahbub, A.S.M. 2016. Effects of Separate Sex Growing on Performance and Metabolic Disorders of Broilers. *Int. J. Anim. Resour.* **1**, 19–26.
- Berg, C. & Yngvesson, J. 2012. Optimal stocking density for broilers—Optimal for whom? In *xxiv World's Poultry Congress, Area: Poultry Welfare and Environment 5–9 August, Salvador, Bahia, Brazil*.
- Bhamare, K.S., Dildeep, V., Senthil, M.S. & Chavan, S.J. 2016. Nutritive evaluation of cashew apple waste in broilers. *Intern. J. Sci. Nat.* **7**, 629–632.

- Buijs, S., Keeling, L., Rettenbacher, S., Van, P.E. & Tuytens, F.A. 2009. Stocking density effects on broiler welfare: identifying sensitive ranges for different indicators. *Poult. Sci.* **88**, 1536–1543.
- Cengiz, Ö., Köksal, B.H., Tatlı, O., Sevim, Ö., Ahsan, U., Üner, A.G., Ulutaş, P.A., Beyaz, D., Büyükyörük, S., Yakan, A. & Önel, A.G. 2015. Effect of dietary probiotic and high stocking density on the performance, carcass yield, gut microflora, and stress indicators of broilers. *Poult. Sci.* **94**, 2395–2403.
- Council Directive 2007/43/EC, Laying down minimum rules for the protection of chickens kept for meat production. *Offic. J. Europ. Uni.* L 182/19.
- Das, H. & Lacin, E. 2014. The effect of different photoperiods and stocking densities on fattening performance, carcass and some stress parameters in broilers. *Israel J. Vet. Med.* **69**, 211–220.
- Estevez, I. 2007. Density allowances for broilers: where to set the limits. *Poult. Sci.* **86**, 1265–1272.
- Farhadi, D., Seyed, M.H. & Bahareh, T.D. 2016. Effect of house type on growth performance, litter quality and incidence of foot lesions in broiler chickens reared in varying stocking density. *J. BioSci. Biotech.* **5**, 69–78.
- Feddes, J.J.R., Emanuel, E.J. & Zuidhoft, M.J. 2002. Broiler performance, body weight variance, feed and water intake, and carcass quality at different stocking densities. *Poult. Sci.* **81**, 774–779.
- Ghosh, S., Debasis, M. & Rupak, G. 2012. Broiler performance at different stocking density. *Indian J. Anim. Res.* **46**, 381–384.
- Guardia, S., Konsak, B., Combes, S., Levenez, F., Cauquil, L., Guillot, F., Moraau, V.C., Lessire, M., Juin, H. & Gabriel, I. 2011. Effects of stocking density on the growth performance and digestive microbiota of broiler chickens. *Poult. Sci.* **90**, 1878–1889.
- Hassanein, H.H.M. 2011. Growth performance and carcass yield of broiler as affected by stocking density and enzymatic growth promoters. *Asi. J. Poult. Sci.* **5**, 94–101.
- Lemons, M.E. & Moritz, J.S. 2016. The effect of feeder space access and crumble or pellet-to-fine ratio on 38 day old broiler performance. *J. Appl. Poult. Res.* **25**:12–20.
- Marcu, A., Ioan, V.O., Marcu, A., Lucia, D., Dorel, D. & Bartolomeu, K. 2013a. The influence of genotype and sex on carcass characteristics at broiler chickens. *Lucrări Științifice-Seria Zootehnie* **59**, 16–21.
- Marcu, A., Vacaru-Opriș, I., Gabi, D., Liliana, P.C., Marcu, A., Marioara, N., Ioan, P., Dorel, D., Bartolomeu, K. & Cosmin, M. 2013b. The Influence of Genetics on Economic Efficiency of Broiler Chickens Growth. *Anim. Sci. Biotech.* **46**, 339–346.
- NRC. 1994. Nutrient Requirements of Poultry. 9<sup>th</sup> revised edition. National Academy Press, Washington, DC.
- Nogueira, W.C.L., Velásquez, P.A.T., Furlan, R.L. & Macari, M. 2013. Effect of dietary energy and stocking density on the performance and sensible heat loss of broilers reared under tropical winter conditions. *Brazilian J. Poult. Sci.* **15**, 53–58.
- Ozturk, E., Sarica, M., Saylam, K. & Yildirim, A. 1998. Effect of sex separated broiler growing on performance and carcass traits. *J. Agric., Fac., O.M.U.* **13**, 33–45.
- Pakage, S., Hartono, B., Fanani, Z. & Nugroho, B.A. 2015. Analysis of technical, allocative and economic efficiency of broiler production using closed house system in Malang District of East Java Indonesia. *Livest. Res. Rural. Dev.* **27**, 174.
- Qaid, M.M., Albatshan, H.A., Shafey, T.M., Hussein, E.O. & Abudabos, A.M. 2016. Effect of stocking density on the performance and immunity of 1- to 14-d- old broiler chicks. *Brazilian J. Poult. Sci.* **18**, 683–692.
- Ravindran, V., Thomas, D.V., Thomas, D.G. & Patrick, C.H.M. 2006. Performance and welfare of broilers as affected by stocking density and zinc bacitracin supplementation. *Anim. Sci. J.* **77**, 110–116.

- Sam, I.M., Akpa, G.N., Alphonsus, C., Iyeghe–Erakpotobor, G.I. & Agubosi, O.C.P. 2010. Effect of sex separation on growth performance and carcass characteristics of broilers raised to maturity. *Continental J. Animal and Veterinary Research* **2**, 35–40.
- Sekeroglu, A., Demir, E., Sarica, M. & Ulutas, Z. 2009. Effects of housing systems on growth performance, blood plasma constituents and meat fatty acids in broiler chickens. *Pak. J. Biol. Sci.* **12**, 631–636.
- Shim, M.Y., Tahir, M., Karnuah, A.B., Miller, M., Pringle, T.D., Aggrey, S.E. & Pesti, G.M. 2012. Strain and sex effects on growth performance and carcass traits of contemporary commercial broiler crosses. *Poult Sci.* **91**, 2942–2948.
- Simitzis, P.E., Kalogeraki, E., Goliomytis, M., Charismiadou, M.A., Triantaphyllopoulos, K., Ayoutanti, A., Niforou, K., Hager-Theodorides, A.L. & Deligeorgis, S.G. 2012. Impact of stocking density on broiler growth performance, meat characteristics, behavioral components and indicators of physiological and oxidative stress. *Brit. Poult. Sci.* **53**, 721–730.
- Terčič, D., Žolger, M. & Pestotnik, M. 2015. Effect of different litter materials on foot pad dermatitis, hock burn and feather coverage in broiler chickens. *Acta Agric. Slove.* **106**, 97–101.
- Tong, H.B., Lu, J., Wang, Q. & Shi, S.R. 2012. Effects of stocking density on growth performance, carcass yield, and immune status of a local chicken breed. *Poult. Sci.* **91**, 667–673.
- Tsiouris, V., Georgopoulou, I., Bazios, C., Pappaioannou, N., Ducatelle, R. & Fortomaris, P. 2015. High stocking density as a predisposing factor for necrotic enteritis in broiler chicks. *Avian Patho.* **44**, 59–66.
- Türkyilmaz, M.K. 2008. The effect of stocking density on stress reaction in broiler chickens during summer. *Turkish J. Vet. Anim. Sci.* **32**, 31–36.
- Uzum, M.H. & Toplu, H.D.O. 2013. Effects of stocking density and feed restriction on performance, carcass, meat quality characteristics and some stress parameters in broilers under heat stress. *Revue Méd. Vétér.* **164**, 546–554.
- Van, I. 2003. Growth and broilers industrialization. *Ed. Ceres, Bucharest.* 235–236.
- Zuowei, S., Yan, L., Yan, L., Jiao, H., Song, Z., Guo, Y. & Lin, H. 2011. Stocking density affects the growth performance of broilers in a sex-dependent fashion. *Poult. Sci.* **90**, 1406–1415.

## **Effect of I<sub>2</sub>/KI water solution to wheat seeds imbibition assessed by image analysis**

J. Lev\* and J. Blahovec

Czech University of Life Sciences Prague, Faculty of Engineering, Department of Physics, Kamýcká 129, CZ165 21 Prague 6, Czech Republic

\*Correspondence: [jlev@tf.czu.cz](mailto:jlev@tf.czu.cz)

**Abstract.** Water plays key role in a seed germination due to its participation in starting of many metabolic processes that accompany the seed germination. Rate of water uptake into seeds is a usual basis for determination of the three germination phases. The water uptake into seeds during their germination was investigated by many researchers who used various methods (e.g. magnetic resonance micro-imaging, near-infrared hyperspectral imaging and visualization with I<sub>2</sub>/KI solution (Lugol's iodine)). The method of using I<sub>2</sub>/KI water solution for this purpose is quite popular for its relatively applicability. In this paper we compared the seed surface area projection and shape development of the seeds imbibed in the I<sub>2</sub>/KI solution and in the pure water via image analysis. It was found that the presence of the I<sub>2</sub>/KI in water changes the increase of seeds volume during germination and the effect is different during the initial imbibition and during the next germination phases. The seed shape development is similar for both variants, pure water and I<sub>2</sub>/KI solution.

**Key words:** imbibition, germination, image analysis, water diffusion.

### **INTRODUCTION**

Seeds germination represents one of the key processes in a plant growth which affects its subsequent grain yield. The water plays important role in this process because it allows the start of the important metabolic activities in the seeds. The germination phases are defined by seed water uptake and it is generally accepted that the process consists of three phases (Bewley, 1997; Weitbrecht et al., 2011). The first phase (denote as the phase I), which is distinguished by the initial rapid absorption of water, is referred to as ‘early imbibition’. For wheat, this takes about 5–7 h (Abenavoli et al., 2006; Dell’Aquila, 2006; Rathjen et al., 2009; Harb, 2013). The early imbibition is followed by a phase, termed phase II, in which water absorption is lowered; in this phase the previous seed increase of volume and mass is nearly stopped. Phase II is finished by a new increase in the seed’s moisture content that indicates start of phase III that is usually termed ‘proper germination’ (Rathjen et al., 2009). The total increase in mass in phases I–III is about 100%, and slightly more than one half of this increase is achieved in the phase I.

Research works dealing with the water uptake in germinating seeds have been regularly appearing for a long time ago (King 1984; Becker 1960; Shull 1913). However, a significant amount of research works was published in the twenty-first century (Alvarado & Bradford, 2002; Wiwart et al., 2006; Finch-Savage et al., 2007; Ribeiro-Oliveira & Ranal, 2017). The water uptake is normally used as a chronological marker in molecular biology and physiological studies focusing on seeds germination (Harb 2013; Ribeiro-Oliveira & Ranal 2017). Dong et al. (2015) presented the dynamic proteome analysis of wheat seed germination. Authors revealed the dynamic changes in the proteome involved in wheat seed germination. Authors also showed a connection between the dynamic changes in the proteome and germination phase.

Many researchers used various methods for the water movement monitoring in the seeds (Kikuchi et al., 2006). One of them consists in monitoring of the morphological changes in the seed under microscope or electron microscope (Dell 1980; McDonald et al., 1988). Nakanishi & Matsubayashi (1997) dealt with nondestructive water imaging by the neutron beam analysis in living plants. Gruwel et al., (2001) used magnetic resonance technique for the water uptake monitoring in barley seeds. Authors were able to distinguish two stages which correspond to the first and the second germination phase. Rathjen et al. (2009) and Kikuchi et al. (2006) used micro-magnetic resonance imaging for water movement monitoring during imbibition. This technique allowed precise monitoring of the water movement during whole imbibition process.

Manley et al. (2011) and Lancelot et al. (2017) used near infrared hyperspectral imaging for monitoring of water diffusion in wheat seeds. Lancelot et al. (2017) presented that the developed system allows real-time monitoring of the diffusion of water into different tissues of the wheat seeds and it can be fully automated.

In many cases a simple and time-saving method is required. These requirements could be complied with the  $I_2/KI$  solution (Lugol's iodine) (Kikuchi et al., 2006; Rathjen et al., 2009). Rathjen et al. (2009) presented that  $I_2/KI$  was found to be the most effective marker for visualizing water uptake into the endosperm, due to its small solute size and the ability to bind with and stain starch tissue.

However, the effect of the  $I_2/KI$  solution is not wholly assessed. The aim of this paper is to compare the effect on parameters of germination in pure water and standard solution of  $I_2/KI$  usually used for visualization of internal changes in the seeds.

## MATERIALS AND METHODS

The imbibition process of two wheat varieties was monitored for 24 h. The seeds were imbibed in pure water and in  $I_2/KI$  solution. The  $I_2/KI$  solution contains 1 g  $I_2$ , 2.5 g KI and 96.5 mL distilled water. Winter wheat (varieties: Tosca and Steffi, supplier: Selgen Plc.) harvested in 2016 was used for the experiments. The moisture content (wet basis) of the seeds was 6–7% (ASABE Standard S358.2, 2006). Data was collected during four measurements, and they took place in December 2017. The imbibition monitoring was performed on three layers of a polyester cloth (white Novolin, area density: 100 g m<sup>-2</sup>). The polyester cloth and seeds were placed into a glass vessel and the cloth was moisturized by 12 g of pure water or 12 g of  $I_2/KI$  solution. For the purpose of minimizing an evaporation the glass vessel was covered by a thin glass plate. The tests with pure water were performed with 49 seeds and the tests with  $I_2/KI$  solution were performed with 42 seeds.

The seeds were monitored with a photographic camera (Canon EOS 750D with lens Canon 18–55 mm) on a stand. The second part of the laboratory setup for seeds monitoring was an illumination LED panel that was used for illumination of the specimens while taking pictures. The LED panel was placed under the glass vessel. The whole laboratory setup was placed in a laboratory incubator (Friocell 111 – EVO, manufacturer: BMT Medical Technology Inc.) that ensure a constant temperature 21 °C during experiment. The camera and LED panel were controlled via computer using a program written in the Python programming language (version 2.7). Images from the camera were stored on a hard disk. The focal length was set to the top surface of the seeds. The interval between each photograph was 120 s. The moisture content of each seed was determined by the standard oven-dried method (ASABE Standard S358.2).

The image processing and the data analysis were partially mentioned in our previous paper (Lev & Blahovec, 2017). The image processing and data analysis were performed by Python 2.7 programming language and by its supporting libraries: OpenCV 2.4.8, NumPy 1.8.2, and Matplotlib 1.3.1. The image processing started with conversion to the grey scale. The blue channel was used for experiments with pure water and the green channel for I<sub>2</sub>/KI solution. The next step was conversion to the binary image using standard threshold method and application of erosion-dilation filter fir noise reduction (Gonzalez & Woods, 2002). Outlines of the individual seeds as well as their areas (surface area projections) were determined on the binary image. Each seed outline was rotated so that its major axis was parallel with the *x*-axis. The angle of orientation can be calculated as follows:

$$\theta = \frac{1}{2} \arctan\left(\frac{2\mu_{1,1}}{\mu_{2,0} - \mu_{0,2}}\right) \quad (1)$$

where  $\theta$  – the angle of the seed outline major axis;  $\mu_{1,1}$ ,  $\mu_{2,0}$  and  $\mu_{0,2}$  – central image moments.

The central image moments (Hu, 1962; Gonzalez & Woods, 2002) are defined by the following equation:

$$\mu_{p,q} = \sum_x \sum_y (x - x_c)^p (y - y_c)^q f(x, y) \quad (2)$$

where  $p, q$  – natural numbers;  $x_c, y_c$  – components of a centroid;  $f(x, y)$  – a digital image.

The last step is determining of the normalized central moment  $\eta_{2,0}$ :

$$\eta_{2,0} = \frac{\mu_{2,0}}{m_{0,0}^2} \quad (3)$$

where  $m_{0,0}$  – the outline area.

For purpose of the  $\eta_{2,0}$  comparison, the data were recalculated by the following formula:

$$n_{2,0} = \eta_{2,0} - \eta_{2,0}^0 \quad (4)$$

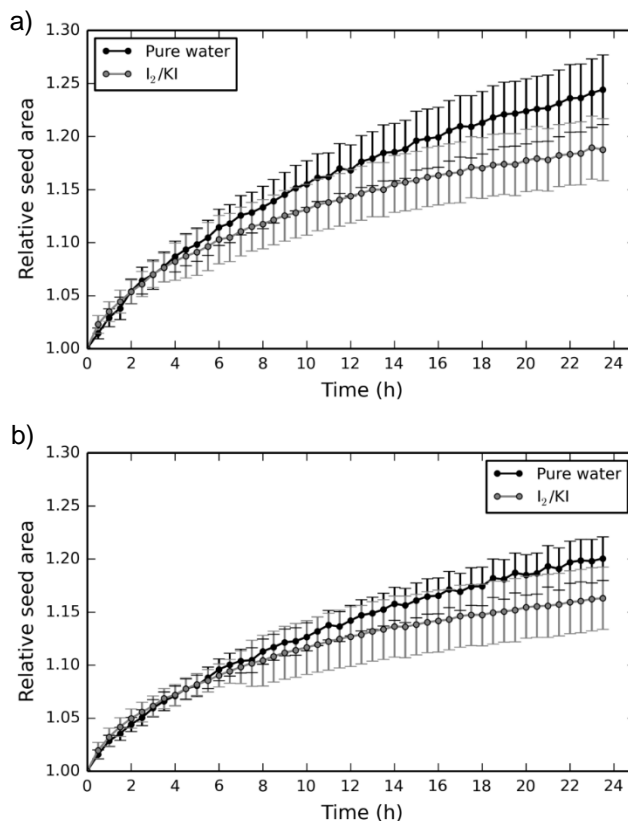
where  $n_{2,0}$  – the moved normalized central moment;  $\eta_{2,0}^0$  – initial normalized central moment.

The time development of the  $n_{2,0}$  contains a local maximum. For purpose of maximum detect, the data were fitted (using the least squares method) by polynomial of fourth degree. The desired maximum then corresponds to a local maximum of the

polynomial function. This procedure was applied for each seed. The increase of seed area (at all time steps), positions of the  $n_{2,0}$  maxima and seeds' moisture contents were compared by the Mann–Whitney–Wilcoxon test (Mann & Whitney, 1947).

## RESULTS AND DISCUSSION

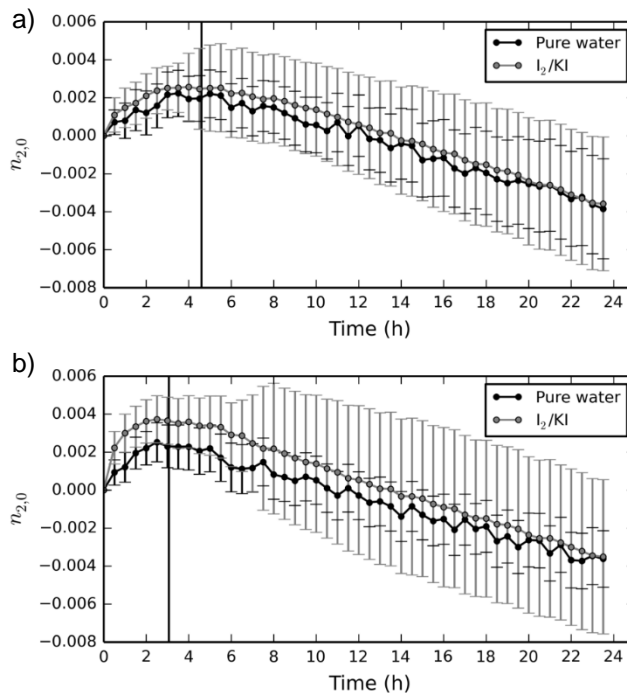
The Fig. 1 shows the time development of the seed area. The Fig. 1 (a) depicts the Tosca variety and the Fig. 1 (b) depicts the Steffi variety. The variants with pure water and  $I_2/KI$  water solution are represented by black and grey colour, respectively. In both cases (Tosca and Steffi variety) the time development of the seed area is possible divide into two stages. The first stage is shown by mildly higher seed area increase for variant with  $I_2/KI$  solution. During the second stage the seed area increase is higher for the variant with pure water. For the Tosca variety the statistically significant differences ( $p$ -value < 0.01) were found between 0.2 and 0.8 h and from 5.8 h for the first and the second stages, respectively. For the Steffi variety the statistically significant differences ( $p$ -value < 0.01) were found between 0.2 and 1.1 h and from 8.6 h for the first and the second stages, respectively.



**Figure 1.** Time development of the relative seed area. The variants with pure water and  $I_2/KI$ s water solution are represented by black and grey colour, respectively. (a) Tosca variety, (b) Steffi variety.

The water enters the wheat seeds through the micropyle and it is known that the water is located mainly in the embryo part during the initial state of the imbibition (Gruwel et al., 2001; Rathjen et al., 2009). We reported in our previous paper (Lev & Blahovec, 2017) that the embryo part where the micropyle is located significantly influences the seed cross area increase during the first hour of imbibition. Then the water enters the other seed parts and around 7 h after the start of the imbibition it enters the seed endosperm (Gruwel et al., 2001; Rathjen et al., 2009). Results in the Fig. 1 show that the embryo and the other seed parts respond differently to the attendance of the I<sub>2</sub>/KI solution. It seems that the endosperm plays important role in this process. This idea is supported by the fact that at the moment when the water or I<sub>2</sub>/KI solution enters into endosperm, the other parts of the seed are almost fully saturated by water and/or I<sub>2</sub>/KI water solution (Rathjen et al., 2009). Also this is supported by our experiments that will be published later.

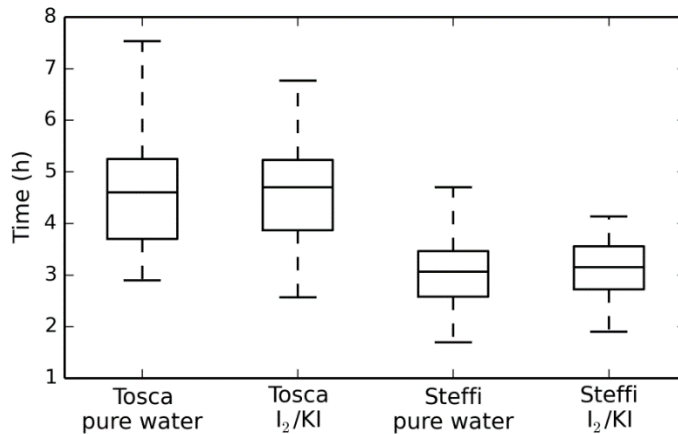
The Fig. 2 depicts the time development of the normalized image moment  $n_{2,0}$ . This parameter corresponds with the ratio of the seed length and width so the increase of the  $n_{2,0}$  corresponds with the relative seed elongation. The time development of the  $n_{2,0}$  is similar for both varieties and for both test variants. For variants with I<sub>2</sub>/KI solution, mildly higher increase during the initial phase of the imbibition is noticeable (primarily for Steffi variety). This is probably due to the higher volume increase of the embryo part in this phase.



**Figure 2.** Time development of the moved normalized central moment  $n_{2,0}$ . The variants with pure water and I<sub>2</sub>/KI water solution are represented by black and grey colour, respectively. (a) Tosca variety, (b) Steffi variety. Vertical line in the plots denotes the mean values of the both  $n_{2,0}$  maxima (the time differences between them is approximately 1 min. for all cases).



The time development of the  $n_{2,0}$  contains a characteristic point (maximum) and the position of the point is with high probability connected with the water movement in the seed (Lev et al., 2017). A comparison of the  $n_{2,0}$  maxima positions is depicted in the Fig. 3. The  $n_{2,0}$  maximum for Tosca variety was approximately 4.5 h and any statistical significant difference was not found ( $p$ -value: 0.30) between the variant with pure water and the variant with  $I_2/KI$  solution. The  $n_{2,0}$  maximum for Steffi variety was approximately at 3 h and any statistical significant difference between the variant with pure water and the variant with  $I_2/KI$  solution was also not found ( $p$ -value: 0.26).



**Figure 3.** Comparison of the  $n_{2,0}$  maximums detected during imbibition. Mean values, quartiles, outside values are depicted in the box-plot.

The behaviour described above could be significantly influenced by amount of water absorbed during imbibition. The seeds moisture content after experiment was assessed for this reason. The average moisture contents for Tosca variety were 29.17% and 28.56% (standard deviations: 1.85% and 2.26%) for the variants with pure water and the  $I_2/KI$  solution, respectively. For Steffi variety they were 27.62% and 27.58% (standard deviations: 1.27% and 1.79%) for the variants with pure water and the  $I_2/KI$  solution, respectively. No statistically significant differences between the variants with pure water and  $I_2/KI$  solution were found. It confirms that the behaviour described above is connected with  $I_2/KI$  solution attendance.

## CONCLUSIONS

The  $I_2/KI$  solution influences the seed swelling during imbibition. The influence is different during initial part of imbibition (appr. 1 h) and again during the next germination phases at 5 and 8 h for Tosca and Steffi, respectively. The changes of the shape and mass during seed germination in both liquids (pure water and  $I_2/KI$  solution) exhibits similar time development.

**ACKNOWLEDGEMENTS.** This work was supported by the Internal Grant Agency of the Czech University of Life Sciences Prague, Project No. 31120/1312/3110, (2017)

## REFERENCES

- Abenavoli, M. R., Cacco, G., Sorgonà, A., Marabottini, R., Paolacci, A.R., Ciaffi, M. & Badiani, M. 2006. The inhibitory effects of coumarin on the germination of durum wheat (*Triticum turgidum* ssp. durum, CV. Simeto) seeds. *J. Chemical Ecology* **32**, 489–506.
- Alvarado, V. & Bradford, K.J. 2002. A hydrothermal time model explains the cardinal temperatures for seed germination. *Plant Cell Environ* **25**, 1061–1069.
- ASABE Standards. 2006. S358.2 Moisture measurement-forages. St. Joseph, Michigan, USA.
- Becker, H.A. 1960. On the Absorption of Liquid Water by the Wheat Kernel. *Cereal Chem.* **37**, 309–323.
- Bewley, J.D. 1997. Seed germination and dormancy. *Plant Cell* **9**, 1055–1066.
- Dell, B. 1980. Structure and function of the strophliar plug in seeds of *Albizia lophantha*. *American Journal of Botany* **67**, 556–563.
- Dell'Aquila, A.D. 2006. Computerized seed imaging: a new tool to evaluate germination quality. *Communications in Biometry Crop Sci.* **1**, 20–31.
- Dong, K., Zhen, S., Cheng, Z., Cao, H., Ge, P. & Yan, Y. 2015. Proteomic analysis reveals key proteins and phosphoproteins upon seed germination of wheat (*Triticum aestivum* L.). *Frontiers in Plant Science* **6**, 1–14.
- Finch-Savage, W.E., Cadman, C.S., Toorop, P.E., Lynn, J.R. & Hilhorst, H.W. 2007. Seed dormancy release in *Arabidopsis Cvi* by dry after-ripening, low temperature, nitrate and light shows common quantitative patterns of gene expression directed by environmentally specific sensing. *Plant Journal* **51**, 60–78.
- Gonzalez, R.C. & Woods, R.E. 2002. Digital image processing. prentice hall, New Jersey, USA.
- Gruwel, M.L.H., Chatson, B., Yin, X.S. & Abrams, S. 2001. A magnetic resonance study of water uptake in whole barley kernels. *Int. J. Food Sci. Technol.* **36**, 161–168.
- Harb, A.M. 2013. Reserve mobilization, total sugars and proteins in germinating seeds of durum wheat (*Triticum durum* Desf.) under water deficit after short period of imbibition. *Jordan J. Biological Sci.* **6**, 67–72.
- Hu, M.K. 1962. Visual pattern recognition by moment invariant. *IRE Trans. Information Theory* **8**, 179–187.
- Kikuchi, K., Koizumi, M., Ishida, N. & Kano, H. 2006. Water uptake by dry beans observed by micro-magnetic resonance. *Imaging Annals of Botany* **98**, 545–553.
- King, R.W. 1984. Water Uptake in Relation to Pre-harvest Sprouting Damage in Wheat: Grain Characteristics. *Aust. J. Agric. Res.* 1984, **35**, 337–345.
- Lancelot, E., Bertrand, D., Hanafi, M. & Jaillais, B. 2017. Near-infrared hyperspectral imaging for following imbibition of single wheat kernel sections. *Vibrational Spectroscopy* **92**, 46–53.
- Lev, J. & Blahovec, J. 2017. Imbibition of wheat seeds: Application of image analysis. *Int. Agrophys.* **31**, 475–481.
- Lev, J., Chalupa, B. & Blahovec, J. 2017. Shape development of wheat seeds during germination. In: *16th International Scientific Conference on Engineering for Rural Development*, Jelgava, Latvia, 1220–1224.
- Manley, M., du Toit, G. & Geladi, P. 2011. Tracking diffusion of conditioning water in single wheat kernels of different hardnesses by near infrared hyperspectral imaging. *Analytica Chimica Acta* **686**, 64–75.
- Mann, H.B. & Whitney, D.R. 1947. On a test of whether one of two random variables is stochastically larger than the other, *The Annals of Mathematical Statistics* **18**, 50–60.
- McDonald, M.B., Vertucci, C.W. & Roos, E.E. 1988. Seed coat regulation of soybean seed imbibition. *Crop Sci.* **28**, 987–992.

- Nakanishi, T.M. & Matsubayashi, M. 1997. Water imaging of seeds by neutron beam. *Bioimages* **5**, 45–48.
- Rathjen, J.R., Strounina, E.V. & Mares, D.J. 2009. Water movement into dormant and non-dormant wheat (*Triticum aestivum* L.) grains. *J. Exp. Botany* **60**, 1619–1631.
- Ribeiro-Oliveira, J.P. & Ranal, M.A. 2017. Sample size and water dynamics on germinating diaspores: the first step for physiological and molecular studies on the germination process. *Plant Biosystems – An International Journal Dealing with all Aspects of Plant Biology*, <https://doi.org/10.1080/11263504.2017.1353551>.
- Shull, C.A. 1913. Semipermeability of seed coats. *Bot Gaz* **56**, 169–199.
- Weitbrecht, K., Müller, K. & Leubner-Metzger, G. 2011. First off the mark: early seed germination. *J. Exp. Botany* **62**, 3289–3309.
- Wiwart, M., Moś, M. & Wójtowicz, T. 2006. Studies on the imbibition of triticale kernels with a different degree of sprouting, using digital shape analysis. *Plant Soil and Environ* **52**, 328–334.

## Effect of nitrogen fertilization on winter wheat yield and yield quality

L. Litke<sup>1,2,\*</sup>, Z. Gaile<sup>1</sup> and A. Ruža<sup>1</sup>

<sup>1</sup>Latvia University of Life Sciences and Technologies, Faculty of Agriculture, Institute of Agrobiotechnology, Lielā iela 2, LV–3001, Jelgava, Latvia

<sup>2</sup>Latvia University of Life Sciences and Technologies, Faculty of Agriculture, Research and Study Farm "Pēterlauki", Lielā iela 2, LV-3001, Jelgava, Latvia

\*Correspondence: linda.litke@llu.lv

**Abstract.** Wheat (*Triticum aestivum* L.) is the most common cereal, which is grown in Latvia. Nowadays, farmers are trying to get high grain yields in line with food quality, at the same time trying to minimize production costs and to use environmentally friendly technologies. The objective of this experiment was to clarify the impact of nitrogen fertilization on winter wheat yield and yield quality under two soil tillage systems and after two forecrops. Trials were conducted at the Research and Study farm 'Peterlauki' of Latvia University of Agriculture (56° 30.658' N and 23° 41.580' E). Researched factors were (1) crop rotation (wheat/wheat and oilseed rape (*Brassica napus* ssp. *oleifera*)/wheat), (2) soil tillage (traditional soil tillage with mould-board ploughing at a depth of 22–24 cm and reduced soil tillage with disc harrowing at a depth below 10 cm), (3) nitrogen fertilizer rate (altogether eight rates: N0 or control, N60, N90, N120(90+30), N150(90+60), N180(90+60+30), N210(90+70+50), and N240(120+60+60)), and (4) conditions of the growing seasons 2014/2015, 2015/2016 and 2016/2017. The results indicate that winter wheat yield has been significantly affected by soil tillage, nitrogen fertilizer rate ( $p < 0.001$ ) and forecrop ( $p < 0.05$ ). Three-year research confirmed significant yield increase until the nitrogen fertilizer rate N180. Significantly higher average grain yield was obtained under traditional soil tillage. Nitrogen fertilizer affected significantly all tested yield quality indicators ( $p < 0.001$ ). Increase of nitrogen fertilizer rate secured significant increase of winter wheat grain quality indices, except starch content, after both forecrops and in both soil tillage variants. Values of yield quality indicators increased significantly enhancing N-rate from N150 up to N210

**Key words:** nitrogen fertilization, grain quality, winter wheat, soil tillage.

### INTRODUCTION

In recent years, wheat areas have increased in Latvia. Winter wheat is the most widely grown winter cereal in Latvia and farms are increasingly striving to use agrotechnical measures that reduce production costs. Nowadays, not only yield amount but also the quality of the produced grain is important, because the quality of the grains determines their direction of use. That is why farmers are trying to get high grain yields in line with food (accepted for bread baking) quality, while minimize production costs and using environmentally-friendly technologies.

One of the most important agro-technical measures is crop rotation. It is mentioned that the unfavourable preceding crops decrease significantly the yield of wheat and yield losses can be up to 10% (Sieling & Christen, 2015). In recent years, wheat growers have increasingly used short crop rotation. Wheat is mostly grown after oilseed rape or repeated several years after wheat, and this short crop rotation is combined with reduced soil tillage. Soil tillage is also an important agrotechnical measure. In literature, information about the effect of soil tillage impact on wheat yield and quality differs. Some authors (e.g. Seibutis et al., 2009) found that the wheat grain yield was not significantly affected by different soil tillage methods (conventional tillage – ploughing at 20–22 cm depth, presowing tillage – shallow seed bed preparation at 4–5 cm depth and reduced tillage – stubble cultivation at 10–12 cm depth were compared), but others (e.g. Šíp et al., 2013) indicated that soil tillage system effect on grain yield is significant (conventional tillage – ploughing to 22 cm depth and reduced soil tillage – surface stubble ploughing at 8–10 cm depth were compared). Moreover, soil tillage can affect nitrogen leaching (Stenberg et al., 1999).

Optimal nutrient provision is an important factor to get high yield with high grain quality. Nitrogen is one of the most important elements of plant nutrition, which often to a great extent determines not only wheat yield level, but especially grain baking quality. It is also one of the most mobile plant nutrients in the soil. Therefore, it is important to evaluate the use of high nitrogen fertilizer rates, because unsuitable nitrogen doses lead to increased nitrate leaching (Huang et al., 2018) which contributes to eutrophication of surface waters. In Latvia, nitrogen influence on wheat yields has been much studied, but with the development of agrotechnology and changing varieties (implementing high yielding ones), the effect of nitrogen fertilization is also changing. Therefore, it is important to look for cost-effective and environmentally friendly rates of N-fertilizers in different tillage systems with different forecrops for winter wheat. The objective of this experiment was to clarify the nitrogen fertilization impact on winter wheat yield and yield quality under two soil tillage systems and after two forecrops.

The research was funded by the ‘State and European Union investment for encouragement in agriculture’ theme ‘Determination of maximal fertilizer norms for crops’.

## MATERIALS AND METHODS

Field trials using equal methodology during all research years were conducted at the Research and Study farm ‘Peterlauki’ of Latvia University of Agriculture (56° 30.658’ N and 23° 41.580’ E). Trials were arranged using split plot design in four replications. The researched factors were: (1) crop rotation (wheat/wheat and oilseed rape (*Brassica napus* ssp. *oleifera*)/wheat), (2) soil tillage (traditional soil tillage with mould-board ploughing at a depth of 22–24 cm and reduced soil tillage with disc harrowing at a depth below 10 cm), (3) nitrogen fertilizer rate (altogether eight rates: N0 or control, N60, N90, N120(90+30), N150(90+60), N180(90+60+30), N210(90+70+50), and N240(120+60+60)), and (4) the conditions of the growing season (trial was repeated three years: 2014/2015, 2015/2016 and 2016/2017).

Trials were conducted in loam soil, *Endocalcaric Abruptic Luvisol* (Cutanic, Hypereutric, Ruptic, Siltic, Protostagnic, Epiprotovetric). Soil agrochemical characteristics were as follows: in 2014/2015 – pH KCL = 6.3 (potentiometrically in

1 M KCl suspension), organic matter content (oxidizing the soil with potassium dichromate ( $K_2Cr_2O_7$ ))  $31 \text{ g kg}^{-1}$ , P content (Egner– Riehm (DL) method)  $30.08 \text{ mg kg}^{-1}$  and K content (Egner – Riehm (DL) method)  $131.14 \text{ mg kg}^{-1}$  of the soil; in 2015/2016 – pH KCL = 6.9; organic matter content  $24 \text{ g kg}^{-1}$ , P content  $107.69 \text{ mg kg}^{-1}$  and K content  $272.24 \text{ mg kg}^{-1}$  of the soil; in 2016/2017 – pH KCL = 7.2; organic matter content  $32 \text{ g kg}^{-1}$ , P content  $74.56 \text{ mg kg}^{-1}$  and K content  $171.81 \text{ mg kg}^{-1}$  of the soil.

Before sowing fertilizer was applied: P  $17.44\text{--}28.34 \text{ kg ha}^{-1}$  and K  $33.2\text{--}51.13 \text{ kg ha}^{-1}$  depending on a year. Weeds were controlled by herbicides in all years, but wheat diseases were controlled by fungicide application once per season (growth stage (GS) 51 – beginning of heading (Lancashire et al., 1991)).

Cultivar ‘Skagen’ was sown at the rate of  $450\text{--}500$  germinable seeds  $\text{m}^{-2}$ . ‘Skagen’ is characterized by a good winterhardiness, which is combined with disease resistance and baking quality, notably high and stable falling number.

In spring, when the vegetation had renewed, nitrogen fertilizer ( $NH_4NO_3$ ; N 34%) was applied for all variants, except the control variant N0. The whole rate of fertilizer was applied once for variants N60 and N90; rate was divided into two applications for variants N120 and N150, but into three applications – for variants N180 – N240. Second top-dressing was done at GS 29 (end of tillering) until 31 (early stem elongation) of winter wheat, but the third – at GS 47 (flag leaf sheet opening) until 51 (beginning of heading).

After harvesting the whole plots, yield was weighted, grain purity and moisture content detected, and yield data was recalculated to standard moisture (14%) and 100% purity. Wheat grain quality indices were analysed at the Grain and Seed Research laboratory of Latvia University of Agriculture using express method and standard methods. Crude protein (CP), gluten and starch content (%), volume weight ( $\text{g L}^{-1}$ ) and Zeleny index were detected by Infratec™ Grain Analyzer 1241 (FOSS); 1,000 grain weight was determined using standard method LVS EN ISO 520; the Hagberg falling number was measured by the Hagberg-Perten method according LVS EN ISO 3093.

Analysis of variance was used for data statistical processing. Bonferroni test was used for comparison of means; the differences were considered statistically significant when  $p < 0.05$ . Significantly different means were labelled with different letters (a,b,c,d,e,f,g,h or A, B) in superscript. Data processing was done using R-studio.

Meteorological conditions in all three trial years differed obviously from the long-term average data. Autumn of 2014 was long and cool, in 2015, it was relatively warm and dry, and similarly in 2016, it was warm and dry. All winters during the trial period were mild and favourable for good wheat overwintering. The vegetation renewed in mid-March in all years. In 2015, spring was moderately warm and wet; however, summer in June and August was dry, but July was characterized by high rainfall. In 2016, spring was warm with a little rainfall, but summer was very rainy. Spring of 2017 was warm with enough moisture; summer was rainy, especially with high rainfall in July, but in August the amount of precipitation decreased.

## RESULTS AND DISCUSSION

**Wheat yield.** The results showed that the average wheat grain yield was very high in all three years of research ( $4.72$  (N0) –  $9.45$  (N240)  $\text{t ha}^{-1}$ ) (Table 1). All the researched factors showed significant effect on grain yield. The effect of the nitrogen

fertilization on the wheat grain yield was the same in all years of study. Increase of nitrogen fertilization rate increased significantly the average grain yield in all years ( $p < 0.001$ ) if compared to control. This result corresponds with the findings of other researchers (Rieger et al., 2008; Sieling & Christen, 2015). All N rates used, from the lowest (N60) to the highest (N240), have a significant effect on grain yield increase if compared to control. In our trial a significant average yield increase was observed until the nitrogen fertilizer rate N180, and further increase of N rate (i.e. till N210 and N240) did not give a significant increase of wheat yield (Table 1).

**Table 1.** Winter wheat yield depending on nitrogen fertilizer rate, forecrop, soil tillage and growing seasons, t ha<sup>-1</sup>

Factors	N-rate, kg ha <sup>-1</sup>								Average
	N0	N60	N90	N120	N150	N 180	N210	N240	
Forecrops ( $p < 0.05$ )									
Oilseed rape	4.77 <sup>a</sup>	6.83 <sup>b</sup>	7.64 <sup>c</sup>	8.46 <sup>d</sup>	8.82 <sup>de</sup>	9.30 <sup>e</sup>	9.46 <sup>e</sup>	9.46 <sup>e</sup>	8.09 <sup>A</sup>
Wheat	4.68 <sup>a</sup>	6.69 <sup>b</sup>	7.34 <sup>c</sup>	8.34 <sup>d</sup>	8.71 <sup>de</sup>	9.18 <sup>ef</sup>	9.38 <sup>f</sup>	9.43 <sup>f</sup>	7.99 <sup>B</sup>
Soil tillage ( $p < 0.001$ )									
Traditional	4.83 <sup>a</sup>	7.05 <sup>b</sup>	7.71 <sup>b</sup>	8.63 <sup>c</sup>	8.87 <sup>cd</sup>	9.36 <sup>d</sup>	8.63 <sup>d</sup>	9.50 <sup>d</sup>	8.18 <sup>A</sup>
Reduced	4.76 <sup>a</sup>	6.57 <sup>b</sup>	7.23 <sup>b</sup>	7.97 <sup>c</sup>	8.36 <sup>cd</sup>	8.72 <sup>d</sup>	8.80 <sup>d</sup>	8.88 <sup>d</sup>	7.71 <sup>B</sup>
Growing seasons ( $p < 0.001$ )									
2014/2015	4.20 <sup>a</sup>	6.40 <sup>b</sup>	7.42 <sup>c</sup>	9.00 <sup>d</sup>	9.09 <sup>d</sup>	9.85 <sup>e</sup>	10.22 <sup>e</sup>	10.23 <sup>e</sup>	8.30 <sup>A</sup>
2015/2016	5.33 <sup>a</sup>	7.10 <sup>b</sup>	7.56 <sup>c</sup>	8.15 <sup>d</sup>	8.80 <sup>d</sup>	9.12 <sup>e</sup>	9.30 <sup>e</sup>	9.36 <sup>e</sup>	8.09 <sup>B</sup>
2016/2017	4.63 <sup>a</sup>	6.78 <sup>b</sup>	7.49 <sup>c</sup>	8.05 <sup>cd</sup>	8.40 <sup>de</sup>	8.74 <sup>e</sup>	8.75 <sup>e</sup>	8.75 <sup>e</sup>	7.72 <sup>C</sup>
Average	4.72 <sup>A</sup>	6.76 <sup>B</sup>	7.49 <sup>C</sup>	8.40 <sup>CD</sup>	8.76 <sup>D</sup>	9.24 <sup>E</sup>	9.42 <sup>E</sup>	9.45 <sup>E</sup>	×

a,b,c,d,e,f,g – yields labelled with different letters are significantly different in rows depending on N-rate; A, B, C – average yields labelled with different letters are significantly different depending on forecrop, soil tillage, growing season, and on average per trial period.

In the trial, soil tillage affected grain yield significantly ( $p < 0.001$ ). Similar results were observed in another study, which demonstrated that the soil tillage had a significant effect on grain yield (Šíp et al., 2013). Our result does not conform to the findings in other studies concluding that the grain yield is not significantly affected by different soil tillage methods (Seibutis et al., 2009; Šíp, 2009), and over the long term, the yield of winter wheat under reduced soil tillage did not differ markedly from that provided by conventional soil tillage (Šíp, 2009). In our trial, the grain yield was significantly lower in variant, where the reduced soil tillage system (4.76–8.88 t ha<sup>-1</sup>) was used if compared with the variant of traditional soil tillage (4.83–9.50 t ha<sup>-1</sup>).

Data mathematical processing shows that the forecrop had a significant ( $p < 0.05$ ) effect on the grain yield. The average grain yield was mostly higher in variant, where oilseed rape was the forecrop (Table 1). Similarly, Sieling et al. (2005) found that the wheat following oilseed rape provided a higher grain yield if compared with variant where wheat was grown after wheat. However, when wheat was grown in repeated sowings in three-year period, the third wheat crop reduced the yield by 20% if compared with the first crop. In our trial, average grain yield was 4.77–9.46 t ha<sup>-1</sup> when wheat was grown after oilseed rape depending on nitrogen rate, but in repeated wheat sowings average grain yield was 4.68–9.43 t ha<sup>-1</sup>. Winter wheat grain yields increased significantly until nitrogen rate N180 after both forecrops. Use of higher N-rates smoothed out yield differences depending of forecrop, e.g. in the variant where N240

was used, yields after oilseed rape and after wheat were almost equal: 9.46 and 9.43 t ha<sup>-1</sup>, respectively.

Despite the fact that winter wheat grain yield was very high on average per all research years (7.72–8.30 t ha<sup>-1</sup>, Table 1) it was significantly ( $p > 0.001$ ) affected by conditions in the growing season. In growing season 2014/2015, the highest grain yield was obtained, but it was the lowest in 2016/2017.

**Crude protein (CP) content.** Grain CP content is the most important indicator of wheat grain quality. CP content ranged from 8.7 to 13.7% on average per all trial years depending on nitrogen fertilizer rate (Table 2). Results show that the nitrogen fertilizer increased significantly ( $p < 0.001$ ) the CP content in grain. It coincides with the results of another study demonstrating that protein was significantly influenced by nitrogen treatment (Weber et al., 2008). Average CP content per trial period in wheat grain increased in correlation with the nitrogen rate increase. The highest protein content was established when the highest fertilizer rates were used. CP content in grain increased significantly until nitrogen fertilizer rate N180 (Table 2), and the next significant increase was observed when N rate was increased until N240. The standards for food quality wheat grain CP content is from 12% (<https://dzirnavnieks.lv/lv/graudu-piegadatajiem>). In our trial, average CP content, which conforms to food quality lowest border, was obtained using nitrogen fertilizer rate N180 (12.8%). Similarly, Weber et al. (2008) concluded from research in Germany, that N-rate 180 kg N ha<sup>-1</sup> is needed to obtained CP content suitable for bread baking.

**Table 2.** Winter wheat crude protein content depending on nitrogen fertilizer rate, forecrop, soil tillage and growing seasons, %

Factors	N-rate, kg ha <sup>-1</sup>								Average
	N0	N60	N90	N120	N150	N180	N210	N240	
Forecrops ( $p < 0.001$ )									
Oilseed rape	9.0 <sup>a</sup>	9.1 <sup>a</sup>	10.0 <sup>ab</sup>	10.7 <sup>b</sup>	12.0 <sup>c</sup>	13.9 <sup>cd</sup>	13.4 <sup>d</sup>	13.7 <sup>d</sup>	11.4 <sup>A</sup>
Wheat	8.5 <sup>a</sup>	8.8 <sup>ab</sup>	9.5 <sup>bc</sup>	10.2 <sup>c</sup>	11.5 <sup>d</sup>	12.6 <sup>e</sup>	13.2 <sup>ef</sup>	13.8 <sup>f</sup>	11.0 <sup>B</sup>
Soil tillage ( $p < 0.001$ )									
Traditional	8.8 <sup>a</sup>	9.1 <sup>a</sup>	9.9 <sup>ab</sup>	10.5 <sup>b</sup>	11.8 <sup>c</sup>	12.9 <sup>cd</sup>	13.4 <sup>d</sup>	13.8 <sup>d</sup>	11.3 <sup>A</sup>
Reduced	7.8 <sup>a</sup>	8.3 <sup>ab</sup>	9.0 <sup>b</sup>	10.0 <sup>c</sup>	11.1 <sup>d</sup>	12.1 <sup>e</sup>	12.4 <sup>ef</sup>	13.1 <sup>f</sup>	10.5 <sup>B</sup>
Growing seasons ( $p < 0.001$ )									
2014/2015	8.0 <sup>a</sup>	8.2 <sup>a</sup>	8.4 <sup>a</sup>	8.8 <sup>a</sup>	10.8 <sup>b</sup>	11.4 <sup>bc</sup>	12.3 <sup>bc</sup>	12.7 <sup>c</sup>	10.0 <sup>A</sup>
2015/2016	9.8 <sup>a</sup>	9.8 <sup>a</sup>	10.6 <sup>ab</sup>	11.0 <sup>b</sup>	12.6 <sup>c</sup>	13.6 <sup>d</sup>	14.1 <sup>de</sup>	14.5 <sup>e</sup>	12.0 <sup>B</sup>
2016/2017	7.9 <sup>a</sup>	8.3 <sup>a</sup>	9.3 <sup>b</sup>	10.3 <sup>c</sup>	11.1 <sup>d</sup>	12.2 <sup>e</sup>	12.7 <sup>ef</sup>	13.2 <sup>f</sup>	10.6 <sup>C</sup>
Average	8.7 <sup>A</sup>	8.9 <sup>A</sup>	9.8 <sup>B</sup>	10.4 <sup>B</sup>	11.8 <sup>C</sup>	12.8 <sup>D</sup>	13.3 <sup>DE</sup>	13.7 <sup>E</sup>	×

a,b,c,d,e,f,g – yields labelled with different letters are significantly different in rows depending on N-rate; A, B, C – average yields labelled with different letters are significantly different depending on forecrop, soil tillage, growing seasons, and on average per trial period.

In the trial, the forecrop affected the wheat CP content significantly ( $p < 0.001$ ). This result does not conform to the findings of other research, where it was found that the choice of forecrops had no significant influence on the CP content of wheat grain (Jankowski et al., 2015). In our trial, oilseed rape was a better forecrop for higher winter wheat CP content formation if compared with repeated sowing wheat after wheat. Soil tillage also had a significant ( $p < 0.001$ ) impact on the protein content. Under traditional



tillage system (8.8–13.8%) the protein content of winter wheat was higher than under reduced soil tillage (7.8–13.2%) in all N-fertilizer variants (Table 2). Conditions of growing season affected significantly ( $p < 0.001$ ) the grain protein content. On average, the highest protein content was observed in growing season 2015/2016 (12.0%). Lower protein content in the grains was observed during the growing season 2014/2015 (10.0%) and 2016/2017 (10.6%). Seasons 2014/2015 and 2016/2017 characterize with a lot of cloudy days, and the lack of sun can be one of reasons of lower grain CP content; in addition, the highest yield was obtained in 2014/2015; thus more N can be used for yield formation.

**Gluten content.** Nitrogen fertilization has a positive impact on gluten content of grain and the content of nitrogen substances is closely related to the gluten content in grain (Kozlovsky et al., 2009). Results of our trial show that the average gluten content in wheat grain was 14.73–28.74% depending on nitrogen rate ( $p < 0.001$ ). Similarly to CP content, also gluten content in grain increased significantly until nitrogen fertilizer rate N180 and the next significant increase was secured by N240. The lowest border of food demand of gluten content was obtained when nitrogen fertilizer rate N180 was used. Forecrop also affected the gluten content in grain significantly ( $p < 0.01$ ). Higher average gluten content was observed when wheat was grown after oilseed rape. This result is similar to the findings of other research showing that significantly lower wet gluten concentrations were obtained when winter wheat was grown in monoculture if compared with winter wheat growing after oil plants (Jankowski et al., 2015). Soil tillage affected the gluten content significantly ( $p < 0.001$ ). Under traditional soil tillage system the gluten content was higher than under reduced soil tillage. Results show that the growing season also had a significant ( $p < 0.001$ ) impact on gluten content, and explanation can be the same as for CP content (see above).

**Table 3.** Average winter wheat quality indicators depending on nitrogen fertilizer rate

Grain quality indicators	N-rate, kg ha <sup>-1</sup>								Average
	N0	N60	N90	N120	N150	N180	N210	N240	
Gluten content (%)	14.73 <sup>a</sup>	14.91 <sup>a</sup>	16.89 <sup>b</sup>	18.78 <sup>b</sup>	22.79 <sup>d</sup>	25.90 <sup>e</sup>	27.47 <sup>ef</sup>	28.74 <sup>f</sup>	21.24
Zeleny index	17.58 <sup>a</sup>	18.52 <sup>a</sup>	22.85 <sup>b</sup>	27.90 <sup>c</sup>	37.14 <sup>d</sup>	45.11 <sup>e</sup>	50.57 <sup>f</sup>	54.61 <sup>f</sup>	34.19
Starch content (%)	70.66 <sup>a</sup>	70.89 <sup>a</sup>	70.39 <sup>ab</sup>	69.69 <sup>b</sup>	68.32 <sup>c</sup>	67.13 <sup>d</sup>	66.63 <sup>de</sup>	66.13 <sup>e</sup>	68.74
Volume weight (kg hl <sup>-1</sup> )	76.13 <sup>a</sup>	76.76 <sup>ab</sup>	77.91 <sup>ac</sup>	78.74 <sup>ad</sup>	79.55 <sup>bcd</sup>	80.24 <sup>cd</sup>	80.57 <sup>cd</sup>	80.33 <sup>cd</sup>	78.77
Falling number, s	314 <sup>a</sup>	334 <sup>ab</sup>	379 <sup>bc</sup>	361 <sup>cd</sup>	371 <sup>c</sup>	382 <sup>d</sup>	383 <sup>d</sup>	381 <sup>d</sup>	363
1,000 grain weight, g	44.84 <sup>a</sup>	46.24 <sup>ab</sup>	46.38 <sup>ac</sup>	46.34 <sup>ad</sup>	47.07 <sup>bcd</sup>	47.73 <sup>bcd</sup>	48.09 <sup>bcd</sup>	47.76 <sup>bcd</sup>	46.80

a,b,c,d,e,f,g – yields labelled with different letters are significantly different in rows depending on N-rate.

**Zeleny index.** Zeleny index determines quantity and quality of gluten proteins (Kozlovsky et al., 2009). Results show that the nitrogen fertilizer increase has a significant ( $p < 0.001$ ) impact on Zeleny index (Table 3). Similar results were obtained in other studies (Weber et al., 2008; Liniņa & Ruža, 2012). In our trial, significant increase of Zeleny index was observed until nitrogen fertilizer rate N210. Zeleny index was affected significantly ( $p < 0.001$ ) by the forecrop. Higher results were observed when wheat was grown after oilseed rape if compared to wheat growing after wheat. In addition, soil tillage had a significant ( $p < 0.001$ ) effect on Zeleny index. Under

traditional soil tillage system Zeleny index was higher than under reduced soil tillage. Growing season affected the Zeleny index significantly ( $p < 0.001$ ). More favourable conditions for higher Zeleny index formation were in 2015/2016 when also the highest average CP content in grain accumulated.

**Starch content.** Starch content was affected significantly ( $p < 0.001$ ) by the use of nitrogen fertilizer, but this indicator decreased with increased nitrogen fertilizer rate. Average starch content was 66.13–70.89% depending on N-rate. Our study confirmed the negative relationship between protein and starch content in grain once more: protein content decreased if starch content increased. In our trial, forecrop ( $p < 0.001$ ), soil tillage ( $p < 0.001$ ) and conditions of growing season ( $p < 0.001$ ) had a significant impact on starch content. All obtained starch results depending on mentioned factors were opposite to CP content. Criterion for starch content is not set for winter wheat if it is used for bread baking.

**Volume weight.** It is an indicator of flour outcome at milling enterprise (Kozlovsky et al., 2009). Higher volume weight indicated better grain maturation and richness with nutrients; more flour can be obtained from such grain during processing as well (Liniņa & Ruža, 2015). Our results showed a significant effect of nitrogen fertilizer on volume weight, which agrees with results of other research (Skudra & Ruža, 2016). Average per trial period volume weight was between 76.13–80.57 kg hl<sup>-1</sup> depending on nitrogen fertilizer rate. Increase of nitrogen fertilizer rate also increased volume weight, and a significant ( $p < 0.001$ ) increase was observed until fertilizer rate N150; applying higher nitrogen fertilizer rates volume weight did not increase significantly. Volume weight demand for food grain is 75.0 kg hl<sup>-1</sup> and above. Our results showed volume weights that were in line with food quality demands even in the control (76.13 kg hl<sup>-1</sup>). Result showed that the forecrop had no significant ( $p = 0.062$ ) impact on volume weight. This result does not agree with other findings stating that the volume weight was significantly affected by different forecrops, and winter wheat grown in monoculture was characterized by significantly lower volume weight (Jankowski et al., 2015). Our results showed significant ( $p < 0.001$ ) impact of soil tillage on volume weight. Under reduced soil tillage volume weight was higher than in variant with traditional soil tillage. Other authors stated that the conditions of growing season had a significant effect on volume weight (Skudra & Ruža, 2016). Our findings agree with it: the highest wheat grain volume weight was produced in 2016/2017, but the lowest – in 2015/2016.

**Falling number.** It indicates the rate of alpha-amylase activity in grain. Falling number increases up to its maximum value at full maturity during maturation. It is mentioned in literature that the falling number value is mostly influenced by genetic and environmental factors, especially weather conditions at maturation stage (Grausruber et al., 2000). In our trial, falling number values ranged between 314–383 s on average per all years. It is mentioned by Perten already in 1964 that bread volume is diminished and a dry crumb results when the falling number is greater than 350 s. Increase of nitrogen fertilizer rate had a significant ( $p < 0.001$ ) impact on falling number increase until nitrogen fertilizer rate N180. This result does not agree with other studies, where significant effect of nitrogen fertilizer application on falling number values was not found (Erekul, 2012; Liniņa & Ruža, 2012). Criterion for falling number in Latvia is 280 s; a slightly higher result was obtained even in control variant with N0 (314 s) in our trial (Table 3). When growing wheat after oilseed rape, we observed a tendency that the falling number was slightly higher than in variant where wheat after wheat was grown.

This coincides with the observations of Jankowski et al. (2015), who found that the wheat grown after winter rapeseed characterizes by higher falling number if compared with winter wheat grown in monoculture. Results show that the soil tillage system had no significant ( $p > 0.05$ ) impact on value of falling number. Growing season affected significantly ( $p < 0.001$ ) the falling number in our trial. This agrees with conclusion of Gooding et al. (2003) that the falling number is a grain quality indicator whose value is affected by climate conditions during the grain filling period, and the precipitation during maturation has a greater impact on falling number than fertilization. Despite the fact that falling number in all trial years exceeded demands for bread baking, it was the highest in 2015/2016.

**1,000 grain weight (TGW)** characterizes the size of seed and is used as one of the parameters for assessing the quality of grain. Grains with higher TGW have better milling quality and ensure better emergence (Protić et al., 2013). At the same time, TGW is also a yield component. Results showed that the nitrogen fertilizer rate affected the TGW significantly ( $p < 0.001$ ). Effect of N-rate on TGW is shown also by Linina & Ruza (2015). The average TGW depending on nitrogen fertilizer rates was 44.84–48.09 g, total increase of 3.24 g was observed; although more expressed TGW increase was observed until nitrogen fertilizer rate N150. Opposite results were obtained by Protic et al. (2007) and Linina & Ruza (2015). Linina & Ruza (2015) found that increase of nitrogen fertilizer rates causes significant decrease of TGW, and the highest TGW was obtained when the nitrogen fertilizer rates N60 and N90 were used. Data mathematical processing showed a significant ( $p < 0.001$ ) effect of forecrop on the TGW. Average TGW decreased when wheat was grown after wheat if compared with growing wheat after oilseed rape. This result agrees with another study stating that the wheat as preceding crop mainly decreased the TGW (Sieling & Christen, 2015). Seibutis et al. (2009) found that soil tillage had no significant impact on TGW, but the slightly higher TGW was found in the wheat monocrop if compared with other forecrops. Our results showed that the soil tillage had a significant ( $p < 0.001$ ) impact on TGW. When comparing the soil tillage systems, it was observed that the higher average TGW was ensured in reduced soil tillage variant (48.88 g) than in traditional soil tillage (46.94 g) variant. Similarly to the results of Skudra & Ruža (2016), the growing season also had a significant ( $p < 0.001$ ) impact on TGW. TGW significantly varied over the years, and the highest average TGW was observed in the growing season 2016/2017 (48.63 g), but the smallest grain was observed in 2015/2016 (44.79 g).

## CONCLUSIONS

Winter wheat yield has been significantly affected by nitrogen fertilizer rate, soil tillage, conditions of growing year ( $p < 0.001$ ) and forecrop ( $p < 0.05$ ). Average yield increased significantly until the nitrogen fertilizer rate N180. Significantly higher average grain yield was obtained under traditional soil tillage and in variants where the forecrop was oilseed rape.

Increase of nitrogen fertilizer rate increased significantly almost all tested yield quality indicators ( $p < 0.001$ ), except starch content, after both forecrops and in both soil tillage variants.

Crude protein and gluten content in grain increased on average until nitrogen fertilizer rate N210. However, protein and gluten content conforming to lowest food quality demands was obtained from nitrogen fertilizer rate N180. Significant increase of volume weight was observed until fertilizer rate N150, but that of falling number – until nitrogen fertilizer rate N180; both quality indicators conform for food quality in all fertilization variants, even in control variant.

ACKNOWLEDGEMENTS. Research was financed by the project ‘Determination of maximal fertilizer norms for crops’ which is carried out within the framework of ‘State and European Union investment for encouragement in agriculture’, but the paper is prepared thanks to funding of the State research program ‘Agricultural Resources for Sustainable Production of Qualitative and Healthy Food in Latvia: project No. 1 ‘Sustainable Use of Soil Resources and Abatement of Fertilisation Risks (SOIL)’.

## REFERENCES

- Ereku, O., Götz, K.-P. & Koca, Y.O. 2012. Effect of sulphur and nitrogen fertilization on bread-making quality of wheat (*Triticum aestivum* L.) varieties under Mediterranean climate conditions. *Journal of Applied Botany and Food Quality* **85**, 17–22.
- Gooding, M.J., Ellis, R.H., Shewry, P.R. & Schofield, J.D. 2003. Effects of Restricted Water Availability and Increased Temperature on the Grain Filling, Drying and Quality of Winter Wheat. *Journal of Cereal Science* **37**, 295–309.
- Grausgruber, H., Oberforster, M., Werteker, M., Ruckebauer, P. & Vollmann, J. 2000. Stability of quality traits in Austrian-grown winter wheats. *Field Crop Research* **66**, 257–267.
- Huang, P., Zhang, J., Zhu, A., Li, X., Ma, D., Xin, X., Zhang, C., Wu, S., Garland, G. & Pereira, E.I.P. 2018. Nitrate accumulation and Leaching potential reduced by coupled water and nitrogen management in the Huang-Huai-Hai Plain. *Sciences of the Total Environment* **610–611**, 1020–1028.
- Jankowski, K. J., Kijewski, Ł. & Dubis, B. 2015. Milling quality and flour strength of the grain of winter wheat grown in monoculture. *Romanian Agricultural Research* **32**, 191–200.
- Kozlovský, O., Balík, J., Černý, J., Kulhánek, M., Kos, M. & Prášilová, M. 2009. Influence of nitrogen fertilizer injection (CULTAN) on yield, yield components formation and quality of winter wheat grain. *Plant Soil Environ* **55**(12), 536–543.
- Lancashire, P.D., Bleiholder, H., Van Den Boom, T., Langeluddeke, P., Stauss, R., Weber, E. & Witzinger, A. 1991. A uniform decimal code for growth stages of crops and weeds. *Ann. appl. Biol.* **119**, 561–601.
- Linīņa, A. & Ruža, A. 2012. Cultivar and Nitrogen Fertiliser Effects on Fresh and Stored Winter Wheat Grain Quality Indices. *The Journal of Latvian Academy of Sciences* **66**, 177–184.
- Linīņa, A. & Ruža, A. 2015. Influence of nitrogen fertilizer and meteorological conditions on winter wheat grain physical indices. In: *Proceedings of the Scientific and Practical Conference Harmonious Agriculture*, Jelgava, LLU, 70–73 (in Latvian).
- Perten, H. 1964. Application of the falling number method for evaluating alpha-amylase activity. *Cereal Chemistry* **41**, 127–140.
- Protic, R., Jovin, P., Protic, N., Janovic, S. & Jovanovic, Ž. 2007. Mass of 1,000 grains in several winter wheat genotypes at different dates of sowing and rates of nitrogen fertilizer. *Romanian Agricultural Research* **24**, 39–42.
- Protic, R., Todoroc, G., Protič, N., Kostič, M., Delic, D., Filipovič, M. & Ugrenovič, V. 2013. Variation of grain weight per spike of wheat depending on variety and seed size. *Romanian Agricultural Research* **30**, 51–55.

- Rieger, S., Richner, W., Streit, B., Frossard, E. & Liedgens, M. 2008. Growth, yield, and yield components of winter wheat and the effects of tillage intensity, preceding crops, and N fertilization. *European Journal of Agronomy* **28**, 405–411.
- Seibutis, V., Deveikyte, I. & Feiza, V. 2009. Effects of short crop rotation and soil tillage on winter wheat development in central Lithuania. *Agronomy Research* **7**, 471–476.
- Sieling, K. & Christen, O. 2015. Crop rotation effects on yield of oilseed rape, wheat and barley and residual effects on the subsequent wheat. *Archives of Agronomy and Soil Science* **61**, 1531–1549.
- Sieling, K., Stahl, C., Winkelmann, C. & Christen, O. 2005. Growth and yield of winter wheat in the first three years of a monoculture under varying N fertilization in NW Germany. *European Journal of Agronomy* **22**, 71–84.
- Šíp, V., Růžek, P., Chrpová, J., Vavera, R. & Kusá, H. 2009. The effect of tillage practice, input level and environment on the grain yield of winter wheat in the Czech Republic. *Field Crops Research* **113**, 131–137.
- Šíp, V., Vavera, R., Chrpová, J., Kusa, H. & Růžek, P. 2013. Winter wheat yield and quality related to tillage practice, input level and environmental condition. *Soil and Tillage Research* **132**, 77–85.
- Skudra, I. & Ruža, A. 2016. Evaluation of the influencing factors for the 1,000 grain weight and grain volume weight of winter wheat. In: *Proceedings of the Scientific and Practical Conference Harmonious Agriculture*. Jelgava, pp. 217–218 (in Latvian).
- Stenberga, M., Aronssonb, H., Lindénc, B., Rydberga, T. & Gustafsonb, A. 1999. Soil mineral nitrogen and nitrate leaching losses in soil tillage systems combined with a catch crop. *Soil and Tillage Research* **50**, 115–125.
- Weber, E.A., Graeff, S., Koller, W.-D., Hermann, W., Merkt, N. & Claupein, W. 2008. Impact of nitrogen amount and timing on the potential of acrylamide formation in winter wheat (*Triticum aestivum* L.). *Field Crops Research* **106**, 44–52.

## **The indigenous arbuscular mycorrhizal fungal colonisation potential in potato roots is affected by agricultural treatments**

K. Loit<sup>1,2,\*</sup>, L. Soonvald<sup>1</sup>, M. Kukk<sup>1</sup>, A. Astover<sup>2</sup>, E. Runno-Paurson<sup>3</sup>,  
T. Kaart<sup>4</sup> and M. Öpik<sup>5</sup>

<sup>1</sup>Estonian University of Life Sciences, Chair of Plant Health, Kreutzwaldi 1, EE51014 Tartu, Estonia

<sup>2</sup>Estonian University of Life Sciences, Chair of Soil Science, Kreutzwaldi 1, EE51014 Tartu, Estonia

<sup>3</sup>Estonian University of Life Sciences, Chair of Crop Science and Plant Biology, Kreutzwaldi 1, EE51014 Tartu, Estonia

<sup>4</sup>Estonian University of Life Sciences, Chair of Animal Breeding and Biotechnology, Kreutzwaldi 1, EE51014 Tartu, Estonia

<sup>5</sup>University of Tartu, Department of Botany, 40 Lai St., EE51005 Tartu, Estonia

\*Correspondence: [kaire.loit@emu.ee](mailto:kaire.loit@emu.ee)

**Abstract.** There is an urgent need to develop novel approaches to enhance sustainable agriculture while not reducing crop yields. Arbuscular mycorrhizal (AM) fungi establish symbiotic associations with most crop plants improving plant performance and soil health. This study investigated the extent of colonisation of potato roots by indigenous AM fungi in the arable soil under conventional and organic farming systems. Potato roots had greater AM fungal colonisation levels under organic than conventional farming, though in general, root colonisation levels were extremely low in both farming systems. Potato root AM fungal colonisation was lower with higher soil P content and higher with higher annual C input. Trap plant root AM fungal colonisation was considerably higher than in field potato roots and showed that soil mycorrhizal inoculum potential was higher in organic than in conventional farming. Thus, the positive impact of manure application in organic fields to the potato AM fungal colonisation can be explained by previous higher total annual C fresh organic matter input and lower soil P content under treatment. Furthermore, the natural AM fungal abundance in the soil was sufficient to colonise trap plant roots, suggesting a low mycorrhizal dependence of the studied potato cultivar.

**Key words:** Cropping systems, Glomeromycota, management practices, *Solanum tuberosum* L.

### **INTRODUCTION**

Arbuscular mycorrhizal (AM) fungi are the most widespread symbionts of plant roots in terrestrial habitats. AM fungi provide a major contribution to plant nutrient uptake in most ecosystems and thus improve plant growth (Smith & Read, 2008; Smith & Smith, 2011). In addition to nutritional benefits, AM fungi protect host plants against root and shoot pathogens, including nematodes, other fungi and viruses, and enhance host plant resistance to various abiotic stresses such as drought, salinity and high heavy

metal concentrations in soil (Augé et al., 2015; Pozo et al., 2015). The ecosystem services provided by AM fungi in agroecosystems make them an important group of soil biota to be managed for crop production both in conventional and sustainable systems (Gianinazzi et al., 2010; Mahmood & Rizvi, 2010; Bender et al., 2016).

Diversity and functioning of AM fungi in agroecosystems are affected by differences in management regimes. Intensive agricultural management approaches are characterised by high N and P inputs (Verbruggen & Kiers, 2010). Environmental conditions with high nutrient input cause a decrease in host plant resource allocation to AM fungi and therefore have a negative impact on AM fungal biodiversity and species richness (Mäder et al., 2000; Verbruggen et al., 2010). The low-input systems, on the other hand, promote AM fungal colonisation, because plants benefit from the AM fungi by increased soil nutrient uptake when these are available at low concentrations (Mäder et al., 2000).

AM fungi are native to agricultural soils and form a mutualistic symbiosis with the majority of crop plants (Douds et al., 2007). Potato is a non-grain crop of global importance but has one of the heaviest production demands for fertilizer and pesticide inputs of all vegetable crops (Wu et al., 2013). For plants such as potato, which have a low root density in soil, the AM symbiosis may be of particular significance in coping with P-deficiency stress in natural ecosystems (McArthur & Knowles, 1993). Previous inoculation studies have reported high levels of AM fungal colonisation in potato roots (Davies et al., 2005; Douds et al., 2007), but the knowledge about AM fungi colonising potato roots in field conditions under common agricultural practices is scarce.

Therefore, it is necessary to determine how the natural AM fungal colonisation in potato roots is affected by different agricultural management regimes.

We specifically asked: (1) How do the conventional and organic farming systems influence potato root AM fungal colonisation? (2) How do the conventional management treatments with different mineral fertilization levels and different organic treatments with cover crop and manure amendment influence potato root AM fungal colonisation? We characterized AM fungal colonisation of potato roots in field grown potato plants and in addition we determined AM fungal inoculum potential (IP) using a trap plant bioassay.

## MATERIALS AND METHODS

### Field experiment

The field site was located in Tartu, Estonia (58°22' N, 26°40' E). This field was established in 2008 as a part of the 5-year crop rotation experiment with two organic (OFS) and four conventional farming systems (CFS). In the crop rotation experiment, red clover (*Trifolium pratense* L.), winter wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.), potato (*Solanum tuberosum* L.) and barley (*Hordeum vulgare* L.) were grown in succession. In both organic management systems, a winter cover crop (CC) for green manure was used. Winter oilseed rape (*Brassica napus* L.) seeds as CC were sown at the rate of 6 kg ha<sup>-1</sup> before the potato cropping in September 2009 and ploughed under in April the year 2010. No cattle manure was added to one treatment (CC), whereas composted cattle manure (M) was added to the second organic treatment (CC+M). No fungicides, herbicides or insecticides were applied under organic systems. Weeds were removed mechanically. In a CFS, four mineral fertilizer treatments were used: N<sub>0</sub>P<sub>0</sub>K<sub>0</sub>,

N<sub>50</sub>P<sub>25</sub>K<sub>95</sub>, N<sub>100</sub>P<sub>25</sub>K<sub>95</sub>, and N<sub>150</sub>P<sub>25</sub>K<sub>95</sub>. Conventional systems were treated with several synthetic pesticides. Field operations and their timings are shown in Table S1.

**Table S1.** Field operations and their timings during year 2010 in the study site

Field operation	Conventional farming system	Organic farming system
Planting date	May 6 <sup>th</sup>	May 6 <sup>th</sup>
Planting rate	3 t ha <sup>-1</sup> 57,000 tubers ha <sup>-1</sup>	3 t ha <sup>-1</sup> 57,000 tubers ha <sup>-1</sup>
Harvest date	August 30 <sup>th</sup>	August 30 <sup>th</sup>
Fertilization	N <sub>0</sub> P <sub>0</sub> K <sub>0</sub> – not fertilized N <sub>50</sub> P <sub>25</sub> K <sub>95</sub> 1) May 4 <sup>th</sup> N <sub>20</sub> P <sub>25</sub> K <sub>95</sub> (Kemira Grow How Power 5:14:28 400 kg ha <sup>-1</sup> ) 2) June 7 <sup>th</sup> N <sub>30</sub> P <sub>0</sub> K <sub>0</sub> (AN* 34:0:0)	April 20 <sup>th</sup> Winter cover crop
	N <sub>100</sub> P <sub>25</sub> K <sub>95</sub> 1) May 4 <sup>th</sup> N <sub>20</sub> P <sub>25</sub> K <sub>95</sub> (Kemira Grow How Power 5:14:28 400 kg ha <sup>-1</sup> ) 2) June 7 <sup>th</sup> N <sub>60</sub> P <sub>0</sub> K <sub>0</sub> (AN* 34:0:0) 3) June 16 <sup>th</sup> N <sub>20</sub> P <sub>0</sub> K <sub>0</sub> (AN* 34:0:0)	April 20 <sup>th</sup> Winter cover crop + composted cattle manure 40 t ha <sup>-1</sup>
	N <sub>150</sub> P <sub>25</sub> K <sub>95</sub> 1) May 4 <sup>th</sup> N <sub>20</sub> P <sub>25</sub> K <sub>95</sub> (Kemira Grow How Power 5:14:28 400 kg ha <sup>-1</sup> ) 2) June 7 <sup>th</sup> N <sub>90</sub> P <sub>0</sub> K <sub>0</sub> (AN* 34:0:0) 3) June 16 <sup>th</sup> N <sub>40</sub> P <sub>0</sub> K <sub>0</sub> (AN* 34:0:0)	
Herbicide application	June 7 <sup>th</sup> Titus 25 DF (50 g ha <sup>-1</sup> ) (containing 12.5 g ha <sup>-1</sup> rimsulfuron)	No herbicides applied
Insecticide application	July 22 <sup>nd</sup> Fastac 50 (0.3 L ha <sup>-1</sup> ) (containing 15 g ha <sup>-1</sup> alpha-cypermethrin) August 6 <sup>th</sup> Decis 2.5 EC (0.2 L ha <sup>-1</sup> ) (containing 5 g ha <sup>-1</sup> deltamethrin)	No insecticides applied
Fungicide application	June 25 <sup>th</sup> Shirlan 500 SC (0.4 L ha <sup>-1</sup> ) (containing 200 g ha <sup>-1</sup> fluazinam) July 8 <sup>th</sup> and 22 <sup>nd</sup> Ridomil Gold MZ 68 (2.5 kg ha <sup>-1</sup> ) (containing 100 g ha <sup>-1</sup> metalaxyl-M + 1.6 kg ha <sup>-1</sup> mancozeb) August 6 <sup>th</sup> Ranman 400 SC (0.15 L ha <sup>-1</sup> ) (containing 60 g ha <sup>-1</sup> cyazofamid)	No fungicides applied

\*AN – ammonium salpeter.

Treatments were arranged in a systematic block design with each plot in four replications. The size of each test plot was 60 m<sup>2</sup>. Organic and conventional plots were separated by an 18 m long section of mixed grasses to avoid contamination with synthetic pesticides and mineral fertilizers. The distance between seed tubers was 27 cm, and the distance between rows was 70 cm. The soil of the trial field was *Stagnic Luvisol* (LVj) with sandy loam texture with a humus layer of 20–30 cm. No irrigation was used. The data of total annual carbon inputs from cover crops, straw, roots of pea, weeds and cattle manure (kg C ha<sup>-1</sup> y<sup>-1</sup>) to the soil before potato were obtained from earlier publications about this field trial (Kauer et al., 2015; Madsen et al., 2016).



Potato root samples were collected in July 2010 from plants of the locally bred potato cultivar 'Reet' (Tsahkna & Tähtjärvi, 2008). Three potato plants were sampled randomly from each of the four replicate plots of all treatments of both farming systems (a total of 72 samples). Roots were sampled at the potato flowering (BBCH60) stage (Hack et al., 2001). Root samples were dried with silica gel and preserved airtight at room temperature as described by Uibopuu et al. (2012).

### **Trap plant bioassay**

Narrowleaf plantain (*Plantago lanceolata* L.), as an AM host plant commonly used in experiments, was used to evaluate the mycorrhizal inoculum potential (IP) of field soil. Three soil samples were collected randomly from each of the four replicate plots of all treatments before soil tillage in spring 2011, and stored in darkness at 10 °C until use. Before the use in experiment, the three soil samples per plot were pooled and handled as one composite sample. Each composite sample was thoroughly mixed with autoclaved sand in a 1:1 v/v ratio to improve drainage and aeration of the soil mixture. *P. lanceolata* seeds were germinated in Petri dishes on moist filter paper, following Uibopuu et al. (2012). Three seedlings per pot were planted on January 2011 in plastic pots (13 x 15 cm, depth x diameter). One seedling was retained per pot after four weeks of growth. Plants were kept in a greenhouse under controlled conditions, watered as needed with tap water and grown for 3 months. At harvest, shoots and roots were separated and handled like described previously. The infectivity bioassay of Moorman & Reeves (1979) was used to quantify the relative density of colonising propagules of AM fungi.

### **Root staining and assessment of AM fungal root colonisation**

Root samples from both field and pot experiment were stained with Trypan blue according to Koske & Gemma (1989). Briefly, roots were cleared in 10% KOH, acidified with 1% HCl and stained with 0.01% trypan blue in lactoglycerol. Root colonisation by AM fungi was estimated using the magnified grid-line intersections method (McGonigle et al., 1990), by scoring 120 fields of view per sample under the compound microscope at 400x magnification, as described in Uibopuu et al. (2012). Total root length colonised was estimated.

### **Soil chemical analysis**

In mid-April 2010, before tillage, soil samples were collected from the bulk soil at a depth of 0–25 cm. Eight sub-samples were taken from each plot and mixed to obtain a composite sample for each plot. Soils were air-dried and sieved through a 2 mm sieve. Soil pH was determined in a 1 M KCl solution (1:2.5). Soil organic carbon ( $C_{org}$ ) was measured using the Tjurin method (Vorobeveva, 1998), and total nitrogen ( $N_{tot}$ ) concentration was measured using the Kjeldahl method (van Reeuwijk, 1995). The concentrations of plant-available nutrients in the soil (P, K, Ca and Mg) were determined by the ammonium lactate (AL) method (Egnér et al., 1960).

### **Statistical data analysis**

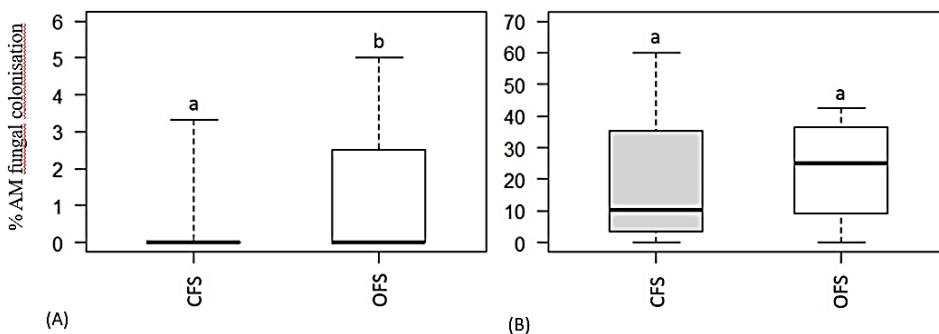
Statistical analysis was performed using R 3.22 (R Core Team 2015) within R-Studio environment (0.99.484; RStudio 2015). First, normal distribution (*Shapiro-Wilk* test) and equality of variances in treatment groups (*Levene* test) of data were explored. Then, the non-parametric *Kruskal-Wallis rank sum* test was conducted to

determine the differences in among treatments followed by the *Dunn's* test for pairwise comparison. For the *Dunn's* test, the *P*-values were adjusted according to the *Bonferroni correction*. When comparing only two groups, the non-parametric *Wilcoxon signed-rank* test was used. To study the relationship between soil parameters and AM fungal colonisation *Pearson's correlation* analysis was carried out.

## RESULTS AND DISCUSSION

### Potato root AM fungal colonisation

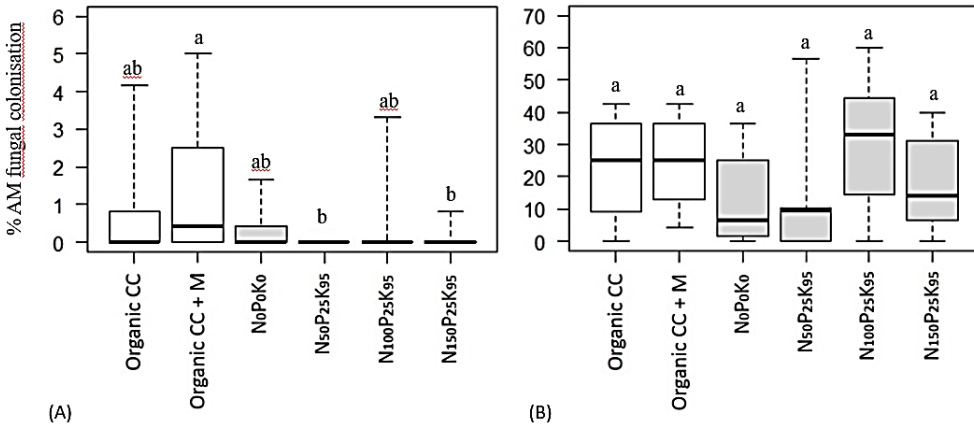
AM fungal colonisation level in roots of the potato cultivar 'Reet' was higher in OFS than CFS, though colonisation levels were very low in both systems (median 0%, maximum 3.3% in CFS and 5.0% in OFS; *Wilcoxon signed-rank* test,  $W = 415.5$ ,  $p = 0.007$ ; Fig. 1, A). Individual treatments influenced root AM fungal colonisation levels differently. AM fungal colonisation was higher in treatment CC+M than treatments N50 and N150 (*Kruskal-Wallis* test,  $\chi^2 = 11.85$ ,  $df = 5$ ,  $p = 0.037$ ; Fig. 2, A). There were no statistically significant differences among other treatments.



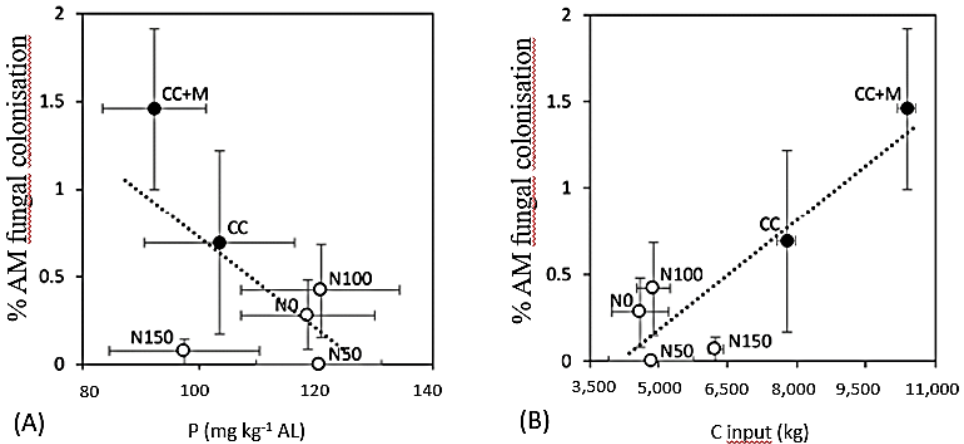
**Figure 1.** Median values ( $\pm$  min, max) of percent of arbuscular mycorrhizal (AM) fungal colonisation of potato roots in conventional (CFS) and organic farming systems (OFS) in the field trial (A) and narrowleaf plantain roots grown in pots in sand inoculated with soil from the same conventional and organic farming systems (B). Box plots indicate median (bold horizontal line), interquartile ranges (box) and minimum and maximum values (whiskers). Different letters above the boxes indicate statistically significant differences at  $P < 0.05$  among treatments (*Wilcoxon rank sum* test). Grey boxes – CFS, open boxes – OFS.

Potato root AM fungal colonisation level was negatively correlated with soil phosphorus content ( $r = -0.50$ ,  $p = 0.012$ , Fig. 3, A; Table S2) and positively with total annual C input to the soil ( $r = 0.46$ ,  $p = 0.023$ ; Fig. 3, B; Table S2). When exploring these relationships separately by treatment groups, the relationship between soil phosphorus content and potato root AM fungal colonisation level remained for OFS ( $r = -0.78$ ,  $p = 0.022$ ; Table S3), but not for CFS ( $r = -0.11$ ,  $p = 0.67$ ; Table S4). In the case of relationship between total annual C input to soil and potato root AM fungal colonisation level, there were no significant relationships neither for OFS ( $r = 0.26$ ,  $p = 0.52$ ; Table S3) nor CFS ( $r = -0.35$ ,  $p = 0.17$ ; Table S4). Exceptionally, in OFS, potato root AM fungal colonisation level was negatively correlated with  $C_{org}$  ( $r = -0.76$ ,

$p = 0.03$ ; Table S3). There were no significant relationships between soil pH,  $C_{org}$ , K, Ca, Mg and total N content, and potato AM fungal colonisation (Table S2, Table S5).



**Figure 2.** Median values ( $\pm$  min, max) of percent of AM fungal colonisation of potato roots in different agricultural treatments in the field trial (A) and narrowleaf plantain roots in greenhouse trial inoculated with soils from the same agricultural treatments in the field (B). Box plots indicate median (bold horizontal line), interquartile ranges (box) and minimum and maximum values (whiskers). Different letters above the boxes indicate statistically significant differences at  $P < 0.05$  among treatments (*Kruskal-Wallis rank sum test*, as post hoc test, was used *Dunn test* with *Bonferroni* correction). Open boxes – organic farming systems, grey boxes – conventional farming systems. CC – farming system with cover crops, CC + M – farming system with cover crops and composted cattle manure,  $N_0P_0K_0$  – control system with no additional fertilizers used,  $N_{50}P_{25}K_{95}$ ,  $N_{100}P_{25}K_{95}$ , and  $N_{150}P_{25}K_{95}$  – systems with different N rates used.



**Figure 3.** Relationships between AM fungal colonisation of potato roots and (A) soil phosphorus content and (B) total annual carbon input in the field trial. The points with whiskers indicate the mean ( $\pm$  standard error) of four replicate samples per treatment; the dotted lines present the linear relationship. Closed circles – organic farming systems, open circles – conventional farming systems. See Fig. 2 legend for treatment coding.

**Table S2.** Linear correlation coefficients between soil characteristics and AMF colonisation in potato roots

	pH KCl	C <sub>org</sub> (%)	P (mg kg <sup>-1</sup> )	K (mg kg <sup>-1</sup> )	Mg (mg kg <sup>-1</sup> )	Ca (mg kg <sup>-1</sup> )	Total N (%)	Total C input (kg ha <sup>-1</sup> y <sup>-1</sup> )	AM fungal colonisation (%)
pH KCl	1								
C <sub>org</sub> (%)	<b>0.57*</b>	1							
P (mg kg <sup>-1</sup> )	<b>0.63*</b>	0.39	1						
K (mg kg <sup>-1</sup> )	0.27	0.33	<b>0.48*</b>	1					
Mg (mg kg <sup>-1</sup> )	<b>0.78*</b>	<b>0.45*</b>	<b>0.56*</b>	0.38	1				
Ca (mg kg <sup>-1</sup> )	<b>0.86*</b>	<b>0.67*</b>	<b>0.59*</b>	0.4	<b>0.85*</b>	1			
Total N (%)	<b>0.55*</b>	<b>0.74*</b>	<b>0.41*</b>	<b>0.54*</b>	<b>0.44*</b>	<b>0.62*</b>	1		
Total C input (kg ha <sup>-1</sup> y <sup>-1</sup> )	0.35	<b>0.50*</b>	-0.24	0.12	0.25	<b>0.43*</b>	<b>0.52*</b>	1	
AM fungal colonisation (%)	-0.04	0.09	<b>-0.50*</b>	-0.18	-0.09	-0.00	0.09	<b>0.46*</b>	1

Statistically significant relationships ( $p < 0.05$ ) are indicated with asterisks.

**Table S3.** Linear correlation coefficients between soil characteristics and AMF colonisation of potato in OFS

	pH KCl	C <sub>org</sub> (%)	P (mg kg <sup>-1</sup> )	K (mg kg <sup>-1</sup> )	Mg (mg kg <sup>-1</sup> )	Ca (mg kg <sup>-1</sup> )	Total N (%)	Total C input (kg ha <sup>-1</sup> y <sup>-1</sup> )	AM fungal colonisation (%)
pH KCl	1								
C <sub>org</sub> (%)	0.09	1							
P (mg kg <sup>-1</sup> )	0.67	0.59	1						
K (mg kg <sup>-1</sup> )	0.53	0.35	0.32	1					
Mg (mg kg <sup>-1</sup> )	<b>0.90*</b>	-0.09	0.51	0.34	1				
Ca (mg kg <sup>-1</sup> )	<b>0.96*</b>	0.25	<b>0.79*</b>	0.53	<b>0.87*</b>	1			
Total N (%)	0.37	0.56	0.44	<b>0.81*</b>	0.10	0.46	1		
Total C input (kg ha <sup>-1</sup> y <sup>-1</sup> )	0.28	0.07	-0.10	<b>0.74*</b>	0.25	0.31	0.67	1	
AM fungal colonisation (%)	-0.31	<b>-0.76*</b>	<b>-0.78*</b>	0.00	-0.31	-0.47	-0.13	0.26	1

Statistically significant relationships ( $p < 0.05$ ) are indicated with asterisks.

**Table S4.** Linear correlation coefficients between soil characteristics and AMF colonisation of potato in CFS

	pH KCl	C <sub>org</sub> (%)	P (mg kg <sup>-1</sup> )	K (mg kg <sup>-1</sup> )	Mg (mg kg <sup>-1</sup> )	Ca (mg kg <sup>-1</sup> )	Total N (%)	Total C input (kg ha <sup>-1</sup> y <sup>-1</sup> )	AM fungal colonisation (%)
pH KCl	1								
C <sub>org</sub> (%)	<b>0.63*</b>	1							
P (mg kg <sup>-1</sup> )	<b>0.82*</b>	<b>0.67*</b>	1						
K (mg kg <sup>-1</sup> )	0.26	<b>0.62*</b>	<b>0.53*</b>	1					
Mg (mg kg <sup>-1</sup> )	<b>0.76*</b>	<b>0.68*</b>	<b>0.87*</b>	<b>0.57*</b>	1				
Ca (mg kg <sup>-1</sup> )	<b>0.81*</b>	<b>0.71*</b>	<b>0.85*</b>	<b>0.57*</b>	<b>0.88*</b>	1			
Total N (%)	<b>0.61*</b>	<b>0.81*</b>	<b>0.72*</b>	<b>0.57*</b>	<b>0.72*</b>	<b>0.62*</b>	1		
Total C input (kg ha <sup>-1</sup> y <sup>-1</sup> )	0.23	0.30	0.11	0.39	0.02	0.17	0.25	1	
AM fungal colonisation (%)	-0.26	0.04	-0.11	-0.18	-0.20	-0.15	-0.22	-0.35	1

Statistically significant relationships ( $p < 0.05$ ) are indicated with asterisks.

**Table S5.** Average ( $\pm$ standard error) soil characteristics by treatments

	Treatment					
	N0	N50	N100	N150	CC	CC $\pm$ M
pH KCl	5.95 ( $\pm$ 0.21)	5.83 ( $\pm$ 0.22)	5.73 ( $\pm$ 0.19)	5.72 ( $\pm$ 0.11)	5.94 ( $\pm$ 0.10)	6.03 ( $\pm$ 0.19)
C <sub>org</sub> (%)	1.22 ( $\pm$ 0.12)	1.17 ( $\pm$ 0.08)	1.30 ( $\pm$ 0.12)	1.34 ( $\pm$ 0.10)	1.46 ( $\pm$ 0.06)	1.43 ( $\pm$ 0.07)
P (mg kg <sup>-1</sup> )	118.6 ( $\pm$ 11.4)	120.6 ( $\pm$ 10.7)	120.8 ( $\pm$ 13.5)	97.5 ( $\pm$ 12.9)	103.4 ( $\pm$ 12.9)	92.3 ( $\pm$ 9.0)
K (mg kg <sup>-1</sup> )	146.4 ( $\pm$ 5.3)	170.8 ( $\pm$ 6.8)	167.4 ( $\pm$ 8.8)	165.0 ( $\pm$ 10.7)	139.1 ( $\pm$ 9.7)	167.1 ( $\pm$ 11.3)
Mg (mg kg <sup>-1</sup> )	127.8 ( $\pm$ 15.1)	147.0 ( $\pm$ 12.6)	147.3 ( $\pm$ 14.6)	120.7 ( $\pm$ 15.0)	143.1 ( $\pm$ 11.3)	161.3 ( $\pm$ 33.4)
Ca (mg kg <sup>-1</sup> )	1,156 ( $\pm$ 143.0)	1,310 ( $\pm$ 133.4)	1,247 ( $\pm$ 84.0)	1,185 ( $\pm$ 105.0)	1377 ( $\pm$ 90.0)	1,452 ( $\pm$ 137.8)
Total N (%)	0.122 ( $\pm$ 0.007)	0.126 ( $\pm$ 0.002)	0.127 ( $\pm$ 0.010)	0.127 ( $\pm$ 0.008)	0.129 ( $\pm$ 0.011)	0.145 ( $\pm$ 0.005)
Total C input (kg ha <sup>-1</sup> y <sup>-1</sup> )	4,604 ( $\pm$ 640.1) <sup>A</sup>	4,841 ( $\pm$ 955.4) <sup>A</sup>	4,900 ( $\pm$ 615.1) <sup>A</sup>	6,241 ( $\pm$ 445.0) <sup>AB</sup>	7,778 ( $\pm$ 195.1) <sup>B</sup>	1,0394 ( $\pm$ 195.1) <sup>C</sup>

Means with different letters are statistically significantly different among treatments ( $p < 0.05$ , *Tukey* test). See Fig. 2 legend for treatment coding.

### **AM fungal inoculum potential of arable soil**

Root AM fungal colonisation of narrowleaf plantain in greenhouse trial was significantly greater ( $p < 0.001$ ) than that of field-grown potato roots in both farming systems (data not shown). The median AM fungal colonisation levels in the narrowleaf plantain roots were 10.6% (range 0–60%) in CFS and 25.0% (range 0–42.5%) in OFS. The AM fungal colonisation of narrowleaf plantain showed an insignificant tendency to be higher in the OFS than in the CFS (*Wilcoxon signed-rank test*,  $W = 451.5$ ,  $p = 0.14$ ; Fig. 1B). AM fungal root colonisation values in soils from individual field treatments varied considerably, but with no significant differences between treatments.

## **DISCUSSION**

By combining a field trial and trap plant greenhouse assay we show that AM fungal colonisation in potato roots in the field conditions was very low, regardless of sufficient amount of AM fungal inoculum in the field soils to support moderate root colonisation levels of trap plant narrowleaf plantain inoculated with these field soils. AM fungal root colonisation tended to be higher in the organic farming systems both in the field grown potato roots and greenhouse-grown plantain roots. The individual fertilizing treatments in the conventional and organic farming systems did not show regular differences in root AM fungal colonisation levels neither in the field potato roots, nor trap plant roots, with the exception of positive effect of manure amendment in organic farming system as compared to inorganic fertilizer addition in the conventional farming system. We found that high soil P content decreased root AM fungal colonisation and higher annual C input to the soil increased root AM fungal colonisation. Therefore, the positive impact of organic farming on the potato AM fungal colonisation in our study system can be explained by previous higher fresh organic matter input and lower soil P content under manure amendment treatment. These results suggest that the potato cultivar studied by us could be a relatively poor AM host, and that AM fungal abundance and functioning is further decreased by higher soil fertility, but can be improved by organic farming practices such as use of manure as fertiliser.

In the present study root mycorrhizal colonisation rate was measured for a locally bred potato cultivar 'Reet'. This relatively new cultivar is described by breeders Tsahkna & Tähtjärvi (2008). Breeding programs are generally conducted in experimental stations under high nutrient levels (Philippot et al., 2013). This has resulted in several crops showing lower root mycorrhizal fungal colonisation and lower mycorrhizal growth response than their wild progenitors, though large variations exist (Martin-Robles et al., 2018). It is therefore conceivable that modern potato varieties may have lower mycorrhizal dependence caused by selective breeding under conditions where plants receive little benefit from mycorrhizal symbiosis. This possibility is supported by evidence that newer cultivated plant varieties tend to have lower AM fungal root colonisation (Lehmann et al., 2012). Similar to our results, very low root colonisations have also been reported in earlier field surveys of potato (Cesaro et al., 2008). Furthermore, potato root AM fungal colonisation can vary to a large degree across plant growth phases (Buysens et al., 2017).

Additionally, soil in our field experiment had high to very high plant-available P levels (Schick et al., 2013), which could be one of the reasons for low observed AM fungal root colonisation. Negative relationship between root AM fungal colonisation and

soil P level as observed in this study, is frequently reported in other agricultural and natural systems (Verbruggen et al., 2013). As P availability increases, plants in return become less dependent on AM fungi and down-regulate their mycorrhiza formation (Smith & Read, 2008). Still, not only phosphorus fertilization negatively affects AM fungi, but previous studies have shown that high-input conventional farming as a whole chemical-dependent system negatively affects AM fungi (Kabir, 2005; Verbruggen et al., 2010; Prosser et al., 2015).

However, our root colonisation data verified the previous findings (Jansa et al., 2006) that manure amendment has beneficial effect on plant colonisation by AM fungi compared to the application of inorganic fertilizers. This is in accordance with findings by Gryndler et al. (2006), who showed that in manured soil the concentration of AM fungal spores and mycelial growth increased with mineral fertilization. Furthermore AM fungal colonisation rate in plant roots might also be influenced by other factors related to farming practices like cropping history. A meta-analysis conducted by Lekberg & Koide (2005) showed that avoiding non-mycorrhizal plants in crop rotation has a positive effect on subsequent mycorrhizal colonisation. The cover crop used in the current study was winter oilseed rape, which is a non-mycorrhizal crop plant. Therefore, low AM fungal colonisation rate in potato roots of our study could in part result from usage of non-mycorrhizal crops as a cover crop, which possibly decreased the positive effect of AM fungi for the following crop.

In comparison to the field crop, potato, the trap plant narrowleaf plantain roots showed higher AM fungal colonisation, indicating that the field soils were not exhausted of AM fungi. It is noteworthy that the higher root colonisation of plantains was obtained on the field soil diluted with sand, effectively reducing the amount of available AM fungal propagules for the host plants compared to that available for potato plants in the field. Narrowleaf plantain is a widely used plant species because of its commonly high mycorrhizal colonisation and responsiveness, as well as its broad range of AM fungal partners (Schnoor et al., 2011; Davison et al., 2015). Our comparison of potato and plantain also confirms that host plant–AM fungal relationships depend on both symbiosis partners (e.g., Bever, 2002), whereby the same fungal inoculum may result in very different plant root colonisation levels, fungal community compositions and plant growth in the case of different host plant species. Host-AM fungal compatibility may also influence potato yield, as shown earlier in inoculation trials of micropropagated potatoes (Duffy & Cassells, 2000). Whether a different AM fungal community would result in a different root colonisation (and ultimately, yield) of the potato cultivar studied by us, requires further testing.

## CONCLUSIONS

Our study demonstrated extremely low AM fungal colonisation rate in the roots of potato in field conditions. At the same time, AM fungal colonisation was higher in organic than in conventional farming system, and was related to higher fresh organic matter input and lower soil phosphorus content. Interestingly, both soils from the conventional and organic field had relatively high AM fungal inoculum potential as detected by trap plant assay with narrowleaf plantain. These results suggest that plant species or cultivar can have a strong influence on AM fungal colonisation levels. Further research is needed to clarify whether other varieties of potato show similarly low levels

of AM fungal colonisation as the cultivar used in this study, and to which degree plant growth phase affects this measure. Furthermore, it is necessary to explore how AM fungal species diversity in field potato roots relates to the root colonisation levels and potato yield of different cultivars under the regionally used potato cropping systems. This would provide guidelines for the most efficient management of AM fungi in these cropping systems with regionally used cultivars, fertilization levels, cover crop and pesticide usages considering optimal potato production at the regional scale.

**ACKNOWLEDGEMENTS.** The study was supported by the Estonian Science Foundation grant No 9432, the European Regional Development Fund (project RESIST 3.2.0701.11–0003), Institutional research funding grants IUT36-2 and IUT20-28 of the Estonian Ministry of Education and Research.

## REFERENCES

- Augé, R.M., Toler, H.D. & Saxton, A.M. 2015. Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. *Mycorrhiza* **25**, 13–24. doi.org/10.1007/s00572-014-0585-4
- Bender, S.F., Wagg, C. & van der Heijden, MGA. 2016. An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends in Ecology & Evolution* **31**, 440–452.
- Bever, J.D. 2002. Host-specificity of AM fungal population growth rates can generate feedback on plant growth. *Plant Soil* **244**, 281–290.
- Buysens, C., Alaux, P.L., Cesar, V., Huret, S., Declerck, S. & Cranenbrouck, S. 2017. Tracing native and inoculated *Rhizophagus irregularis* in three potato cultivars (Charlotte, Nicola and Bintje) grown under field conditions. *Applied Soil Ecology* **115**, 1–9.
- Cesaro, P., van Tuinen, D., Copetta, A., Chatagnier, O., Berta, G., Gianinazzi, S. & Lingua, G. 2008. Preferential colonization of *Solanum tuberosum* L. roots by the fungus *Glomus intraradices* in arable soil of a potato farming area. *Applied and Environmental Microbiology* **74**(18), 5776–5783
- Davies, Jr, F.T., Calderón, C.M. & Huaman, Z. 2005. Influence of Arbuscular Mycorrhizae Indigenous to Peru and a Flavonoid on Growth, Yield, and Leaf Elemental Concentration of 'Yungay' Potatoes. *HortScience* **40**, 381–385.
- Davison, J., Moora, M., Öpik, M., Adholeya, A., Ainsaar, L., Ba, A., Burla, S., Diedhiou, A.G., Hiiesalu, I., Jairus, T., Johnson, N.C., Kane, A., Koorem, K., Kochar, M., Ndiaye, C., Pärtel, M., Reier, Ü., Saks, Ü., Singh, R., Vasar, M., Zobel, M. 2015. Global assessment of arbuscular mycorrhizal fungus diversity reveals very low endemism. *Science* **349**, 970–973.
- Douds, D.D., Jr., Nagahashi, G., Reider, C. & Hepperly, P.R. 2007. Inoculation with Arbuscular Mycorrhizal Fungi Increases the Yield of Potatoes in a High P Soil. <http://handle.nal.usda.gov/10113/21020>
- Duffy, E.M. & Cassells, A.C. 2000. The effect of inoculation of potato (*Solanum tuberosum* L.) microplants with arbuscular mycorrhizal fungi on tuber yield and tuber size distribution. *Appl. Soil Ecol.* **15**, 137–144.
- Egnér, H., Riehm, H. & Domingo, W. 1960. *Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. II. Chemische Extraktionsmethoden zur Phosphor- und Kaliumbestimmung.* (Studies on the chemical soil analysis as a basis for the assessment of the nutrient status of soils. II. Chemical extraction methods for phosphorus and potassium determination). *Kungliga Lantbrukshögskolans Annaler*, 26, 199–215 (in German).



- Gianinazzi, S., Golotte, A., Binet, M.N., van Tuinen, D., Redecker, D. & Wipf, D. 2010. Agroecology: The key role of arbuscular mycorrhizas in ecosystem services. *Mycorrhiza* **20**(8), 519–530. doi.org/10.1007/s00572-010-0333-3
- Gryndler, M., Larsen, J., Hršelová, H., Řezáčová, V., Gryndlerová, H. & Kubát, J. 2006. Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment. *Mycorrhiza* **16**, 159–166. doi 10.1007/s00572-005-0027-4
- Hack, H., Gal, H., Klemke, T., Klose, R., Meier, U., Strauss, R., Witzemberger, A. & Witzemberger, A. 2001. The BBCH scale for phenological growth stages of potato (*Solanum tuberosum* L.). In: Meier U, ed. Growth stages of mono- and dicotyledonous plants, BBCH Monograph. Berlin, Braunschweig, Germany: Federal Biological Research Centre for Agriculture and Forestry, 7–16.
- Jansa, J., Wiemken, A. & Frossard, E. 2006. The effects of agricultural practices on arbuscular mycorrhizal fungi. Geological Society, London, Special Publications, **266**(1), 89–115. doi.org/10.1144/GSL.SP.2006.266.01.08
- Kabir, Z. 2005. Tillage or no-tillage: Impact on mycorrhizae. *Canadian Journal of Plant Science* **85**(1), 23–29. doi.org/10.4141/P03-160
- Kauer, K., Tein, B., Sanchez de Cima, D., Talgre, L., Eremeev, V., Loit, E. & Luik, A. 2015. Soil carbon dynamics estimation and dependence on farming system in a temperate climate. *Soil and Tillage Research* **154**, 53–63. doi.org/10.1016/j.still.2015.06.010
- Koske, R.E. & Gemma, J.N. 1989. A modified procedure for staining roots to detect VA mycorrhizas. *Mycological Research* **92**(4), 486–488. doi.org/10.1016/S0953-7562(89)80195-9
- Lehmann, A., Barto, E.K., Powell, J.R. & Rillig, M.C. 2012. Mycorrhizal responsiveness trends in annual crop plants and their wild relatives—a meta-analysis on studies from 1981 to 2010. *Plant and Soil* **355**, 231–250.
- Lekberg, Y. & Koide, R.T. 2005. Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. *New Phytologist* **168**, 189–204. doi: 10.1111/j.1469-8137.2005.01490.x
- Mäder, P., Edenhofer, S., Boller, T., Wiemken, A. & Niggli U. 2000. Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. *Biology and Fertility of Soils* **31**, 150–156.
- Madsen, H., Talgre, L., Eremeev, V., Alaru, M., Kauer, K. & Luik, A. 2016. Do green manures as winter cover crops impact the weediness and crop yield in an organic crop rotation? *Biological Agriculture & Horticulture* **32**, 182–191. doi. 10.1080/01448765.2016.1138141
- Mahmood, I. & Rizvi, R. 2010. Mycorrhiza and organic farming. *Asian Journal of Plant Sciences*, **9**, 241–248. doi.org/10.3923/ajps.2010.241.248
- Martin-Robles, N., Lehmann, A., Seco, E., Aroca, R., Rillig, M.C. & Milla, R. 2018. Impacts of domestication on the arbuscular mycorrhizal symbiosis of 27 crop species. *New Phytologist*, doi: 10.1111/nph.14962
- McArthur, D.A.J., Knowles, N.R. 1993. Influence of species of vesicular-arbuscular mycorrhizal fungi and phosphorus nutrition on growth, development, and mineral nutrition of potato (*Solanum tuberosum* L.). *Plant Physiology* **102**, 771–782. doi.org/10.1104/pp.102.3.771
- McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L. & Swan, J.A. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **115**, 495–501. doi.org/10.1111/j.1469-8137.1990.tb00476.x

- Moorman, T. & Reeves, B.F. 1979. The role of endomycorrhizae in revegetation practices in the semi-arid west. II. A bioassay to determine the effect of land disturbance on endomycorrhizal populations. *American Journal of Botany* **66**, 14–18.  
<http://www.jstor.org/stable/2442619>. Accessed 20 June 2017
- Philippot, L., Raaijmakers, J.M., Lemanceau, P. & van der Putten, W.H. 2013. Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews. Microbiology* **11**(11), 789–799. doi.org/10.1038/nrmicro3109
- Pozo, M.J., Lopez-Raez, J.A., Azcon-Aguilar, C. & Garcia-Garrido, J.M. 2015. Phytohormones as integrators of environmental signals in the regulation of mycorrhizal symbioses. *New Phytologist* **205**, 1431–1436.
- Prosser, R., Lissemore, L., Shahmohamadloo, R. & Sibley, P. 2015. Effect of biosolids-derived triclosan and triclocarban on the colonization of plant roots by arbuscular mycorrhizal fungi. *Science of the Total Environment* **508**, 427–434. doi.org/10.1016/j.scitotenv.2014.12.014
- Schick, B.J., Haneklaus, S., Rückamp, D., Astover, A., Busch, S., Kuligowski, K., Rossner, H., Sarvi, M., Ylivainio, K., Ziolkowski, M. & Schnug, E. 2013. Report on P status in agricultural soils of relevant areas of the Baltic Sea Region Report on P status in agricultural soils of relevant areas of the Baltic Sea Region.  
[http://www.balticmanure.eu/download/Reports/bm\\_report\\_on\\_p\\_status\\_in\\_agricultural\\_soils\\_web.pdf](http://www.balticmanure.eu/download/Reports/bm_report_on_p_status_in_agricultural_soils_web.pdf). Accessed 15 June 2017
- Schnoor, T.K., Lekberg, Y., Rosendahl, S. & Olsson, P.A. 2011. Mechanical soil disturbance as a determinant of arbuscular mycorrhizal fungal communities in semi-natural grassland. *Mycorrhiza* **21**, 211–220.
- Smith, A.F. & Smith, S.E. 2011. What is the significance of the arbuscular mycorrhizal colonization of many economically important crop plants? *Plant Soil* **348**, 63–79. doi.org/10.1007/s11104-011-0865-0
- Smith, S.E. & Read, D.J. 2008. *Mycorrhizal Symbiosis*. Amsterdam: Academic Press.
- Tsahkna A., Tähtjärv, T. 2008. The new potato variety ‘Reet’. *Latvian Journal of Agronomy* **11**, 159–164.
- Uibopuu, A., Moora, M., Öpik, M. & Zobel, M. 2012. Temperate forest understorey species performance is altered by local arbuscular mycorrhizal fungal communities from stands of different successional stages. *Plant and Soil* **356**, 331–339.
- Van Reeuwijk, L.P. 1995. *Procedures for soil analysis*, fifth ed. ISRIC Technical Paper 9, Wageningen, Netherlands.
- Verbruggen, E., Rölting, W.F., Gamper, H.A., Kowalchuk, G.A., Verhoef, H.A. & van der Heijden, M.G.A. 2010. Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. *New Phytologist* **186**, 968–979.
- Verbruggen, E., van der Heijden, M.G.A., Rillig, M.C. & Kiers, T.E. 2013. Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success. *New Phytologist* **197**(4), 1104–1109. doi.org/10.1111/j.1469-8137.2012.04348.x
- Verbruggen, E. & Kiers, E.T., 2010. Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. *Evol. Appl.* **3**, 547–560.
- Vorobeva, L.A. 1998. *Chemical analysis of soils*. Moscow University Press, Moscow, 272 pp. (in Russian).
- Wu, F., Wang, W., Ma, Y., Liu, Y., Ma, X., An, L. & Feng, H. 2013. Prospect of beneficial microorganisms applied in potato cultivation for sustainable agriculture. *African Journal of Microbiology Research* **7**, 2150–2158. doi.org/10.5897/AJMRx12.005

## **Comparison of two sowing systems for CTF using commercially available machinery**

M. Macák\*, V. Rataj, M. Barát and J. Galambošová

Slovak University of Agriculture in Nitra, Faculty of Engineering, Department of Machines and Production Biosystems, Tr. A. Hlinku 2, SK949 76 Nitra, Slovakia

\*Correspondence: [miroslav.macak@uniag.sk](mailto:miroslav.macak@uniag.sk)

**Abstract.** The crop establishment belongs to crucial technology operations. The quality of sowing is the basis for obtaining efficiency of production. Controlled Traffic Farming (CTF) is a technology which prevents excessive soil compaction and minimizes compacted area to the smallest possible area of permanent traffic lanes (PTL). There were two sowing systems compared, namely row and band sowing when growing winter barley. Sowing parameters as well as all other field operations were identical for both compared systems. Measurements were conducted at an experimental field on non-compacted and traffic lane areas where CTF system was introduced in 2009, with 64% of compacted and 36% of non-compacted soil. Six crop parameters were analysed. Generally, it can be concluded that the band sowing performed better in yield (by 9.3% in non-compacted area; by 3.8% in traffic lane), ear number (by 5.2% in non-compacted area; by 10.1% in traffic lane) and grain number (by 6.3% in non-compacted area; by 8.1% in traffic lane) as well as crop height (by 6.6% in non-compacted area; and by 2.4% in traffic lane). The only parameter performing worse was TGW with decrease of 6.6% in non-compacted area and decrease 2.8% in traffic lane for band system. Differences in number of grain per ear were negligible.

**Key words:** band sowing, drill, CTF, soil compaction, cereals.

### **INTRODUCTION**

Controlled Traffic Farming (CTF) system currently belongs to modern methods that are progressively becoming part of the efficient management of crop production in precision farming (Rataj et al., 2014).

The essence of this system is to concentrate the individual machinery paths into one trajectory with respect to the integer multiples of the working width of individual machines, in order to reduce the trafficked area and consequently improve growing condition compared to RTF (Random Traffic Farming) system. The reason for putting this system into practice is a long-term trend in continuously increasing size and especially weight of agricultural machinery (Kutzbach, 2000).

Chaiman (2015), Goodwin et al. (2015), Kroulík et al. (2011), Kumhála et al. (2013) and others state, that the primary effect of implementing the CTF system is to improve soil structure. According to the quoted authors this system can be used as a long-term tool to prevent soil compaction. As a result of the decreasing soil compaction, the pore volume and water infiltration capacity are increased, and the soil bulk density is decreased (Kovář et al. 2016; Chyba et al. 2017). Improvement of these physical-mechanical properties of the soil is subsequently reflected in the increase of crop yields.

Vermeulen et al. (2010) states, that within CTF system, besides the above benefits, there is also a more effective use of agricultural machinery. Consolidated tracks allow access to the field even in wetter soil conditions. There is a decrease in energy intensity, a lower number of operations, shallower cultivation, and lower tractor power requirement.

The CTF system is closely associated with conservation tillage systems eventually direct drilling. CTF creates two zones: non-trafficked crop beds and cropped or non-cropped traffic lanes (Chamen, 2015). This system can be established also with ordinary machinery without special adjustment, with different percentage proportion of non-trafficked area up to 68% (Gutu, 2015).

If the farmer decides to seed the CTF trafficked lanes, it is necessary to increase intensity of soil preparation and seeding requirements. This is required, because these lanes are caused by centralized traffic of all machinery during the growing season and the soil is more compacted than soil located in non-trafficked areas (Arslan et al., 2015, Kroulík et al. 2016).

Alternatively, it is possible to use special seed drills (combined seed drills with soil preparation or seed drill for direct drilling), that are able to place seed into the required depth evenly over the entire working width, including compacted CTF traffic lanes.

The aim of this work was to compare differences between two seeding systems in CTF technology through selected crop parameters with emphasis on different intensity of soil compaction.

## **MATERIALS AND METHODS**

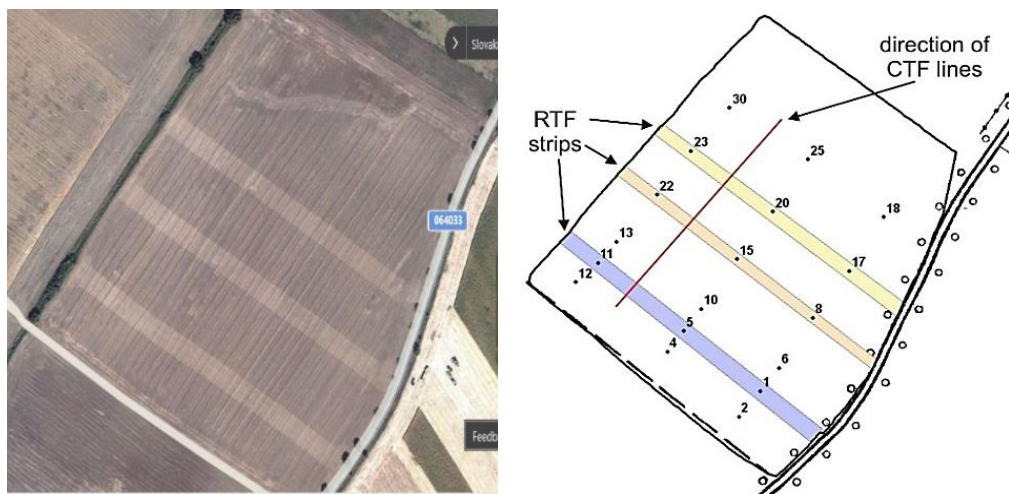
### **Experiment design**

In 2010, a long-term field experiment was launched with the technology of Controlled Traffic Farming (CTF) at Slovak University of Agriculture in Kolíňany, Slovakia. This technology is used on a 16 ha field. Soil texture class on top (0–35 cm) is silty loam (51% silt, 30% sand, 19% clay) – analyses were conducted based on Slovak standards (Hrivňáková et al., 2011). Type of CTF system is 6 m OutTrack (64% non-compacted soil, 36% compacted soil). Commercially available machinery with standard wheel spacing were used for work operations – as they were supplied by the manufacturer. Field was cultivated within soil conservation tillage (without ploughing) up to depth of 15 cm. More detailed information can be found in other publications (e.g. Goodwin et al., 2015; Galambošová et al., 2017; Barát et al., 2017).

The possibility of using direct seed drill with breaker tines for band sowing in CTF system was studied in comparison with row sowing system during one-year experiment in growing season 2016–2017.

It was necessary to respect the long-term experiment during setting up a sampling layout, especially direction of CTF track lanes and simulated RTF strips on the research field.

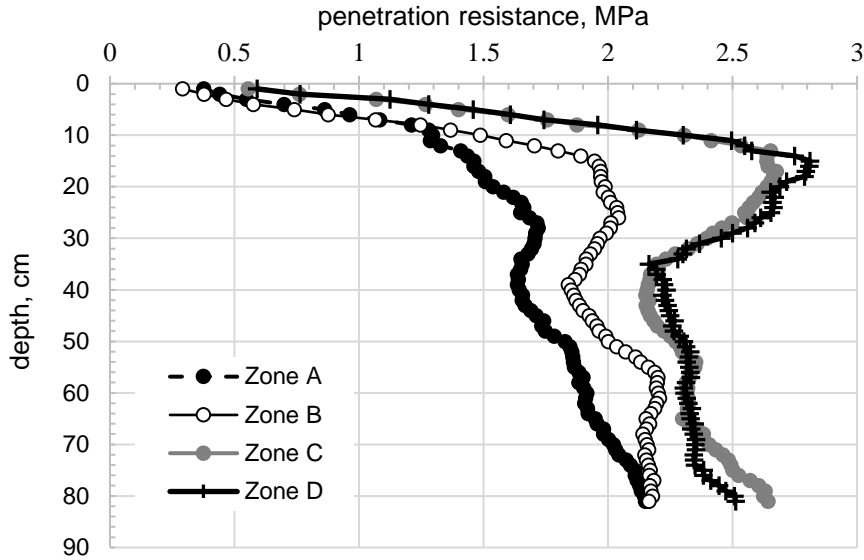
To solve the established aim of this paper, 18 monitoring points were designated, of which 9 points were located in three RTF strips (see Fig. 1). The RTF strips are areas trafficked wheel by wheel once a year with tractor John Deere 8230 (tire pressure 2.0 bar), perpendicularly to the direction of CTF.



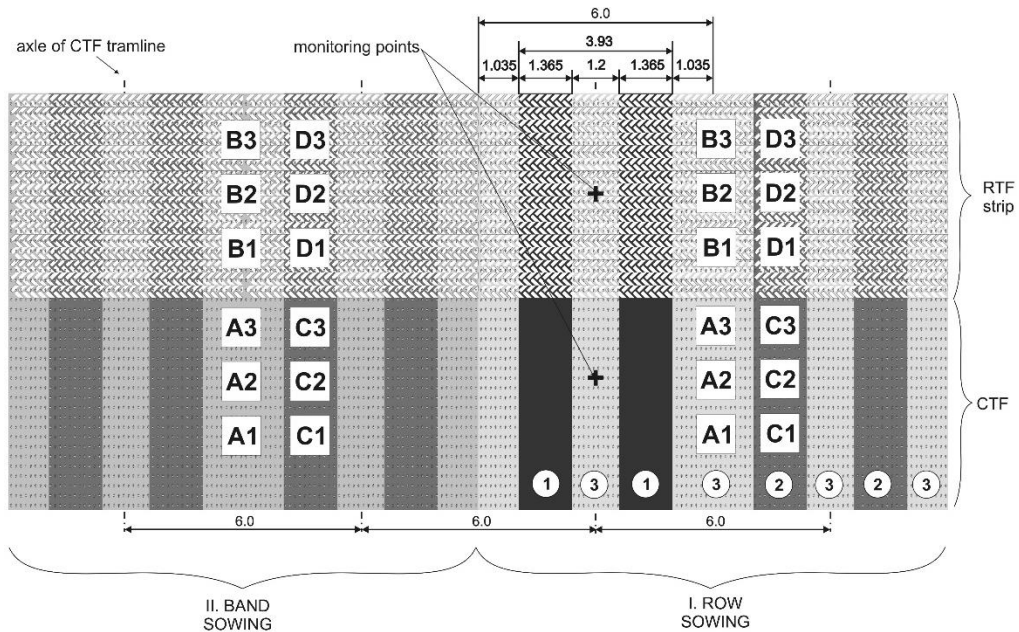
**Figure 1.** Satellite image showing 1x passed compacted area for RTF simulation, direction of CTF lanes and monitoring points (no. 1–0) sampling layout.

The resulting matrix of experiment was based on 4 zones marked A, B, C, D, which represented areas with 4 different compaction intensities. The least compacted soil was in zone A, the most compacted in zone D. The soil compaction intensity was measured as a penetration resistance in the vertical axis, with a device manufactured by Eijkelkamp. The values of penetration resistance (Fig. 2) show different intensity of soil compaction, as well as the depth of long-term soil cultivation.

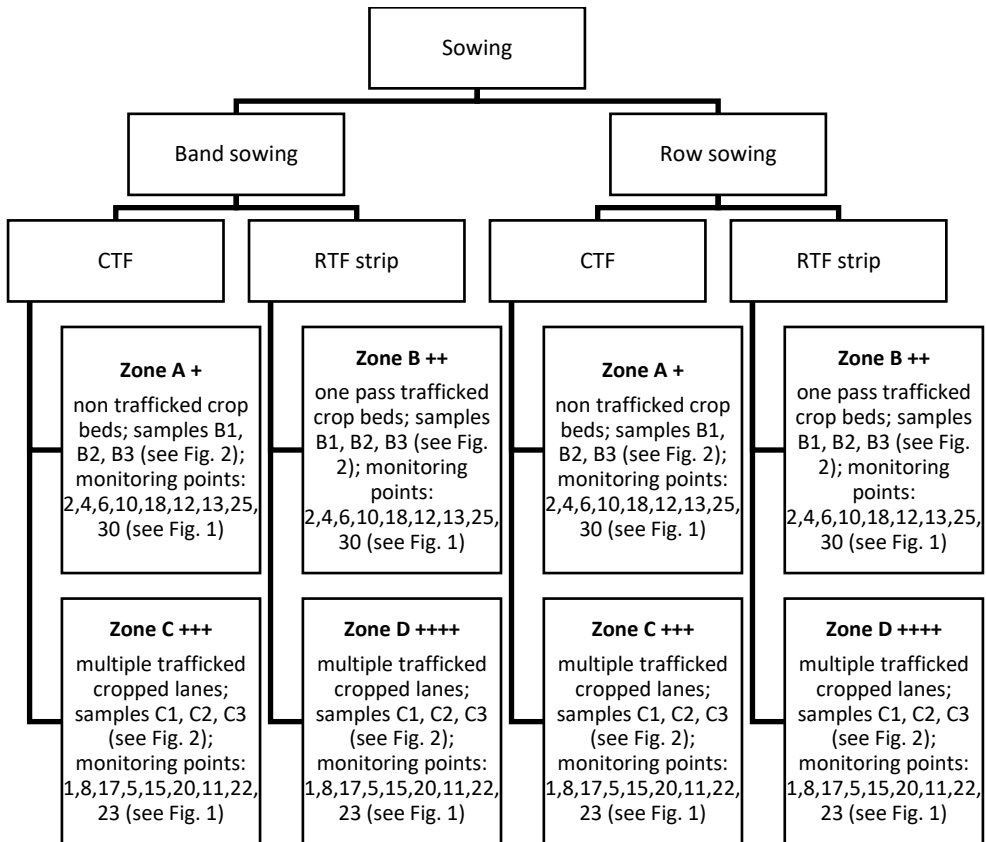
The seeding was carried out alternately in the direction of CTF lanes – two passes with standard row seed drill, and two passes with combined direct drill for band sowing (see Fig. 3). The four treatments (zones A, B, C, D), from within which measurements were taken, were denoted as shown in Fig. 4. The samples were taken from each monitoring point in three replications in each zone. Each sample consisted of crops cut nearly above ground from 1 m<sup>2</sup> area. Together 12 samples (6 from row sowing and 6 from band sowing area) were taken in each monitoring point with the mentioned process (see Fig. 3).



**Figure 2.** Intensity of soil compaction in experimental zones represented by vertical penetration resistance (measured in 2015).



**Figure 3.** Experiment design – example of taking yield sampling layout on two monitoring points; 1 – non-cropped multiple-trafficked lanes – sprayer lanes (each 24 m), 2 – cropped multiple-trafficked lanes (location of samples: C1, C2, C3 and samples D1, D2, D3 in RTF strip), 3 – non-trafficked crop beds (location of samples A1, A2, A3); and one pass trafficked crop beds (location samples B1, B2, B3 in RTF strip), (the values given in the picture are t in meters).



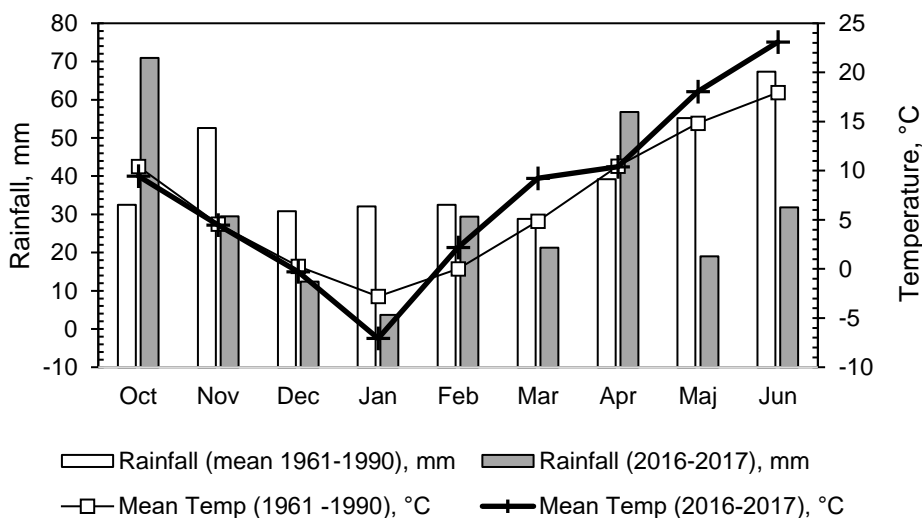
**Figure 4.** Matrix of experiment variants ('+' represents intensity of soil compaction from low + to high level ++++).

### Seasonal and long-term experiment conditions

Within agricultural production processes on experimental field, conservation tillage is used together with growing crops as cereals, oil seed rape, and corn. Temperature and rainfall in the 2016/2017 growing season and long-term averages are shown in Fig. 5.

In one-season experimental assignment it was hypothesized, that the 6 crop parameters could be increased using the band sowing system in comparison to the row sowing system in different soil compaction zones specified in CTF system. Experiment conditions and all seasonal agrotechnical operations, were same for both sowing systems.

Row sowing was done by John Deere 8100 tractor and Lemken Solitair 9 universal seed drill with conventional row spacing of 12.5 cm. Band sowing was established by Kirovec K7484 tractor and Claydon Hybrid T6 combined direct seed drill with partial soil tillage, with band spacing of 30 cm (15 cm seed band + 15 cm free band without seed). The depth of breaker tines was set to 15 cm to keep the cultivation depth the same for both systems. Type of the seed, parameters of sowing and used drills are showed in Table 1.



**Figure 5.** Rainfall represented monthly as sum of precipitations and monthly means of air temperature records for Koliňany, Slovakia (Slovak University of Agriculture in Nitra, 2017).

**Table 1.** Parameters of seed, sowing and used drills

Parameter	SEED	
Botanical name	Hordeum vulgare L. (winter barley)	
Variety	WINTMALT	
Generation	C1	
Seed disinfectant	Lamardor 400 FS	
Certified by	Central Control and Testing Institute in Agriculture – Slovakia	
Parameter	DRILLS	
	Lemken Solitair 9	Claydon Hybrid T6
Swath width, m	6	6
Number of sowing tines, pcs	48	19
Seed row/band spacing, cm	12.5	15
Weight, kg	1,600	6,500
Front breaker tines, pcs	NA	19
Maximum depth of breaker tines, mm	NA	300
Parameter	SOWING	
	row sowing	band sowing
Sowing rate, kg $ha^{-1}$	220	220
Depth of sowing, mm	30–40	30–40
Depth of breaker tines, mm	NA	150

Note: NA – not available.

The following six parameters were analysed: ear number, yield, TGW – Thousand Grain Weight, crop height, grain number per 1 m<sup>2</sup>, grain number per ear. Yield and TGW were recalculated to uniform moisture content 14%.



Parameters were determined using the methodology contained in the following standards – STN EN ISO 520, STN EN ISO 24333/AC, STN EN ISO 712, STN 46 1025. Pairwise comparison of monitored sowing systems was carried out according to *Fisher's LSD test* (Least Significant Difference) statistical method.

## RESULTS AND DISCUSSION

The assessment of band and row sowing systems was carried out in the CTF experimental field. The aim was to find out, if there is a statistically significant difference between the band and row sowing system with respect to 6 crop parameters. Calculated mean values and their standard deviations of measured crop parameters are shown in Table 2. Final results of *Fisher's LSD test* for all measured parameters are shown in Table 3.

**Table 2.** Means and standard deviations of crop parameters recorded for row and band sowing system, ( $n = 9$ ; each value was calculated as the average of the area of 3 m<sup>2</sup>)

Parameter	Zone	Row sowing, mean ± STD	Band sowing, mean ± STD	diff. between means of band and row sowing,	diff. between means of band and row sowing *, %
Ear number, pcs m <sup>-2</sup>	A	913.6±180.1	961.3±118.2	47.7	5.2%
	B	806.5±108.3	1,040.4±155.9	233.9	29.0%
	C	884.9±105.0	974.7±98.7	89.8	10.1%
	D	845.6±79.9	993.3±106.4	147.7	17.5%
Yield (14%), t ha <sup>-1</sup>	A	8.0±1.6	8.7±1.0	0.7	9.3%
	B	6.9±1.1	8.8±1.2	2.0	28.8%
	C	7.8±0.7	8.1±0.7	0.3	3.8%
	D	7.2±1.0	8.3±1.0	1.0	14.3%
TGW (14%), g	A	47.7±1.8	44.6±6.8	-3.1	-6.6%
	B	46.3±1.8	45.4±3.2	-0.9	-2.0%
	C	46.3±1.9	45.1±3.7	-1.3	-2.8%
	D	48.3±4.3	43.3±4.0	-5.0	-10.4%
Crop height, cm	A	78.1±4.9	83.3±3.0	5.1	6.6%
	B	76.1±6.2	83.2±4.8	7.1	9.3%
	C	82.8±4.4	84.9±2.0	2.0	2.4%
	D	77.0±5.3	82.4±3.0	5.3	6.9%
Grain number, pcs m <sup>-2</sup>	A	16,987.7±3,497.3	18,055.7±2479.3	1,068.0	6.3%
	B	15,059.3±2,211.3	19,436.9±3618.5	4,377.6	29.1%
	C	16,547.1±1,970.8	17,893.5±1950.6	1,346.4	8.1%
	D	15,712.6±2,055.2	18,369.1±2717.7	2,656.5	16.9%
Grain number per ear, pcs/ear	A	18.6±0.7	18.8±1.5	0.2	1.3%
	B	18.7±0.8	18.6±1.3	-0.04	-0.2%
	C	18.8±1.4	18.4±1.1	-0.4	-2.1%
	D	18.6±1.6	18.4±1.1	-0.1	-0.7%

diff. = difference; STD = standard deviation; \* row sowing is interpreted in calculation as 100% accounting basis; pcs = pieces.

**Table 3.** Differences between means and significance of pairwise comparisons; asterisks indicate differences larger than *LSD* at  $p < 0.1$  (\*) *LSD* and  $p < 0.05$  (\*\*); ns = not significant

Crop parameter	Zone A	Zone B	Zone C	Zone D
Ear number pcs.m <sup>-2</sup>	<i>LSD</i> <sub>0.05</sub> = 161.44	<i>LSD</i> <sub>0.05</sub> = 142.24	<i>LSD</i> <sub>0.05</sub> = 108.04	<i>LSD</i> <sub>0.05</sub> = 99.74
	<i>LSD</i> <sub>0.1</sub> = 132.95	<i>LSD</i> <sub>0.1</sub> = 117.14	<i>LSD</i> <sub>0.1</sub> = 88.97	<i>LSD</i> <sub>0.1</sub> = 82.14
	<b>47.70 ns</b>	<b>233.93 **</b>	<b>89.78 *</b>	<b>147.70 **</b>
Yield, t ha <sup>-1</sup>	<i>LSD</i> <sub>0.05</sub> = 1.45	<i>LSD</i> <sub>0.05</sub> = 1.25	<i>LSD</i> <sub>0.05</sub> = 0.73	<i>LSD</i> <sub>0.05</sub> = 1.08
	<i>LSD</i> <sub>0.1</sub> = 1.19	<i>LSD</i> <sub>0.1</sub> = 1.03	<i>LSD</i> <sub>0.1</sub> = 0.60	<i>LSD</i> <sub>0.1</sub> = 0.89
	<b>0.74 ns</b>	<b>1.97 **</b>	<b>0.30 ns</b>	<b>1.04 *</b>
TGW, g	<i>LSD</i> <sub>0.05</sub> = 5.31	<i>LSD</i> <sub>0.05</sub> = 2.72	<i>LSD</i> <sub>0.05</sub> = 3.15	<i>LSD</i> <sub>0.05</sub> = 4.39
	<i>LSD</i> <sub>0.1</sub> = 4.38	<i>LSD</i> <sub>0.1</sub> = 2.24	<i>LSD</i> <sub>0.1</sub> = 2.60	<i>LSD</i> <sub>0.1</sub> = 3.62
	<b>-3.14 ns</b>	<b>-0.92 ns</b>	<b>-1.28 ns</b>	<b>-5.05 **</b>
crop height, cm	<i>LSD</i> <sub>0.05</sub> = 4.27	<i>LSD</i> <sub>0.05</sub> = 5.86	<i>LSD</i> <sub>0.05</sub> = 3.60	<i>LSD</i> <sub>0.05</sub> = 4.55
	<i>LSD</i> <sub>0.1</sub> = 3.52	<i>LSD</i> <sub>0.1</sub> = 4.82	<i>LSD</i> <sub>0.1</sub> = 2.96	<i>LSD</i> <sub>0.1</sub> = 3.75
	<b>5.13 **</b>	<b>7.09 **</b>	<b>2.03 ns</b>	<b>5.34 **</b>
Grain number pcs.m <sup>-2</sup>	<i>LSD</i> <sub>0.05</sub> = 3,213.06	<i>LSD</i> <sub>0.05</sub> = 3,178.35	<i>LSD</i> <sub>0.05</sub> = 2,078.29	<i>LSD</i> <sub>0.05</sub> = 2,553.79
	<i>LSD</i> <sub>0.1</sub> = 2,646.17	<i>LSD</i> <sub>0.1</sub> = 2,617.59	<i>LSD</i> <sub>0.1</sub> = 1,711.61	<i>LSD</i> <sub>0.1</sub> = 2,103.22
	<b>1,068.00 ns</b>	<b>4,377.59 **</b>	<b>1,346.39 ns</b>	<b>2,656.52 **</b>
Grain number per ear pcs/ear	<i>LSD</i> <sub>0.05</sub> = 1.26	<i>LSD</i> <sub>0.05</sub> = 1.16	<i>LSD</i> <sub>0.05</sub> = 1.30	<i>LSD</i> <sub>0.05</sub> = 1.50
	<i>LSD</i> <sub>0.1</sub> = 1.04	<i>LSD</i> <sub>0.1</sub> = 0.96	<i>LSD</i> <sub>0.1</sub> = 1.07	<i>LSD</i> <sub>0.1</sub> = 1.23
	<b>0.25 ns</b>	<b>-0.04 ns</b>	<b>-0.40 ns</b>	<b>-0.14 ns</b>

The obtained differences between the average yields, the number of ears, the number of grain per 1 m<sup>2</sup> and the crop height were positive for the benefit of band sowing in each zone (A, B, C and D).

Differences in ear number between band and row sowing system ranged from 5.2% to 29% (Table 2). The differences were significant ( $p > 0.05$ ) in zone B and D, and in zone C (at  $p > 0.1$ ). In zone A, the differences were not significant as is shown in Table 3. Based on this, it can be concluded that placing seed grain to strips, supported tillering of sown cereal (winter barley). Next factor which supported tillering, was the use of breaker tine placed before each seed coulter on combined drill. This breaker tine creates trench in the axis of the seeding strip. Working depth (15 cm) of breaker tine corresponds with maximum depth of soil tillage during season.

Increase of ear number influenced the grain number per 1 m<sup>2</sup> as well as yield. Differences in grain number per 1 m<sup>2</sup> ranged from 6.3% to 29.1%. The differences were significant ( $p > 0.05$ ) in zone B and D. Differences in yield ranged from 3.8% to 28.8% and were significant ( $p > 0.05$ ) in zone B and in zone D ( $p > 0.1$ ).

The authors Chamen (2015) and Kroulík et al. (2011) state, that CTF system concentrates all machines passes to the track lanes. Soil compaction in these track lanes causes increase of soil bulk density (limit value ~ 1.45 t m<sup>-3</sup>; Lhotský et al., 1991). On this basis, it can be concluded that plants have problems with root growth.

Therefore, for CTF systems, the potential to increase the yield, or to reduce the yield penalty, depends on the area ratio of permanent traffic lanes to permanent crop beds and on tillage intensity of cropped traffic lanes (Galambošová et al., 2017).

Based on this study, it is important to pay attention to the soil tillage and quality of sowing i.e. sowing depth, seed spacing in row or in band, mainly in cropped traffic lanes.

Although for band sowing no yield differences were obtained between cropped traffic lanes (zone C and D) and non-compacted soil (zone A), but this system seems to support tillering of growing cereal (winter barley) as it was found in other experimental zones where higher yields were obtained.

*LSD test* confirmed, that these yield differences were significant mostly in zones with higher level of soil compaction.

Based on this it can be concluded, that band sowing performed by combined direct seed drill with partial soil tillage is suitable way, to care for the cropped traffic lanes in the CTF system.

For band sowing, the higher values of crop height were recorded in all experimental zones. Mean values of this parameter ranged from 2.4% in zone C to 9.3% in zone B. The *LSD test* confirmed, that the differences in this parameter were significant ( $p > 0.05$ ) in zones A, B, and D.

Means of TGW were lower for band sowing in all experimental zones. As stated above, the ear number was larger for band sowing than for row sowing. However, the grains in ears from band sowing were smaller than grains in ears cropped in row sowing zones.

Statistically significant differences of TGW ( $p > 0.05$ ) were confirmed only in zone D, which represented the highest soil compaction.

Measured data do not show statistically significant differences between grain number per ear in band and row sowing.

## CONCLUSIONS

Aim of this paper was to evaluate the band and row sowing carried out on soil with different intensity of compaction in the CTF system. The conditions during the experiment were the same for both sowing systems in each zone.

The yield of winter barley was higher at band sowing in all areas with different levels of traffic intensity. However, the differences in yield between the band and row sowing were significant mostly in zones with higher level of soil compaction. The basic influence on the higher yield in band sowing resulted from better tillering. Next factor was the use of breaker tine placed on combined drill before seed couler.

Based on this knowledge, we can recommend band sowing performed by combined seed drill with partial soil tillage, as one of the possibilities to care for the cropped traffic lanes in the CTF system. The depth of breaker tine can vary, according to depth of the compacted soil pan. The depth of this compacted soil pan depends on the depth of soil tillage during the season or on some other factor.

If it is possible to adjust the depth of breaker tines individually, this depth could be different - e.g. in CTF system: lower or zero depth for non-trafficked areas and higher depth for cropped traffic lanes. Then we could use a tractor with lower power and cut down the tractor fuel consumption. Following this, we could predict, that the yields between cropped traffic lanes and non-compacted areas in the CTF system will be equivalent.

**ACKNOWLEDGEMENTS.** This article was prepared in the framework of a research project funded by the European Union entitled: 'ITEPAg: Application of information technologies to increase the environmental and economic efficiency of production agro-system' (ITMS no.

26220220014) and 'Building the Research Centre AgroBioTech' (ITMS no. 26220220180). The authors are grateful to staff at the University Farm in Kolinany (Slovakia) for technical and operational support to conduct this research.

The authors are grateful to staff at the University Farm in Kolinany (Slovakia) and company TOKO AGRI in Rudice (Czech Republic) for technical and operational support to conduct this research.

## REFERENCES

- Arslan, S., Misiewicz, P., Smith, E., Underbill, T., Franklin, K., White, D. & Godwin, R.J. 2015. The effect of tire tracks on draft force requirements of soil tillage and field traffic systems. In American Society of Agricultural and Biological Engineers Annual International Meeting 2015, **5**, pp. 3753-3762. DOI: 10.13031/aim.20152189648. Available on web site: <https://www.scopus.com/record/display.uri?eid=2-s2.0-84951788616&origin=resultslist&sort=plf-f&cite=2-s2.0-84951788616&refeid=2-s2.0-33748857187&src=s&imp=t&sid=cf4b71ef9d1989bcbf3cc0f4f088d8f3&sot=cite&sdt=a&sl=0&relpos=0&citeCnt=0&searchTerm>
- Barát, M., Rataj, V., Týr, Š., Macák, M. & Galambošová, J. 2017. Effect of controlled traffic farming on weed occurrence. *Agronomy Research* **15**(4), 1484–1490. <https://doi.org/10.15159/AR.17.005>
- Chamen, T. 2015. Controlled Traffic Farming – from worldwide research to adoption in Europe and its future prospects. *Acta Technologica Agriculturae* **3**, 64–73.
- Chyba, J., Kroulík, M., Křištof, K. & Misiewicz, P.A., 2017. The influence of agricultural traffic on soil infiltration rates. *Agronomy Research* **15**(3), 664–673. [http://agronomy.emu.ee/wp-content/uploads/2017/05/Vol15Nr3\\_Chyba.pdf#abstract-5453](http://agronomy.emu.ee/wp-content/uploads/2017/05/Vol15Nr3_Chyba.pdf#abstract-5453)
- Galambošová, J., Macák, M., Rataj, V., Antille, D.L., Godwin, R.J. Chamen, W.C.T. Žitňák, M. Vitázková, B., Ďud'ák, J. & Chlplík, J. 2017. Field Evaluation of Controlled Traffic Farming in Central Europe Using Commercially Available Machinery. In *Transactions of the ASABE* **60**(3), 657–669, ISSN 2151-0032. DOI: <https://doi.org/10.13031/trans.11833>
- 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* **3**, 57–63.
- Gutu, D. 2015. Changes in traffic systems on arable land in agricultural company. *PhD Thesis*. 2015. 158 p. (in Czech).
- Hrivňáková, K., Makovníková, J., Barančíková, G., Bezák, P., Bezáková, Z., Dodok, R. & Širáň, M. 2011. Integrated system of soil analysis techniques (In Slovak: Jednotné pracovné postupy rozborov pôd). Bratislava, Slovakia: VÚPOP, 136 pp.
- Kovár, S., Kovaříček, P., Novák, P. & Kroulík, M. 2016. The effect of soil tillage technologies on the surface of the infiltration speed of water into the soil. *Agronomy Research* **14**(2), 434–441. [http://agronomy.emu.ee/wp-content/uploads/2016/05/Vol14-\\_nr2\\_Kovar.pdf#abstract-4181](http://agronomy.emu.ee/wp-content/uploads/2016/05/Vol14-_nr2_Kovar.pdf#abstract-4181)
- Kroulík, M., Kvíz, Z., Kumhála, F., Hůla, J. & Loch, T. 2011. Procedures of soil farming allowing reduction of compaction. *Precision Agriculture* **12**(3), 317–333. <http://dx.doi.org/10.1007/s11119-010-9206-1>
- Kroulík, M., Hůla, J., Rybka, A. & Honzík, I. 2016. Seed passage speed through short vertical delivery tubes at precise seeding. In *Agronomy Research* **14**(2), 442–449, [http://agronomy.emu.ee/wp-content/uploads/2016/05/Vol14-\\_nr2\\_Kroulik.pdf#abstract-4184](http://agronomy.emu.ee/wp-content/uploads/2016/05/Vol14-_nr2_Kroulik.pdf#abstract-4184)
- Kumhála, F., Gutu, D., Hůla, J., Chyba, J., Kovaříček, P., Kroulík, M., Kvíz, Z., Mašek, J. & Vlášková, M. 2013. Technology of Controlled Traffic Farming on fields. *Certificated methodology*, 40 p. ISBN 978-80-213-2425-1. (in Czech).

- Kutzbach, H.D. 2000. Trends in power and machinery. *J. Agric. Eng. Res.* **76**(3), 237–247. <http://dx.doi.org/10.1006/jaer.2000.0574>
- Lhotský, J., Beran, P., Paris, P. & Valigurská, L. 1991. Degradation of soil by increasing compression. *Soil Till. Res.* **19**(2–3), 287–295. [http://dx.doi.org/10.1016/0167-1987\(91\)90096-G](http://dx.doi.org/10.1016/0167-1987(91)90096-G)
- Rataj, V., Galambošová, J., Macák, M. & Nozdrovický, L. 2014. Precision Agriculture: System – machines – experiences. Praha: ProffiPress. pp.160 ISBN 978-80-86726-64-9. (in Slovak)
- STN 46 1025: 2001. Moisture meters for cereal grains and oilseeds. Technical requirements.
- STN EN ISO 24333/AC: 2010. Cereals and cereal products. Sampling.
- STN EN ISO 520: 2011. Cereals and pulses - Determination of the mass of 1000 grains.
- STN EN ISO 712: 2010. Cereals and cereal products. Determination of moisture content. Reference method.
- Vermeulen, G.D., Tullberg, J.N. & Chamen, W.C.T. 2010. Controlled Traffic Farming. In: D. Athanasios & T. Bartzanas, eds. *Soil Engineering*, Springer Verlag, Soil Biology series, ISSN 1613-3382, ed. Prof. Dr. Ajit Varma, 101–120 pp.

## Specialty types of waste paper as an energetic commodity

J. Malat'ák\*, J. Velebil and J. Bradna

Czech University of Life Sciences Prague, Faculty of Engineering, Department of Technological Equipment of Buildings, Kamýcká 129, CZ165 00, Prague 6 – Suchbátka, Czech Republic

\*Correspondence: malatak@tf.czu.cz

**Abstract.** The collection and recycling rate of paper and paper packaging material has been on a rise. From 2010 to 2016 in Czech Republic, the recycled amount of all paper went up by 32%, while the share of energy use in waste paper utilization decreased from 5.5% to 3.8%. However, not every paper and cardboard product can be recycled, and some are rejected from the recycling stream. Recycling specialty types of paper with other grades of recyclable paper is often not possible and their production is not high enough for their separate recycling to be feasible. If material utilization is not feasible then within the waste hierarchy the next best treatment is their energy utilization. Therefore, this article evaluates selected types of specialty paper for their energy content. They were silicone coated papers, polymer coated papers, and paper cores. For all samples proximate, elemental and calorimetric analyses were determined and based on them stoichiometric combustion calculations were performed. Silicon coated papers fared generally well having small to reasonable ash content 1–10% and net calorific value from 15.10 to 17.10 MJ kg<sup>-1</sup> on dry basis. Polymer coated papers had ash content around 6% and net calorific value from value from 16.29 to 22.98 MJ kg<sup>-1</sup> on dry basis. With the exception of paper cores and self-copying paper, all evaluated paper types could be recommended as a component in refuse derived fuels. The least suitable samples were paper cores with nearly 20% wt. of ash and net calorific value 12.45 MJ kg<sup>-1</sup> on dry basis.

**Key words:** non-recyclable paper, proximate analysis, stoichiometric calculations, heating value.

### INTRODUCTION

Paper has the advantage of being both made from renewable resources as well as being able to be recycled to yield its constituent fibres (Venditti et al., 2000). Recycling waste paper normally means using it as a secondary raw material for the production of new paper (Voronych et al., 2016). Waste paper is converted back to fibres that are usually mixed with a portion of virgin fibres and new paper is produced from this mixture (Ekvall & Finnveden, 2000). Papers with functional layers are generally more difficult to recycle and they may not be suitable to mix with the most prevalent grades of sorted paper (Hess et al., 2001).

Paper fibres cannot be recycled indefinitely, but they can go through the process four to six times (Villanueva & Wenzel, 2007). After the fibres have been recycled, they are gradually deformed and shortened until the last possible product is manufactured e.g. paper cores or moulded pulp. Papers that are difficult to recycle or unprofitable for

recycling often contain specific chemicals that hinder the recycling process. This category includes waterproof papers, coated with PE foil, waxed, with adhesive layer, etc. These types of paper are for example silicone coated papers, polymer coated paper, self-copying paper and paper tubing. These types of paper may come from recycling bins or from industry. Recycling of these types of paper with other grades of recyclable paper is not possible. They may be used, for example, to make insulation materials (Vochozka et al., 2016). However, in a paper sorting line these papers will mostly be discarded and will generally be used in a waste-to-energy plant (Leyskens et al., 2014).

Therefore, it is advisable to find the optimal uses for these types of paper, including the energy or construction industry (Mucahit & Sedat, 2009). It is also possible to compost paper (Saludes et al., 2008; Alvarez et al., 2009; Torkashvand, 2009) or produce biogas (Teghammar et al., 2010; Steffen et al., 2016; Rodriguez et al., 2017).

One of the transformation possibilities when using waste paper as an energy source is briquetting, ideally with biomass (Kers et al., 2010; Gado et al., 2014). Briquetting has some advantages over direct combustion technologies, mainly increasing the energy density, avoiding dust emissions during handling and often enabling cleaner combustion in small combustion devices (Malat'ák et al., 2017). Co-combusting of unwanted paper with biomass-based materials can be expected to produce emission benefits because this type of paper contains a low percentage of sulphur and a very low nitrogen content compared to biomass fuels (Boavida et al. 2003; Salvador et al., 2004; Leyskens et al., 2014).

According to the Ministry of the Environment of the Czech Republic (2017) the recycled amount of all paper and cardboard in Czech Republic went up by 32% from 2010 to 2016, while the share of energy use in waste paper utilization decreased from 5.5% to 3.8%. However, the energy use still amounted to 17,000 t.

In the view of these facts, present paper deals with the issue of using specialty papers as a source of energy. Main aim of experimental testing was to determine the energy potential of chosen types of paper and evaluate the suitability for combustion as a standalone solid fuel or as an additive to other wastes or biomass in production of derived fuels.

## **MATERIALS AND METHODS**

### **The types of specialty papers**

Samples of various specialty papers were evaluated by elemental and proximate analysis and based on them stoichiometric combustion calculations were performed. The samples were obtained from companies producing these papers.

- Self-copying paper (SC) allows to make copies without the use of office equipment making it indispensable in some situations. It consists of two layers one of which is coated with microcapsules which burst under the pressure of a pen and colour the contacting layer.
- Greaseproof polymer coated paper (GPC) with low permeability for fats is used for food packaging or other packaging purposes. It is made from bleached sulphite and sulphate pulps.
- Silicone release paper (SR1) is white, smoothed, wood-free paper with one-sided silicone coating. It is used as release layer for sticky tapes, envelopes etc.

- Silicone release paper (SR2) was discarded release paper with the adhesive layer removed. It is coated on one side with a non-adhesive silicone layer. It is white, smoothed, wood-free paper.
- Silicone release paper (SR3) was the same as SR2 release paper with the adhesive sticker layer present.
- Silicone coated baking paper (BP) is a white, wood-free paper with silicone coating on both sides. It is designed to be hygienically safe at high temperatures, resistant up to 220 °C for single use.
- Packaging paper with one sided polyethylene coating (PEP) mostly used for production of paper bags. It is designed to protect packaged goods against dust, moisture and grease. According to the manufacturer, the unit weight of the paper is 70 g m<sup>-2</sup> and of the coating 20 g m<sup>-2</sup>.
- Paper cores (PC) are paper tubes of mostly cylindrical shape intended to hold strips of sheet material such as papers, foils, textiles, etc. The paper cores used were all spirally wound with layers glued together. There was a variety of sizes.

### **Material preparation and laboratory equipment**

Prior to collection, all samples were kept indoors. After collection, the samples spent at least two weeks in laboratory climate (19–23 °C, relative humidity 40–55%). To produce analytical samples the papers were firstly shredded in an office paper shredder to 4 mm by 35 mm strips. The paper cores were shredded in a cutting mill Retsch SM100 on a 6 mm screen. For each material three 100 g samples were taken from the shredded materials and dried at 105 °C until constant weight for at least 24 hours to find the moisture in the original materials. The shredded samples were then milled in a rotor mill Fritsch Pulverisette 14 under 1 mm size to produce analytical samples.

The proximate analysis was performed in a thermogravimetric analyser (LECO TGA 701). The temperature programme first dried the samples at 105 °C to constant weight to determine the analytical moisture. Ash content was determined after burning the samples in oxygen at 550 °C until constant weight.

Elemental composition was analysed in an instrument LECO CHN628+S with helium as carrier gas to find carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) contents. The analyser operates by analysing the flue gas from samples burned in oxygen. C, H and S are measured in infrared absorption cells; N is measured by a thermal conductivity cell. Oxygen was determined as difference from 100% of the sum of these elements and ash in dry state.

Gross calorific value was measured in an isoperibol calorimeter (LECO AC 600). The samples were pressed into pellets and burned in calorimetric bomb filled to 3 MPa. The reference temperature was 28 °C. The correction for the creation of nitric and sulphuric acid were not determined, otherwise the procedure and conversion to net calorific value was done according to ČSN ISO 1928:2010. At least three reliable results were acquired for all samples.

The results of the analyses were converted to the original state and to dry state of the materials.



### Stoichiometric calculation

The theoretical amount of oxygen  $O_{2,min}$  ( $m^3 \text{ kg}^{-1}$ ) is based on the equation:

$$O_{2,min} = V_m(O_2) \left( \frac{C}{M(C)} + \frac{H}{M(2 \cdot H_2)} + \frac{S}{M(S)} - \frac{O}{M(O_2)} \right) \quad (1)$$

where  $C$ ,  $H$ ,  $S$ , and  $O$  are contents of carbon, hydrogen, sulphur and oxygen in the sample (% wt.);  $V_m(O_2) = 22.39 \text{ m}^3 \text{ kmol}^{-1}$  is the molar volume of oxygen gas at normal conditions and  $M(X)$  ( $\text{kg kmol}^{-1}$ ) are molar masses of hypothetical species  $X$  that combine with  $O_2$ .

Where the theoretical amount of dry air  $L_{min}$  ( $m^3_N \text{ kg}^{-1}$ ) is determined from the equation:

$$L_{min} = O_{2,min} \cdot \frac{100}{C_{atm}(O_2)} \quad (2)$$

where  $C_{atm}(O_2) = 20.95\%$  vol. is volumetric concentration of oxygen in air.

The theoretical amount of dry flue gases  $v_{fg,min}$  ( $m^3 \text{ kg}^{-1}$ ) is based on the equation:

$$v_{fg,min} = \frac{V_m(CO_2)}{M(C)} \cdot C + \frac{V_m(SO_2)}{M(S)} \cdot S + \frac{V_m(N_2)}{M(N_2)} \cdot N + \frac{C_{atm}(N_2)}{100} \cdot L_{min} \quad (3)$$

where  $V_m(X)$  ( $m^3 \text{ kmol}^{-1}$ ) are the molar volumes of flue gas components;  $C_{atm}(N_2) = 78.05\%$  vol. is the concentration of  $N_2$  in air.

The theoretical amount of emission concentrations of  $CO_{2,max}$  ( $m^3_N \text{ kg}^{-1}$ ) is based on the equation:

$$CO_{2,max} = \frac{M(C) \cdot C}{V_m(CO_2) \cdot v_{fg,min}} \cdot 100 \quad (4)$$

Volumetric amounts of combustion products:

$$v(CO_2) = \frac{V_m(CO_2)}{M(C)} \cdot C + \frac{C_{atm}(CO_2)}{100} \cdot L \quad (5)$$

$$v(H_2O) = \frac{2 \cdot V_m(H_2O)}{2 \cdot M(H_2)} \cdot H + \frac{V_m(H_2O)}{M(H_2O)} \cdot W \quad (6)$$

$$v_{N_2} = \frac{V_m(N_2)}{M(N_2)} \cdot N + O_{2,min} \cdot \frac{C_{atm}(N_2)}{C_{atm}(O_2)} \quad (7)$$

where  $W$  (% wt.) is the moisture content in the fuel.

Adiabatic combustion temperature  $t_a$  ( $^{\circ}\text{C}$ ) assumes stoichiometric amount of combustion air and all released heat to be kept in the combustion products. Adiabatic combustion temperature is expressed as:

$$t_a = \frac{NCV}{v_{fg,min} \cdot C_{sp}} \quad (8)$$

where  $NCV$  is the net calorific value of the fuel ( $\text{kJ kg}^{-1}$ );  $c_{sp}$  is the specific heat capacity of the flue gas ( $\text{kJ kg}^{-1} \text{ K}^{-1}$ ).

Adiabatic combustion temperature in excess of air  $t_a$  ( $^{\circ}\text{C}$ ) is used to compare different fuels at the same combustion conditions given by excess air coefficient  $n$ , fuel enthalpy and combustion enthalpy. In this case, the chosen value was  $n = 2.11$ . The theoretical combustion temperature is given by the equation:

$$t_t = \frac{NCV + Q_p + Q_{air}}{V_{fg} \cdot c_{sp}} \quad (9)$$

where  $Q_p$  is the enthalpy of the fuel ( $\text{kJ kg}^{-1}$ );  $Q_{air}$  is the enthalpy of the combustion air ( $\text{kJ kg}^{-1}$ );  $V_{fg}$  is the volume of flue gas ( $\text{m}^3_{\text{N}} \text{kg}^{-1}$ ). Since the specific heat capacity of flue gas is dependent on temperature, the combustion temperatures were determined using iterative calculation. Calculations start using expected temperature and are iterated until two consecutive results differ by less than 0.001 K.

## RESULTS AND DISCUSSION

The results of the elemental analysis of the paper samples (see Table 1) show a high concentration of ash, especially in PC, where the ash content was 19.69% wt. in the dry matter. Such a large amount of ash significantly reduces the calorific value. High contents of ash have detrimental effect on the combustion process as well as the operation combustion plant, e.g. clogging of parts of incinerator by ash (Malat'ák & Passian, 2011). The ash contents were generally high compared to biomass fuels, e.g. herbal biomass is 7.8% wt. (Vassilev et al., 2010; Thy et al., 2006) and pure wood biomass averaging 0.5% wt. (Tao et al., 2012). In this regard, only GPC. SR2, BP and PEP with ash content around 1% correspond roughly to the amount of ash in wood biomass (Gürdil et al., 2009). In general, high ash contents lead to high concentration of particulate matter in flue gas (Niu et al., 2016). With plant biomass it has been shown that the amount and composition of fly ash can be affected by operational parameters of a combustion device, e.g. the excess air amount (Bradna et al., 2016; Bradna et al., 2017).

**Table 1.** Proximate, elemental and calorimetric analysis of specialty papers, values are given in original and dry state

	<i>W</i>	<i>Ash</i>	<i>C</i>	<i>H</i>	<i>N</i>	<i>S</i>	<i>O</i>	<i>GCV</i>	<i>NCV</i>
	% wt.	% wt.	% wt.	% wt.	% wt.	% wt.	% wt.	$\text{MJ kg}^{-1}$	$\text{MJ kg}^{-1}$
<i>Self-copying p. (SC)</i>	5.30	13.36	37.34	4.96	0.17	0.05	38.82	14.33	13.12
<i>dry state</i>		14.11	39.43	5.24	0.18	0.06	40.99	15.13	13.99
<i>Greaseproof p. (GPC)</i>	6.16	1.04	42.79	5.48	0.02	0.06	44.45	16.49	15.14
<i>dry state</i>		1.11	45.60	5.84	0.02	0.07	47.36	17.57	16.29
<i>Silicone p. 1 (SR1)</i>	5.38	9.40	39.03	5.35	0.05	0.12	40.67	15.46	14.16
<i>dry state</i>		9.94	41.25	5.65	0.05	0.13	42.98	16.33	15.10
<i>Silicone p. 2 (SR2)</i>	6.51	0.59	42.01	5.48	0.00	0.04	45.37	16.54	15.18
<i>dry state</i>		0.63	44.93	5.87	0.00	0.04	48.53	17.69	16.41
<i>Silicone p. 3 (SR3)</i>	5.20	9.41	42.97	5.49	0.03	0.03	36.88	17.40	16.08
<i>dry state</i>		9.92	45.32	5.79	0.03	0.03	38.90	18.36	17.10
<i>Baking p. (BP)</i>	6.10	1.19	41.27	5.57	0.00	0.06	45.80	16.60	15.23
<i>dry state</i>		1.27	43.96	5.94	0.00	0.06	48.77	17.68	16.38
<i>Packaging p. (PEP)</i>	6.04	0.75	52.14	7.27	0.00	0.08	33.71	23.16	21.44
<i>dry state</i>		0.80	55.50	7.74	0.00	0.08	35.88	24.67	22.98
<i>Paper cores (PC)</i>	5.54	18.60	34.24	4.02	0.20	0.08	37.32	12.64	11.63
<i>dry state</i>		19.69	36.25	4.25	0.21	0.08	39.50	13.38	12.45
<i>Office paper – dry state*</i>		13.17	37.73	4.84	0.06	0.04	44.15	13.49	12.44
<i>Printed paper – dry state*</i>		21.31	36.86	4.49	0.09	0.03	37.22	13.77	12.80
<i>Cardboard – dry state*</i>		12.17	41.34	5.12	0.14	0.05	41.18	15.46	14.34

*W* – moisture; *GCV* – gross calorific value; *NCV* – net calorific value; \*values from (Balada et al., 2016).

The moisture content of the samples in their original state is low and averages to 5.82% by weight. In biomass such low moisture can only be kept after it has undergone further treatment, for example pelletizing (Malat'ák & Bradna, 2017).

The amount of carbon, as the main carrier of calorific value was mainly influenced by the high ash concentration. In samples of SC and PC, the carbon content in dry matter was less than 40% wt. On the other hand, in PEP it reached up to 55.5% wt.

The resulting heat and calorific values of the materials correspond to the proportion of combustible matter. Above all, a large amount of ash reduced the net calorific value of PC to 12.45 MJ kg<sup>-1</sup> in dry matter. Samples of SC, GPC, SR1, SR2, SR3 and BP achieved net calorific values comparable to wood biomass (Vassilev et al., 2010). The highest net calorific value of all samples was found in PEP with 22.98 MJ kg<sup>-1</sup> in dry matter. Compared to ordinary recycling paper grades, i.e. discarded office paper, printed magazine paper and cardboard (Balada et al., 2016), the presently studied papers, except for PC and SC papers, are more favourable for energy use both in heating value and ash content.

Sulphur content in a fuel has a direct effect on the formation of H<sub>2</sub>SO<sub>4</sub> and therefore also on the service life of the combustion device. Someshwar (2015) shows that addition of sulphur containing fuel may lead to increase in HCl concentration in the flue gas. On the other hand, the presence of SO<sub>2</sub> in flue gas reduces the corrosion of metals by alkali metal halides (Paneru et al., 2103). Highest sulphur concentrations of 0.13% wt. in dry matter were determined in SR1. Sulphur concentrations in other samples correspond to wood (Vassilev et al., 2010). Relatively higher contents of nitrogen were found in PC. However, the maximum concentration of 0.21% wt. in dry matter corresponds to usual levels in plant biomass (Vassilev et al., 2010).

**Table 2.** Stoichiometric volumes of combustion air and gaseous products, adiabatic temperatures under stoichiometric and excess air conditions

	$L_{min}$ m <sup>3</sup> <sub>N</sub> kg <sup>-1</sup>	$v_{fg,min}$ m <sup>3</sup> <sub>N</sub> kg <sup>-1</sup>	$v(CO_2)$ m <sup>3</sup> <sub>N</sub> kg <sup>-1</sup>	$V(H_2O)$ m <sup>3</sup> <sub>N</sub> kg <sup>-1</sup>	$V(N_2)$ m <sup>3</sup> <sub>N</sub> kg <sup>-1</sup>	$CO_{2max}$ % vol.	$t_a$ °C	$t_t$ °C
<i>Self-copy. p. (SC)</i>	3.33	3.30	0.69	0.75	2.60	21.00	2,310	1,068
<i>Greaseproof p. (GPC)</i>	3.77	3.74	0.79	0.84	2.94	21.24	2,350	1,089
<i>Silicone p. 1 (SR1)</i>	3.53	3.48	0.72	0.80	2.75	20.80	2,360	1,088
<i>Silicone p. 2 (SR2)</i>	3.67	3.64	0.78	0.84	2.86	21.39	2,410	1,115
<i>Silicone p. 3 (SR3)</i>	4.04	3.95	0.80	0.84	3.15	20.18	2,360	1,092
<i>Baking p. (BP)</i>	3.61	3.59	0.77	0.84	2.82	21.34	2,450	1,132
<i>Packaging p. (PEP)</i>	5.43	5.21	0.97	1.10	4.24	18.57	2,380	1,093
<i>Paper cores (PC)</i>	2.86	2.87	0.64	0.63	2.24	22.12	2,350	1,095

$L_{min}$  – Stoichiometric volume of combustion air;  $v_{fg,min}$  – Stoichiometric volume of flue gas;  $v(CO_2)$  – Stoichiometric volume of CO<sub>2</sub>;  $v(H_2O)$  – Stoichiometric volume of H<sub>2</sub>O;  $v(N_2)$  – Stoichiometric volume of N<sub>2</sub>;  $CO_{2max}$  – Stoichiometric concentration of CO<sub>2</sub> in dry flue gas;  $t_a$  – Adiabatic temperature in stoichiometric combustion;  $t_t$  – Adiabatic temperature in combustion with excess air.

The elemental contents affect not only the calorific value, but also the behaviour during combustion. In particular, high content of fuel oxygen will affect the stoichiometric consumption of combustion air and the total amount of flue gas produced (Malat'ák et al., 2017). The resulting stoichiometric analysis (see Table 2) shows large differences in these properties, especially between PEP and PC. These samples show higher stoichiometric need for combustion air and therefore higher quantities of flue gas

compared to other samples. The values determined for PEP and SR3 are comparable to e.g. wood biomass (Malat'ák et al., 2013). On the other hand, PC was on the opposite end with low stoichiometric volumes due to low carbon content. These large differences in stoichiometric values of air consumption and flue gas production affect the selection and adjustment of the combustion plant depending on their representation in the fuel stream. Large differences were also found at the maximum carbon dioxide concentration, where PEP is at 18.57% vol. CO<sub>2</sub> and PC 22.12% vol. CO<sub>2</sub>. The resulting stoichiometric parameters have a major impact on the actual efficiency of combustion plants (Malat'ák & Bradna, 2017) and on the total exhaust gas concentration. Similar results were obtained when evaluating the potential of waste biomass in (Brunerová et al., 2017).

Table 2 gives the results of the adiabatic combustion temperatures under stoichiometric and excess combustion air conditions. The highest adiabatic stoichiometric combustion temperature was determined for BP and SR2 samples at 2,450 °C. Since this temperature is limited to stoichiometric amount of air, the adiabatic combustion temperature with excess air is more useful for practical comparison. The highest and lowest values were determined for BP ( $t_i = 1,132$  °C) and SC ( $t_i = 1,068$  °C), respectively. Higher combustion temperature has a positive effect on complete combustion and therefore also on lowering CO emissions (Eskilsson et al., 2004), but on the other hand higher combustion temperature increases NO<sub>x</sub> emission production (Díaz-Ramírez et al., 2014). The problem of lower combustion temperatures may in practice be amended by co-combustion. There have been positively evaluated tests of firing pellets made of cardboard paper and wood sawdust (Leysens et al., 2014) where the results showed that such pellets lead to a very good combustion and boiler efficiency. Boavida et al. (2003) showed that co-combustion of paper with coal and plastics improves the process of combustion increasing the combustion temperature and improving emissions of CO, CO<sub>2</sub> and NO<sub>x</sub>.

## CONCLUSIONS

The use of specialty types of waste paper for energy recovery e.g. in refuse derived fuel can be recommended as long as material recovery is not effective. In practice, according to EN 15359:2011 most of tested types of paper are suitable for addition to refuse derived fuels up to class 3 without detrimental influence judged by their net calorific value above 15 MJ kg<sup>-1</sup> and ash content sufficiently low. Especially good were silicone release paper 2, greaseproof paper, baking paper and polymer coated packaging paper which had both high calorific value and low ash content. On the other end were paper cores and self-copying paper which have calorific value corresponding to class 4, although they could still be mixed for example with biomass to produce class 3 refuse derived fuel. Sufficiently low chlorine content is another demand on refuse derived fuel. As chlorine content was not analysed low level was only assumed, although in theory it could pose some issue with chlorine-bleached papers.

The limiting factor for direct incineration, especially in smaller combustion devices, is the high percentage of ash in some of the specialty paper types. In pressed fuels made solely from paper the unburned remains often effectively prevent the combustion of the inside portion of the pellet or briquette. A large amount of ash, the highest being 19.69% wt. in the dry matter, can cause major problems during combustion, e.g. fouling of the combustion equipment, failure of the fuel to be burn out, etc. For a complete assessment

of the effect of ash on combustibility, it would be necessary to determine its composition and the ash fusion temperatures as e.g. some inorganic species will react beneficially with components of flue gas or, on the other hand, some will melt below combustion temperatures causing the necessity of increased maintenance. To improve combustion properties, it is advisable to burn paper together with plant biomass.

ACKNOWLEDGEMENTS. The article was financially supported by the Internal Grant Agency of the Czech University of Life Sciences Prague, Czech Republic, Grant No. 20183001.

## REFERENCES

- Alvarez, J.V.L., Larrucea, M.A., Bermúdez, P.A. & Chicote, B.L. 2009. Biodegradation of paper waste under controlled composting conditions. *Waste Manage* **29**(5), 1514–1519.
- Balada, I., Altmann, V. & Šařec, P. 2016. Material waste paper recycling for the production of substrates and briquettes. *Agronomy Research* **14**(3), 661–671.
- Boavida, D., Abelha, P., Gulyurtlu, I. & Cabrita, I. 2003. Co-combustion of coal and non-recyclable paper and plastic waste in a fluidised bed reactor. *Fuel* **82**(15–17), 1931–1938.
- Bradna, J., Malat'ák, J. & Velebil, J. 2017. Impact of differences in combustion conditions of rape straw on the amount of flue gases and fly ash properties. *Agronomy Research* **15**(3), 649–657.
- Bradna, J., Malat'ák, J. & Hájek, D. 2016. The properties of wheat straw combustion and use of fly ash as a soil amendment. *Agronomy Research* **14**(4), 1257–1265.
- Brunerová, A., Malat'ák, J., Müller, M., Valášek, P. & Roubík, H. 2017. Tropical waste biomass potential for solid biofuels production. *Agronomy Research* **15** (2), 359–368.
- Díaz-Ramírez, M., Sebastián, F., Royo, J. & Rezeau, A. 2014. Influencing factors on NOX emission level during grate conversion of three pelletized energy crops. *Appl. Energy* **115**, 360–373.
- Ekvall, T. & Finnveden, G. 2000. The application of Life Cycle Assessment to integrated solid waste management. Part 2 - Perspectives on energy and material recovery from paper. *Process Saf. Environ. Protect.* **78**(4), 288–294.
- Eskilsson, D., Rönnbäck, M., Samuelsson, J. & Tullin, C. 2004. Optimisation of efficiency and emissions in pellet burners. *Biomass Bioenerg.* **27**, 541–546.
- Gado, I.H., Ouiminga, S.K., Daho, T., Yonli, A.H., Sougoti, M. & Koulidiati, J. 2014. Characterization of Briquettes Coming From Compaction of Paper and Cardboard Waste at Low and Medium Pressures. *Waste Biomass Valorization* **5** (4), 725–731.
- Gürdil, G.A.K., Malat'ák, J., Selvi, K.C. & Pinar, Y., 2009. Biomass utilization for thermal energy. *AMA-Agric. Mech. Asia Afr. Lat. A.* **40**(2), 80–85.
- Hess, J., Sena-Gomes, R., Davie, L. & Sykes, M. 2001. Recyclability of mixed office waste papers containing pressure sensitive adhesives and silicone release liners. In: *Pulping Conference, Proceedings of the Technical Association of the Pulp and Paper Industry*, Seattle, WA, 989–996 (United States).
- Kers, J., Kulu, P., Aruniit, A., Laurmaa, V., Križan, P., Šooš, L. & Kask, Ü. 2010. Determination of physical, mechanical and burning characteristics of polymeric waste material briquettes. *Estonian Journal of Engineering* **4**, 307–316.
- Leyssens, G., Trouvé, G., Caplain, I., Schönnenbeck, C. & Cazier, F. 2014. Energetic performances and environmental impact of the combustion of cardboard/sawdust in a domestic boiler. *Fuel* **122**, 21–27.
- Malat'ák, J. & Bradna, J. 2017. Heating and emission properties of waste biomass in burner furnace. *Research in Agricultural Engineering* **63**(1), 16–22.
- Malat'ák, J., Bradna, J., Kučera, M., Černý, D. & Passian, L. 2013. Analysis of selected wood biofuels and evaluation of their thermal emission properties. In: *Conference Proceeding - 5th International Conference, TAE 2013: Trends in Agricultural Engineering 2013*, CULS Prague, pp. 402–408 (Czech Republic).

- Malat'ák, J., Bradna, J. & Velebil, J. 2017. The dependence of CO<sub>x</sub> and NO<sub>x</sub> emission concentrations on the excess air coefficient during combustion of selected agricultural briquetted by-products. *Agronomy Research* **15**(1), 1084–1093.
- Malat'ák, J. & Passian, L. 2011. Heat-emission analysis of small combustion equipments for biomass. *Research in Agricultural Engineering* **57**(2), 37–50.
- Ministry of the Environment of the Czech Republic. 2017. *Statistical Yearbook of the Environment of the Czech Republic 2016*. 521 pp. ISBN 978-80-87770-30-6 (in Czech)
- Mucahit, S. & Sedat, A. 2009. The use of recycled paper processing residue in making porous brick with reduced thermal conductivity. *Ceram. Int.* **35**, 26–31.
- Niu, Y., Tan, H. & Hui, S. 2016. Ash-related issues during biomass combustion: Alkali-induced slagging, silicate melt-induced slagging (ash fusion), agglomeration, corrosion, ash utilization, and related countermeasures. *Prog. Energy Combust. Sci.* **52**, 1–61.
- Paneru, M., Stein-Brzozowska, G., Maier, J. & Scheffknecht, G. 2013. Corrosion mechanism of alloy 310 austenitic steel beneath NaCl deposit under varying SO<sub>2</sub> concentrations in an oxy-fuel combustion atmosphere. *Energy Fuels* **27**(10), 5699–5705.
- Rodriguez, C., Alaswad, A., El-Hassan, Z. & Olabi, A.G. 2017. Mechanical pretreatment of waste paper for biogas production. *Waste Manage.* **68**, 157–164.
- Saludes, R.B., Iwabuchi, K., Miyatake, F., Abe, Y. & Honda, Y. 2008. Characterization of dairy cattle manure/wallboard paper compost mixture. *Bioresour. Technol.* **99**(15), 7285–7290.
- Salvador, S., Quintard, M. & David, C. 2004. Combustion of a substitution fuel made of cardboard and polyethylene: Influence of the mix characteristics - Experimental approach. *Fuel* **83**(4–5), 451–462.
- Someshwar, A.V. 2015. The role of SO<sub>2</sub> in determining hydrogen chloride emissions from combination bark-fired boilers. *NCASI Technical Bulletin* (**1032**), 1–102.
- Steffen, F., Requejo, A., Ewald, C., Janzon, R. & Saake, B. 2016. Anaerobic digestion of fines from recovered paper processing - Influence of fiber source, lignin and ash content on biogas potential. *Bioresour. Technol.* **200**, 506–513.
- Tao, G., Lestander, T.A., Geladi, P. & Xiong, S. 2012. Biomass properties in association with plant species and assortments I: A synthesis based on literature data of energy properties. *Renew. Sust. Energ. Rev.* **16**(5), 3481–3506.
- Teghammar, A., Yngvesson, J., Lundin, M., Taherzadeh, M.J. & Horváth, I.S. 2010. Pretreatment of paper tube residuals for improved biogas production. *Bioresour. Technol.* **101**(4), 1206–1212.
- Thy, P., Jenkins, B.M., Grundvig, S., Shiraki, R. & Leshner, C.E., 2006. High temperature elemental losses and mineralogical changes in common biomass ashes. *Fuel* **85**(5–6), 783–795.
- Torkashvand, A.M. 2009. Influence of the molasses and office paper as carbonic amendments in municipal compost production. *Biotechnology* **8**(1), 114–119.
- Vassilev, S.V., Baxter, D., Andersen, L.K. & Vassileva, C.G. 2010. An overview of the chemical composition of biomass. *Fuel* **89**(5), 913–933.
- Venditti, R., Gilbert, R., Zhang, A. & Abubakr, S. 2000. The effect of release liner materials on adhesive contaminants, paper recycling and recycled paper properties. In: *Proceedings of the 2000 TAPPI Recycling Symposium 2*, Washington, DC, 579–591 (United States).
- Villanueva, A. & Wenzel, H. 2007. Paper waste - Recycling, incineration or landfilling? A review of existing life cycle assessments. *Waste Manage.* **27**(8), S29–S46.
- Vochozka, M., Maroušková, A., Straková, J. & Váchal, J. 2016. Techno-economic analysis of waste paper energy utilization. *Energy Sources Part A-Recovery Util. Environ. Eff.* **38**(23), 3459–3463.
- Voronych, O., Kurta, S., Starchevskyy, V. & Fedorchenko, S. 2016. Technology of recycling, properties and use of polyvinylchloride-coated paper waste. *Chemistry and Chemical Technology* **10**(2), 219–2226.

## **Evaluation of break-even point and gross margin economic risks in producing winter oilseed rape**

M. Mimra<sup>1,\*</sup>, M. Kavka<sup>1</sup> and P. Markytán<sup>2</sup>

<sup>1</sup>Czech University of Life Sciences Prague, Faculty of Engineering, Department of Machinery Utilisation, Kamýcká 129, CZ–16521 Prague 6 – Suchbát, Czech Republic

<sup>2</sup>Union of Oilseeds Growers and Processors, Na Fabiánce 146, CZ–18200 Prague 8–Březiněves, Czech Republic

\*Correspondence: [mimra@tf.czu.cz](mailto:mimra@tf.czu.cz)

**Abstract.** The economic result of growing winter oilseed rape is influenced by a number of variables, such as natural influences, input prices and the used technology. One of the ways to improve the business's profitability is to use the experience and knowledge provided by consulting companies. This paper analyses two data series covering the period of 5 to 10 years regarding specific selected key parameters for companies using the counselling services of the Union of Oilseeds Growers and Processors in Prague (UOGP) and some other companies that make no use of these services (OTHERS).

For the selected key parameters, the risk analysis of reaching the gross margin and the break-even point was conducted with the aid of the Monte Carlo stochastic simulation method. The results of the calculations show that the companies using UOGP consulting achieve on average, at the same level of risk, a gross margin higher by 30% and their break-even point is lower by 11%. Taking advantage of the knowledge and services provided by a consulting company has positive economic benefits, and it increases the competitiveness of companies.

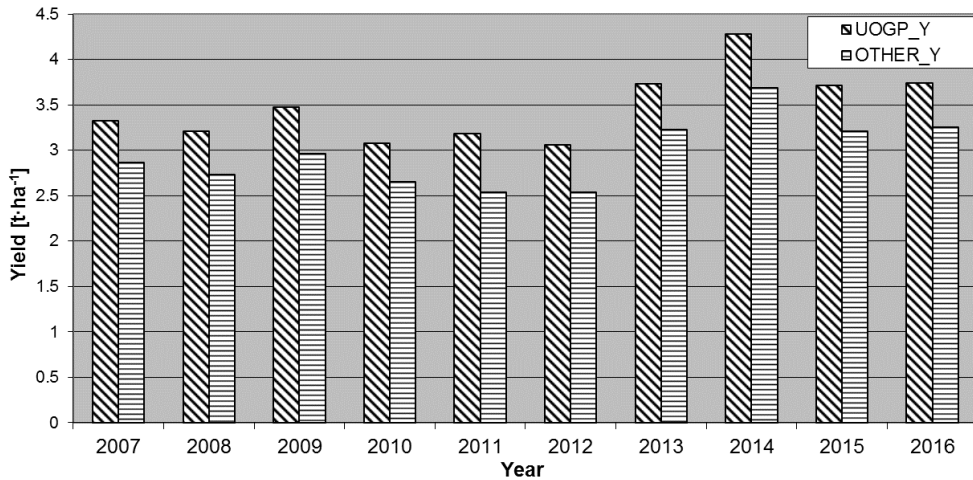
**Key words:** risk analysis, Monte Carlo method, counselling and consulting services.

### **INTRODUCTION**

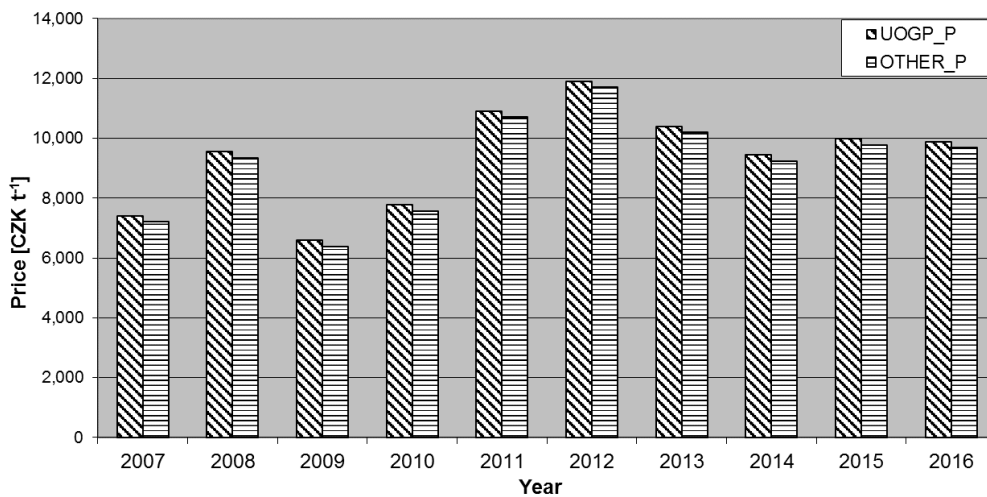
Oilseed rape is one of the most important agricultural crops in the Czech Republic. Winter oilseed rape on arable land in the production year 2015–2016 amounted to 359,243 hectares (Volf & Zeman, 2016), which meant on average 14.4% of the arable land. The high amount of winter oilseed rape and its ongoing increase are mainly due to its market attractiveness. This, on the one hand, means a higher market production, mainly due to higher yields and farmer prices. On the other hand, it is necessary to use more intensive growing technologies, which results in higher costs (Markytán, 2016).

Farmer prices (Fig. 1) and yields (Fig. 2) drive the market production. Both components of market production are under the influence of the market environment, the influence of weather and the level of compliance with the technological discipline in the respective company. Strict adherence to technological discipline also has an impact on input prices and thus on costs (Janotová, 2016; also see Fig. 3), concerning items that the farmer generally cannot influence (such as purchase prices, taxes, fees) as well as the

items which depend on his decisions (such as number of operations, machine sets, etc.). In addition, the impact of consultancy in the Czech Republic leads to the positive production results of winter oilseed rape, which are at a rather high level due to the services provided by Union of Oil Seeds Growers and Processors Prague (hereinafter referred to as ‘UOGP’). Quality advice means better adherence to technological discipline and a better position for farmers to sell winter oilseed rape on the domestic Czech market or on foreign export markets. Quality advice concerning farmer prices for winter oilseed rape considers many factors. Of course, better compliance with technological discipline also means higher unit costs.

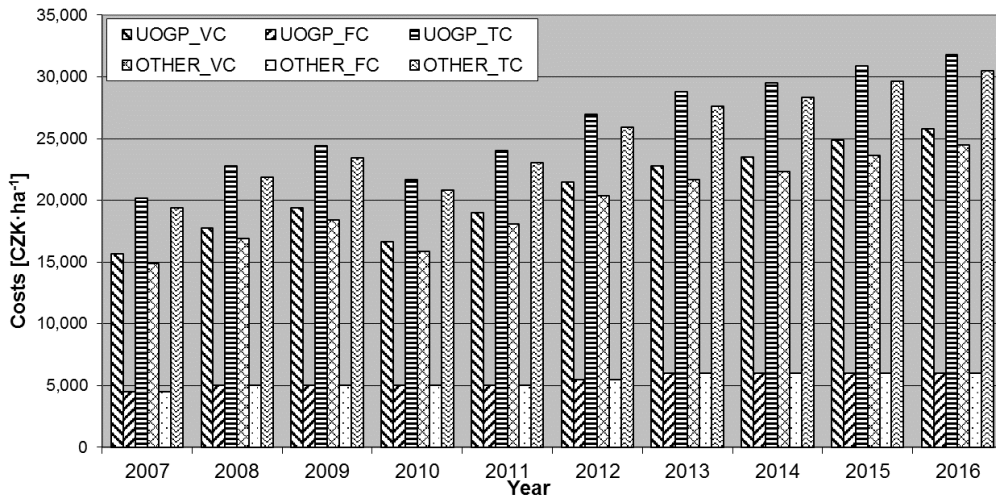


**Figure 1.** Development of winter oilseed rape yields (Source: UOGP Prague).



**Figure 2.** Development of farmer prices for winter oilseed rape (Source: UOGP Prague).





**Figure 3.** Development of the costs (Source: UOGP Prague and [www.agroconsult.cz](http://www.agroconsult.cz)): (VC = variable costs; FC = fixed costs; TC = total costs).

The prosperity and competitiveness of the production depends on the mutual relation between costs, prices and revenues in the market environment. For managerial decision-making, it is therefore essential to analyse constantly the available information and to evaluate the degree of feasibility of the managerial targets (Wolke, 2008), which includes evaluating risks.

In order to outline model risky situations in the production of sugar beet, Gleißner & Berger (2004) used the algorithm of generating random numbers based on predetermined conditions and statistical distribution. When examining crop yield efficiency, Koenker & Zhao (1996) found out that there is a quite a high number of possible risk situations which can occur (e.g. technical, technological, economic and market risks). Therefore, it is recommended to determine the type of distribution by using the quantile method. Quantiles express the degree of random distribution probability of the random variable. They shape the points in which the distribution functions of a random variable intersect a given value. Therefore, according to Koenker & Hallock (2001), it is necessary to establish a pessimistic and an optimistic estimates of possible, expected situations concerning winter oilseed rape, and then to apply the triangular distribution for modelling.

Producing winter oilseed rape is influenced by a number of factors, which are intertwined. Unfortunately, greater attention has not been given to the benefits of the expert experience from companies providing counselling on the economic results of winter oilseed rape yet. Against this background, this paper evaluates the economic risks of growing winter oilseed rape based on UOGP's statistical data recorded over the last 5 to 10 years in order to quantify these risks using simulation models. The results are divided into two groups: for members of the UOGP and for non-members of the UOGP (hereinafter referred to as 'OTHERS'). The reason for such a segmentation is the above-mentioned potential differences between these two groups. In the group of the UOGP oilseed growers, data obtained from 47 agricultural enterprises were included; in the group of OTHERS, data obtained from 41 agricultural enterprises were included. The

total number of hectares on basis of what the average yield was calculated changed depending on crop rotation and the number of sown hectares.

In this paper, the values given in the calculations are in CZK (Czech crowns). Please note that the recent conversion rate of CZK to EUR is 25.50 CZK €<sup>-1</sup>.

## MATERIALS AND METHODS

Modelling is based on the principle of generating random values within boundary conditions for their triangular statistical distribution (Evans et al., 2000). The input parameters are always based on optimistic and pessimistic estimations of the parameter and its most frequent occurrence, which is a so-called distribution peak (Table 1). The risk analysis was conducted with the aid of the stochastic Monte Carlo simulation method's algorithm; its principle was described by Kroese et al. (2011), concerning generating a pseudo-random variable for input parameters. The calculation principle is based on simulating a critical variable using 100,000 simulations (of risk situations) and constructing a two-sided frequency distribution interval at a materiality level of 0.05. The mathematical model created in Microsoft Excel using the Crystal Ball Ad-In is utilised to determine the mean value of a magnitude that results from a random sample. Consequently, data obtained through simulations can be statistically evaluated.

**Table 1.** Marginal conditions used for modelling

Indicators	UOGP			OTHERS		
	P	ML	O	P	ML	O
Yield, t ha <sup>-1</sup>	3.5	3.7	3.9	3	3.2	3.4
Farmer price, CZK ha <sup>-1</sup>	9,760	10,000	10,750	9,560	9,880	10,750
Total costs, CZK ha <sup>-1</sup>	30,700	29,700	28,400	29,500	28,500	27,300
Variable costs, CZK ha <sup>-1</sup>	24,700	23,700	22,600	23,470	22,500	21,450

Legend: P – pessimistic estimate; ML – most likely estimate; O – optimistic estimate.

Parameters, which are likely to change, were selected in order to be considered in the modelling. With regard to market production, the parameters concern changes in the oilseed yields and farmer prices related to one hectare of winter oilseed rape. On the cost side, they concern changes in variable costs (such as labour, materials, maintenance of machines), changes in outputs depending on the fluctuating demand each year, in price changes or in total costs (= variable costs increased by fixed costs, e.g. overhead costs, annual depreciation, insurance) related to one hectare of winter oilseed rape. As a reference parameter, the value of gross profit (GP – see relation 2) and contribution to the gross margin (GM – relation 3), which were reached per hectare, have been selected. In order to compare the results reached by the group of farmers who were members of the UOGP and a group of non-members, the parameter of an achieved break-even point (BEP – relation 4) was selected.

Yield values were generated based on the input analysis in Fig. 1 and on the boundary conditions in Table 1; values of the farm price, according to Figs 2 and 4, and cost values, according to Fig. 3. For the calculation of an approximate probability distribution, the so-called tree-point estimation technique was used. As the top of triangular distribution (the 'most likely' value), an average value of the years 2012 to 2016 was determined. The 'pessimistic estimation' was determined by using the

minimum from the year 2012. An ‘optimistic estimation’ reflects the potential possibility of including new species under favourable cultivation conditions. Final values shown in this paper were calculated based on data provided by UOGP experts, allowing for their estimations and opinions, too.

Market production (MP) is set as:

$$MP = Y \cdot P, \text{ (CZK ha}^{-1}\text{)} \quad (1)$$

where  $Y$  – yield, t ha<sup>-1</sup>;  $P$  – price, CZK t<sup>-1</sup>.

Gross profit (GP) is set as:

$$GP = MP - TC, \text{ (CZK ha}^{-1}\text{)} \quad (2)$$

Where  $MP$  – market production, CZK ha<sup>-1</sup>;  $TC$  – total costs, CZK ha<sup>-1</sup>.

Gross margin (GM) is set as:

$$GM = MP - VC, \text{ (CZK ha}^{-1}\text{)} \quad (3)$$

where  $VC$  – variable costs, CZK ha<sup>-1</sup>.

Break-even point (BEP), is the variant of relation 2 when:

$$GP = 0 \quad (4)$$

Subsequently, this question was determined for the model: ‘Which risk can be expected when a certain value of gross margin is reached by changing the parameters?’ (Table 1). The variation of this question was assessing the risk of reaching a break-even point where the  $GP = 0$ , or at what farmer price of rapeseed this break-point is reached.

Interpretation of the risk is also reflected in the results of the calculation. In general, the interpretation of risk (Smejkal & Rais, 2009) does not follow a clear rule. The limit of the permissible risk of the project depends on the manager's attitudes as well as on the risks, which cannot be influenced like, for example, the development of world market prices. The risk margins of one commodity often correspond to the risk level of other commodities, which are included in the portfolio of the managerial entity. Risk estimates (i.e. pessimistic, optimistic and unexpected) applied in the analysis of economic risks of winter oilseed rape production were based on a qualified analysis of the production and market situation in the Czech Republic. When interpreting the issue of crop risk, it is appropriate to use a classification where the risk up to 20% is rated as low, 21 to 40% as acceptable, 41 to 60% as high, and above 60% as very high (= unacceptable).

For the risk analysis, the gross profit (excluding overheads, taxes, etc.), gross margin and break-even point values were used; all of them are important indicators for managerial decision-making. The gross profit criterion is based on the principle of neoclassical economic theory, which focuses on profit maximisation when taking decisions. Planting technologies are also affected by natural influences and market conditions, which the agricultural company cannot control itself. Therefore, special attention should be paid to the point at which planting becomes profitable, as well as to the gross margin analysis. In companies with a higher share of fixed total costs, the same increase in sales will result in a higher gain in revenues than in enterprises with a lower share of fixed total costs.

## ANALYSIS OF THE PARAMETERS FOR CALCULATION

### 1. Yield (Y)

According to the results monitored by **UOGP** (=Union of Oilseeds Growers and Processors) in Prague, the average yield of winter oilseed rape (Fig. 1) for the last 10 years amounted to 3.48 t ha<sup>-1</sup> for UOGP members and to 2.97 t ha<sup>-1</sup> for non-UOGP members (hereinafter referred to as 'OTHERS'). According to an analysis of the last 5 years, the average yield for UOGP members was 3.70 t ha<sup>-1</sup> and for non-members 3.18 t ha<sup>-1</sup>. Based on the comparison of the average yields over the last 10 and 5 years, an increasing tendency was noticeable. Therefore, the marginal conditions (Table 1) of the average values from the last 5 years have been used for modelling.

### 2. Farmer price (P)

The farmer price (which in this paper means their selling price) for winter oilseed rape is directly dependent on the growing year, on the EUR to CZK exchange rate, the situation on the commodity exchange MATIF and the initial sales strategy of each agricultural company. Under such market conditions, larger companies, which are generally involved with the UOGP have a competitive advantage.

For the analysis, the average prices tracked by UOGP were also taken into consideration. By analysing these data (Fig. 2), the marginal conditions for the triangular statistical division as per Table 1 were determined.

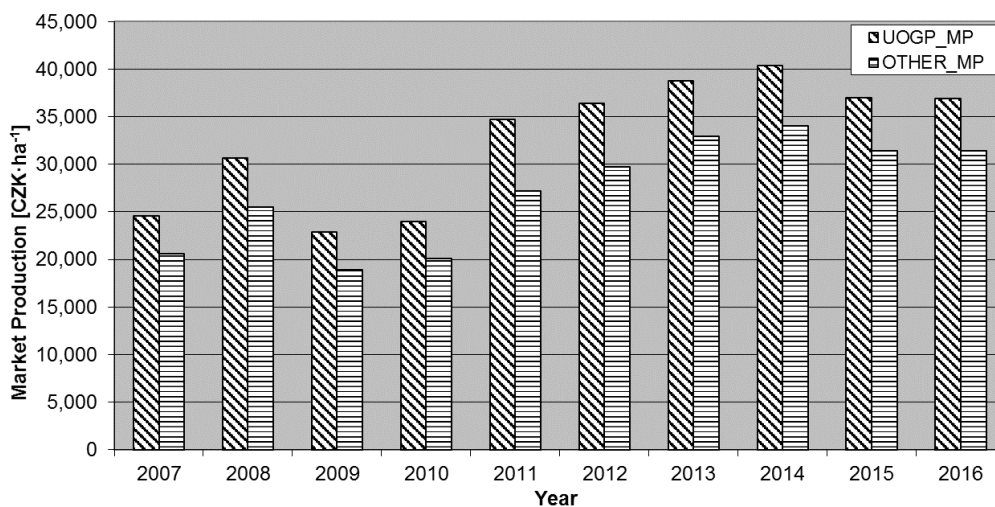
### 3. Costs

The value of the costs was broken down into variable (VC), fixed (FC) and total costs (TC). It was first analysed on the basis of the data monitored by UOGP Prague and then on the basis of an expert calculation done by the AgroConsult advisory system. The value of the costs showed an increasing tendency, which corresponded with the development of input prices and with the compliance with the technological discipline.

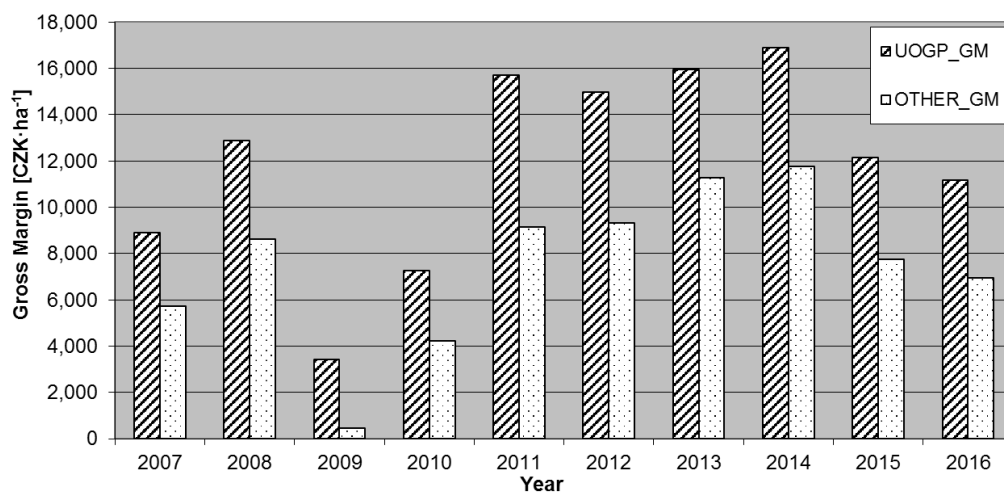
During the last 10 years, the average cost was 9,380 CZK t<sup>-1</sup> for members of UOGP and 9,280 CZK t<sup>-1</sup> for OTHERS. In view of the slightly rising trend, the marginal conditions (Table 4) were set based on the data from the last 5 years, where the average price for UOGP members was 10,317 CZK t<sup>-1</sup> and for OTHERS 10,117 CZK t<sup>-1</sup>.

### 4. Market production (MP) and gross margin (GM)

The results of calculations concerning market production (Fig. 4) and gross margin (Fig. 5) for UOGP members and OTHERS are shown in Table 2. The resulting values were calculated by multiplying the relevant input parameters according to the equation 1 to 3. The SAPS subsidies were not included in calculating the market output; this fact is important for the subsequent assessment of the risk connected to producing given that the SAPS subsidies were gradually reduced by the EU. The tables depict a comprehensive view of the situation in the winter oilseed rape production economy.



**Figure 4.** Market production of winter oilseed rape.



**Figure 5.** Gross margin of winter oilseed rape.

**Table 2.** Average MP, GM and BEP values

Indicators	UOGP	OTHERS
Market production, CZK ha <sup>-1</sup>	37,000	31,616
Gross margin, CZK ha <sup>-1</sup>	13,300	9,116
Break-even point, t ha <sup>-1</sup>	2.97	2.88

## RESULTS AND DISCUSSION

Based on the modelling of the input parameters using the built-in model, the following results were obtained. The results of the risk analysis are shown using the probability distribution graphs of the gross margin and the break-even point. The results

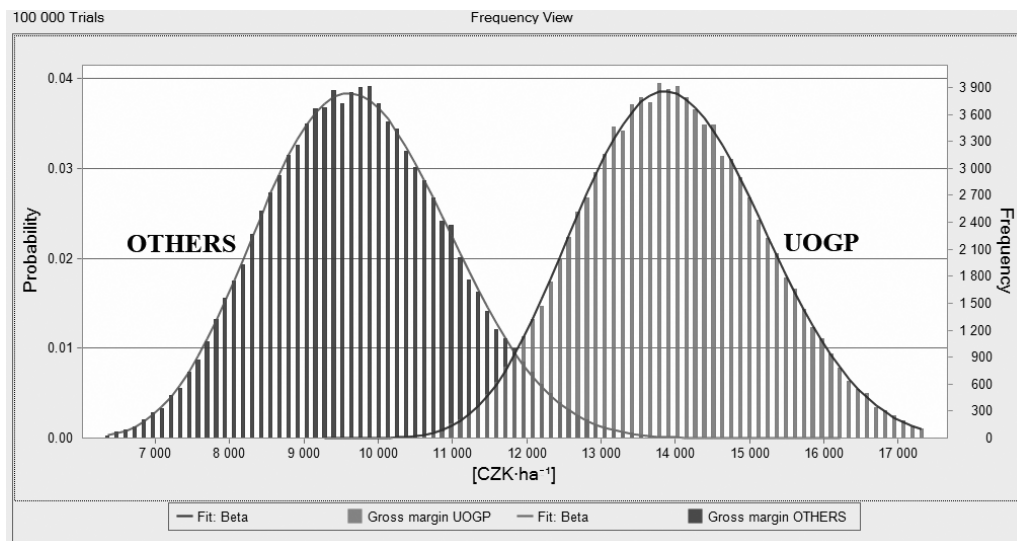
obtained from the risk situations were statistically evaluated using descriptive statistics. For interpretation of the simulation results, a frequency curve was used. The frequency curve displays the frequency of the occurrence of the generated values in the scope of the selected range (between the minimum and the maximum value). Based on this range, the variance distribution can be analysed. Each analysed value (parameter) represents the result of one possible situation.

The form of the value distribution in the frequency curve indicates the nature of the risk of the analysed parameter. The smaller the height of the curve and the range of the minimum and maximum values, the smaller is also the probable risk connected to the parameter being analysed.

The distribution curve displays the cumulative frequency of occurrence of the analysed parameter. By means of the distribution curve, it is possible to determine the probability with which the occurrence of individual generated values can be expected. In this interval, therefore, the probability of occurrence of any (whichever) value of this parameter can be analysed. The inverted value of probability determines the risk that the values can exceed.

### Gross margin

The overlay graph in Fig. 6 shows the distribution of the probability rates of the pay-out amount reached for UOGP members and OTHERS. The statistical characteristics of both values are shown in Table 3. Concerning UOGP members and OTHERS, the same maximum value was reached in order to achieve the maximum contribution rate of 3.9%. For OTHERS, the minimum was 5,788 and the maximum was 14,159 CZK·ha<sup>-1</sup>. For UOGP members, the minimum was 9,926, and the maximum was CZK 18,715 ha<sup>-1</sup>.

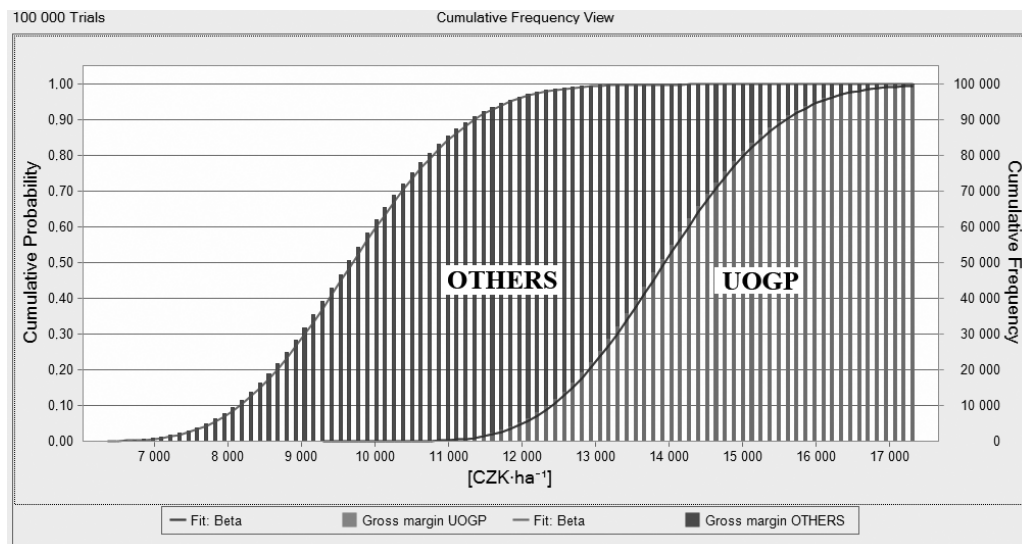


**Figure 6.** Distribution curves and distribution of the probability of reaching a gross margin.

**Table 3.** Characteristics of statistical indicators GM and BEP

Indicators	Gross margin (GM)		Break-even point (BEP)	
	CZK ha <sup>-1</sup>		CZK t <sup>-1</sup>	
	UOGP	OTHERS	UOGP	OTHERS
Trials	100,000.00	100,000.00	100,000.00	100,000.00
Base Case	13,300.00	9,116.00	8,027.03	8,906.25
Mean	13,967.68	9,727.26	8,004.56	8,890.72
Median	13,933.97	9,689.89	8,002.62	8,884.52
Stand. Deviation	1,221.70	1,218.53	217.47	267.59
Variance	1,492,539.00	1,484,818.00	47,292.00	71,606.00
Skewness	0.16	0.17	0.05	0.10
Spikiness	2.78	2.79	2.65	2.65
Coeff. of Variation	0.09	0.125	0.027	0.03
Minimum	9,926.10	5,778.02	7,327.50	8090.02
Maximum	18,715.48	14,158.95	8,712.05	9778.99
Range Width	8,789.38	8,380.93	1,384.56	1,688.96
Mean Std. Error	3.86	3.85	0.69	0.85

For OTHERS, the arithmetic average was 9,727 CZK ha<sup>-1</sup>. Its value was slightly higher than the median of 9,689 CZK · ha<sup>-1</sup> and it had a slight movement of 0.17 – which means that the distribution of the values around the centre is asymmetrical, with slight deviation to the right. The basic payment allowance based on an expert estimate of 9,116 CZK ha<sup>-1</sup> is achieved with a probability of 68%. The standard deviation, expressing the probability of deviation from the arithmetic mean, was 1,219 CZK ha<sup>-1</sup>. The variation coefficient, which expresses the variability of the contribution to the risk reimbursement, was 0.13. Spikiness of 2.79 indicates that the values are distributed more densely and steeply around the centre than in the normal distribution.



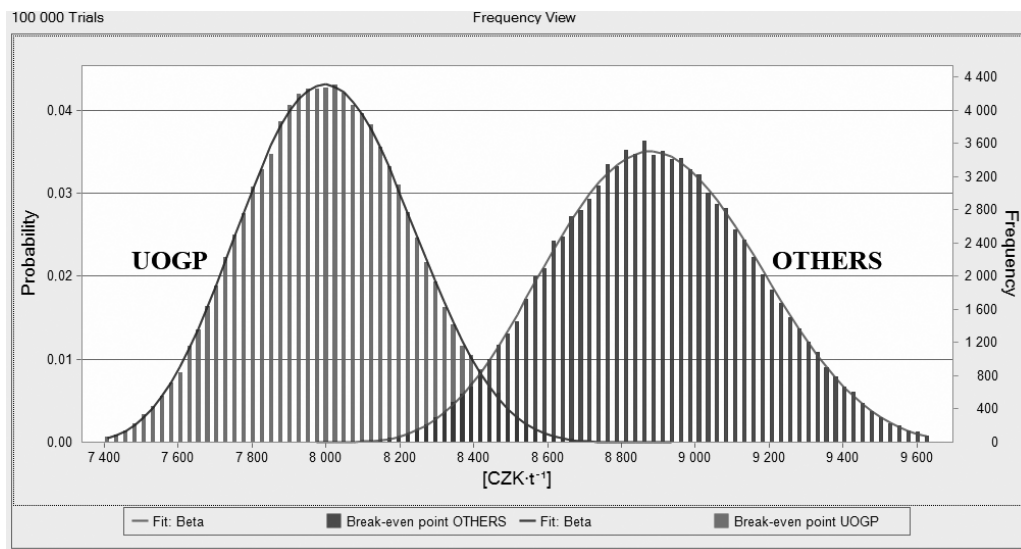
**Figure 7.** Cumulative frequency graph of the probability of reaching gross margin.

For UOGP, the arithmetic average value was 13,967 CZK ha<sup>-1</sup>; the median, 13,933 CZK ha<sup>-1</sup>, and the movement, 0.16. Spikiness 2.78 shows that the values are distributed densely and more steeply around the centre. The basic payment allowance, based on an expert estimate of 13,300 CZK ha<sup>-1</sup>, is achieved with a probability of 69%.

Fig. 7 shows the cumulative frequency of the probability of obtaining a contribution to cover fixed costs for UOGP members and OTHERS. Both distributions of the likelihood of achieving the gross margin show similar data distribution characteristics. These distributions were shifted jointly by 4,184 CZK ha<sup>-1</sup>. The UOGP members will then retain variable costs of 4,184 CZK ha<sup>-1</sup> for fixed costs. Thus, UOGP members can achieve higher profits than OTHERS by expanding their production. Moreover, UOGP members will reach the same probability of a higher gross margin, and there is a lower risk of not meeting the prescribed contribution amount in order to cover the costs.

### Break-even point

The break-even point determines the specific amount of production, which causes neither profit nor loss. This point also determines the amount of farmer price that an agricultural company must achieve so that its economic outturn is not negative. The overlay graph in Fig. 8 shows the breakdown of frequency in achieving farmer price, which makes the company neither profitable nor unprofitable. For OTHERS this value was 8,906 CZK t<sup>-1</sup> (obtained with a probability of 47%); for UOGP members it amounted to 8,027 CZK t<sup>-1</sup> (obtained with a probability of 46%). The absolute difference between these two groups totalled 879 CZK t<sup>-1</sup>.

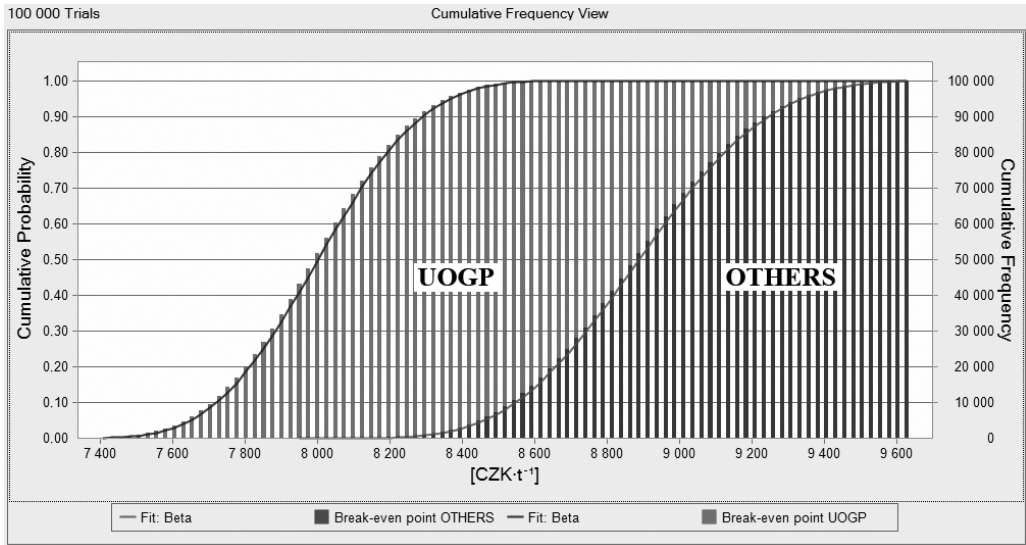


**Figure 8.** Distribution curves and distribution of the probability of reaching a break-even point.

UOGP members achieved a higher frequency of the mean value of 4.2%, compared to OTHERS for whom the probability of the mean value was 3.5%. Both distributions of frequencies reached almost an even distribution of data around the centre when the arithmetic mean and median of the individual distributions had almost the same values. The standard deviation was for OTHERS 268 and for UOGP members 217 CZK t<sup>-1</sup>. A



higher value of the skewness was reached for OTHERS (0.09) compared with 0.05 for UOGP members. They had a difference of minimum and maximum values of more than 304.4 CZK t<sup>-1</sup>. This is also evident from the values of standard deviation, which for OTHERS was 267.59 and for UOGP members only 217.47 CZK t<sup>-1</sup>. Statistical characteristics of distribution of both values for the reference parameters are shown in Table 3. Fig. 9 depicts a cumulative frequency probability of reaching the break-even point. UOGP members achieve a lower BEP at the same level of probability, i.e. they make a profit earlier when growing winter oilseed rape. The predicted BEP values for each 10% interval rate (= 0%, 10%, 20%, 30%, ...) are shown in Table 4.



**Figure 9.** Graph of the cumulative frequency of probability of reaching a break-even point.

**Table 4.** Prediction of probability distribution of GM and BEP

Indicators	Gross margin (GM) CZK ha <sup>-1</sup>		Break-even point (BEP) CZK t <sup>-1</sup>	
	UOGP	OTHERS	UOGP	OTHERS
Percentile				
100%	9,926.10	5,778.02	7,327.50	8,090.02
90%	12,402.29	8,172.03	7,721.63	8,545.18
80%	12,905.59	8,670.27	7,813.26	8,655.90
70%	13,286.12	9,045.66	7,883.00	8,740.39
60%	13,615.14	9,375.31	7,944.50	8,813.98
50%	13,933.97	9,689.86	8,002.62	8,884.52
40%	14,255.34	10,004.37	8,060.00	8,956.90
30%	14,598.93	10,352.95	8,122.83	9,033.50
20%	15,007.48	10,762.66	8,193.30	9,123.81
10%	15,577.92	11,342.61	8,291.26	9,245.64
0%	18,715.48	14,158.95	8,712.05	9,778.99

## CONCLUSIONS

According to Rayburn (2009), the possible benefits of using the stochastic methods are an improved performance and better economic results of the agricultural company. Marin et al. (2017) applied the stochastic methods in order to model the crop yields. The results of the evaluation of economic risks in producing winter oilseed rape using the stochastic methods can lead to the following conclusions:

Companies which are taking advantage of the knowledge and services provided by a consultancy company and which comply with a good technological discipline achieve better economic results in winter oilseed rape production, despite the higher costs for the planting technologies. In general, it can be said that the higher the gross profit the company plans to achieve, the higher is the risk of reaching this target. Homolka & Mydlar (2011) found out in their research that profit is significantly influenced by the changes in the farmer prices of winter oilseed rape. When interpreting the issue of crop production risk, it is possible to use a classification where the risk up to 20% is rated as low, 21 to 40% as acceptable, and 41 to 60% as high and above 60% as very high (unacceptable).

Companies that are members of UOGP achieve the same likelihood higher gross margin (the average rank difference is 4,241 CZK ha<sup>-1</sup>). An increasing range of winter oilseed rape production reduces unit fixed costs, and this results in growing profits. In addition, these companies achieve lower values of the break-even point (the average value difference is 886 CZK t<sup>-1</sup>). This allows them at a lower purchase price of winter rapeseed not to be in the red, in comparison with non-UOGP members.

The members of UOGP reach the value of the contribution to the fixed costs of CZK 13,300 per hectare with a probability of 69%, and the OTHERS reach a fixed cost allowance of 9,116 CZK ha<sup>-1</sup> with a probability of 68%. The amount of the gross margin is sufficient to cover the fixed costs of both above-mentioned groups. Accordingly, it enables them to further development their companies.

The presented method of modelling the economic risks of growing winter oilseed rape can be applied to other crops as well. The accuracy of the modelling results depends on the accuracy of the input parameters of the assessed agricultural company and the growing region. In other words: In order to obtain the most accurate and appropriate results, it is highly recommended not to evaluate the average values collected within a large area (such as an entire EU-country), but rather to apply this method precisely to the input parameters specific for each agricultural company (considering its production technology, used material and machinery, selling price, costs, etc.) or to smaller regions with similar cultivation conditions.

### List of used abbreviations:

BEP – break-even point, CZK t<sup>-1</sup>

FC – fixed costs, CZK ha<sup>-1</sup>

FP – farmer price, CZK ha<sup>-1</sup>

GM – gross margin, CZK ha<sup>-1</sup>

MP – market production, CZK ha<sup>-1</sup>

TC – total costs, CZK·ha<sup>-1</sup>

VC – variable costs, CZK ha<sup>-1</sup>

Y – yield, t

ACKNOWLEDGEMENTS. This paper was completed as part of the project no. MSM6046070905, supported by the Czech Ministry of Education, Youth and Sports.

## REFERENCES

- Evans, M., Hastings, N. & Peacock, B. 2000. *Statistical distributions* – third edition (p. 187). New York, John Wiley & Sons Inc., 220 pp.
- Gleißner, W. & Berger, T. 2004. *Auf nach Monte Carlo: Simulationsverfahren zur Risiko-Aggregation*. In German (The Monte Carlo: Simulation method for risk aggregation) *Risknews* **1**(1), 30–37.
- Homolka, J. & Mydlar, R. 2011. *Efficiency evaluation in intensive growing of winter rape*. *Agricultural Economics*. Vol. 57, Is. 5, 247-257, ISSN: 0139570X.
- Janotová, B. 2016. *Ekonomika pěstování řepky a její vývoj pro rok 2015 v ČR. (Rape Growing Economy and its Development for 2015 in the Czech Republic)*. In Rapeseed production system, sunflower production system, 33rd evaluation seminar, 23.-21.11.2016, Hluk. Hluk: UOSP Praha, 2016. 95–99 (in Czech).
- Koenker, R. & Hallock, K. 2001. Quantile Regression: An Introduction. *Journal of Economic Perspectives* **15**, 143–156.
- Koenker, R. & Zhao, Q. 1996. Conditional Quantile Estimation and Inference for Arch Models. *Econometric Theory*. Cambridge University Press, Vol. 12, No. 5 (Dec., 1996), 793–813.
- Kroese, D.P., Taimre, T. & Botev, Z.I. 2011. *Handbook of Monte Carlo Methods*. New York, John Wiley & Sons. 772 pp. ISBN 0-470-17793-4.
- Marin, F.J., Jones, W., Boote, K.J. 2017. A Stochastic Method for Crop Models: Including Uncertainty in a Sugarcane Model. *Agron. J.* **109**, 483–495. doi:10.2134/agronj2016.02.0103.
- Markytán, P. 2016. *Ekonomika pěstování řepky. (Rape Growing Economy)*. In *Rapeseed production system, sunflower production system, 33rd evaluation seminar, 23.-21.11.2016, Hluk*. Hluk: UOSP Praha, 2016. pp. 100–113 (in Czech).
- Rayburn, E.B. 2009. Estimating Economic Risk Using Monte Carlo Enterprise Budgets. *Forage and Grasslands* **7**. doi:10.1094/FG-2009-0415-01-MG.
- Smejkal, V. & Rais, K. 2009. *Řízení rizik ve firmách a organizacích*. (Risk management in firms and companies). Prague, Grada, 360 pp. (in Czech).
- Volf, M. & Zeman, J. 2016. *Výsledky pěstování řepky v České republice. (Results of rape growing in the Czech Republic)*. In *Rapeseed production system, sunflower production system, 33rd evaluation seminar, 23.-21.11.2016, Hluk*. Hluk: UOSP Praha, 2016. pp. 1–34 (in Czech).
- Wolke, T. 2008. *Risikomanagement*. München, Oldenburg Wissenschaftsverlag GmbH, 134 pp.

## **Productive performance of broilers at the final stage of breeding submitted to different levels of metabolizable energy in different thermal environments**

K.P. Oliveira<sup>1</sup>, C.F.S. Ferreira<sup>1</sup>, I.F.F. Tinoco<sup>1</sup>, R.R. Andrade<sup>1</sup>, M. Barbari<sup>2,\*</sup>,  
V.M.F. Cruz<sup>3</sup>, F.J.F. Baptista<sup>3</sup>, M.F.A. Vieira<sup>4</sup>, L. Conti<sup>2</sup> and G. Rossi<sup>2</sup>

<sup>1</sup>University of Viçosa, Department of Agricultural Engineering of Federal, Av. Peter Henry Rolfs, s/n Campus University of Viçosa, CEP: BR36570-900, Viçosa, Brazil

<sup>2</sup>University of Firenze, Department of Agricultural, Food and Forestry Systems, Via San Bonaventura, 13, IT50145 Firenze, Italy

<sup>3</sup>University of Évora, Department of Rural Engineering, Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Núcleo da Mitra, Évora, Portugal

<sup>4</sup>Federal Rural University of Amazonia – UFRAPA-256, s/n – Nova Conquista, Paragominas – PA, 68627-451

\*Correspondence: [matteo.barbari@unifi.it](mailto:matteo.barbari@unifi.it); [kelle.oliveira@ufv.br](mailto:kelle.oliveira@ufv.br)

**Abstract.** The Brazilian poultry industry is an activity in constant development due to the high indices of productive efficiency. The accelerated evolution of poultry production has allowed to obtain early and very efficient broilers able to convert different foods into animal protein. However, due to this intensive breeding system, a series of metabolic and management problems appeared, with emphasis on thermal stress. The objective of this work was to evaluate the physiological responses of broiler chickens in the final stage of breeding (21 to 42 days of life), submitted to two thermal conditions, one representative of the thermoneutrality situation (T1) and one giving a situation of cyclic stress by heat (T2). For each experimental thermal condition, the birds were submitted to different levels of metabolizable energy of 3,050, 3,125, 3,200, 3,275 kcal kg<sup>-1</sup>. At 28, 35 and 42 days, the birds and the feed leftovers were weighed to measure the performance variables: CR (feed intake), GP (weight gain) and CA (feed conversion), viability of the rearing (Vb), productive efficiency index (PEI).

As conclusions, the GP was 13.6% higher for the birds maintained at the thermoneutrality situation T1. The PEI was 32.5% higher for the birds maintained in T1 condition, when compared to those kept in T2. However, both in thermoneutral and in heat stress conditions, the increase in the level of metabolizable energy in the diet did not influence the performance and the productive efficiency index of broiler chickens aged between 21 and 42 days of age.

**Key words:** broilers, feed, metabolizable energy, productivity, thermal stress.

### **INTRODUCTION**

The Brazilian poultry industry is an activity in constant development thanks to the indexes of productive efficiency with the largest and most advanced technological collection of the agricultural sector. The accelerated evolution of poultry breeding

resulted in the production of early and very efficient broilers able to convert different foods into animal protein. However, due to this intensive process, a series of metabolic and animal management problems emerged, with emphasis on thermal stress. Broilers have a large mass, so they produce great quantities of pollutants (Aarnink et al., 2009; Kic & Růžek, 2014) and metabolic heat. The thermal environment inside the barn is a major factor influencing the performance, health and welfare of broilers in intensive farms. Due to their considerable feed intake and the subsequent high metabolism, broilers produce a high level of surplus heat (Syafwan et al., 2011) that can bring to heat stress (Reiter & Bessei, 2000). High ambient temperature has a negative effect on broiler production efficiency (Sandercock et al., 2001). Broilers exposed to high temperature and relative humidity find it difficult to maintain their core body temperature (Borges et al., 2007). To improve the thermal conditions inside the building in hot season it is necessary to apply an intensive ventilation and sometimes a cooling of supplied air, but also passive systems can be effective (Kic, 2016).

Feeding, besides influencing productive performance and carcass characteristics, is the factor that affects the production costs of broilers. For this reason rations providing the best cost/benefit ratio are explored. Dietary energy needs for maintenance increase when broiler chickens are subjected to environmental temperature above their thermal neutral zone (Hurwitz et al., 1980). Apparent metabolizable energy needs of broilers are influenced by ambient temperature (Dozier III et al., 2007). Considering that birds voluntarily reduce feed intake as the ambient temperature rises above the thermal comfort range, a ration formulated for thermoneutral conditions would not be adequate to meet the energy requirement of birds in a heat stress environment.

The utilization of metabolizable energy in growing broilers can be estimated by using an energy balance technique (Lopez & Leeson, 2007). Metabolizable energy intake is well documented to influence body composition (Morris, 2004) and performance of growing broilers (Leeson et al., 1996). The determination of the nutritional requirement of metabolizable energy is fundamental in the different phases of broiler breeding, as the digestibility increases with the age of the bird due to the development of the digestive tract, which leads to the improvement of its capacity to assimilate nutrients and energy from feed (Mello et al., 2009).

According to Bou et al. (2005), the inclusion of vegetable oil in rations of birds kept under heat stress reduces the depressive effects of temperature on its performance. The beneficial effect of the addition of oil in the rations of animals submitted to heat stress is associated to changes in gastrointestinal physiology and to the lower caloric increment verified during the digestion, absorption and assimilation of nutrients of the rations containing higher oil content (Bertechini, 2012).

On the basis of the above consideration, the objective of the present research was to evaluate the effect of different levels of metabolizable energy in the diet of broilers, raised under two levels of thermal conditions on performance and productive efficiency index. In one case the temperature was maintained inside the thermoneutral zone and in the other one conditions of cyclic heat stress were realized.

## MATERIALS AND METHODS

The experiment was conducted in four climatic chambers, located in the experimental area of the Centre for Research in Environment and Engineering of Agroindustrial Systems (AmbiAgro), belonging to the area of Rural Buildings and Environment of the Department of Agricultural Engineering of the Federal University of Viçosa, Minas Gerais, Brazil.

Each climate chamber has the dimensions of 2.5 x 3.5 x 2.5 m (respectively height, length and width), equipped with an electric resistance air heater (2,000 W of power), an air conditioner of the hot / cold split type of 3,500 W, an air humidifier with a capacity of 4.5 L and a mist flow rate of 300 mL h<sup>-1</sup>.

The heater and humidifier were operated by an electronic controller MT-531 R i Plus of temperature and humidity (0.1 °C resolution, control humidity 20 to 85% RH, with 0.1% RH resolution).

Ventilation applied inside the climatic chambers was obtained through axial exhausters, AMB, Model FD 08025S1M DC 12V 0.15A, with automatic activation, during the whole experimental period. The ventilation was controlled in order to maintain the desired temperatures and the quality of the air in accordance with the norms described in the Protocol of Good Practices of Production of Chickens (Brazilian Poultry Association, 2008). The axial fans were able to guarantee twice renews of air per hour in the climatic chambers.

The experiment was carried out in the final stage of breeding, between the 21<sup>st</sup> and 42<sup>nd</sup> day of life of the birds. For the trials 280 broilers of the Cobb 500 line, with an average initial weight of 1,267 kg ( $\pm$  5%), were used. The birds were put in cages with the following dimensions: 1.0 m wide x 0.5 m long x 0.5 m high (Fig. 1). To replicate the field conditions each cage contained seven birds, with a density of 14 birds.m<sup>-2</sup>. A new 5 cm thick bed of woodchips was provided at the beginning of the trials.



**Figure 1.** View of cages used for the trials. Each cage (1.0 m wide x 0.5 m long x 0.5 m high) contained 7 broilers.

Two temperatures were applied, one representative of the thermoneutrality situation (T1) and the other of the situation of cyclic stress by heat (T2), as determined by Pareja (2014). In condition T1 the birds were submitted to a fixed temperature of 25 °C, during 24 hours a day throughout the experimental period. For condition T2, the birds were subjected to 12 hours of heat stress from 7:00 AM to 7:00 PM at 31 °C while during the other 12 hours they were kept at 25 °C, as shown in Table 1.

**Table 1.** Experimental treatments, defined on the basis of air temperatures (°C), for broilers between 21 and 42 days

Temperature	Situation	Temperature (°C)	
		7:00h to 19:00h	19:00h to 07:00h
T1	Thermoneutrality	25	25
T2	Heat Stress	31	25

The air relative humidity (RH) was controlled during the experimental period in both treatments, in the range from 55 to 65%, through the automatic system present in the climatic chambers.

For each imposed thermal condition, the birds were submitted to four levels of metabolizable energy, 3,050, 3,125, 3,200 and 3,275 kcal kg<sup>-1</sup>, according to the recommendations of Rostagno et al. (2011), presented in Table 2.

**Table 2.** Composition of rations used during experimental trials

Ingredients	Metabolizable Energy kcal kg <sup>-1</sup>			
	3,050	3,125	3,200	3,275
Corn grain	65.6327	63.8867	62.1406	60.3946
Soybean meal 45%	27.1138	27.4137	27.7135	28.0134
Meat and bone meal 40%	3.8260	3.8392	4.5412	5.9846
Soybean oil	1.6544	3.0978	3.8524	3.8655
Limestone	0.4177	0.4125	0.4073	0.4021
Common salt	0.3886	0.3891	0.3896	0.3901
DL-methionine (99% purity)	0.3119	0.3135	0.3151	0.3167
L-lysine HCL (99% purity)	0.3040	0.2979	0.2918	0.2856
Vitamin supplement <sup>1</sup>	0.2500	0.2500	0.2500	0.2500
Mineral supplement <sup>2</sup>	0.2500	0.2500	0.2500	0.2500
L-threonine (99% purity)	0.0920	0.0918	0.0916	0.0914
Choline chloride 60%	0.0500	0.0500	0.0500	0.0500
L-tryptophan (99% purity)	0.0088	0.0078	0.0069	0.0059
	100,00	100,00	100,00	100,00
<b>Calculated composition</b>				
Metabolizable energy (kcal kg <sup>-1</sup> )	3,050.0	3,125.0	3,200.0	3,275.0
Crude protein (%)	19.8000	19.8000	19.8000	19.8000
Vegetable fat (%)	4.9015	6.2739	7.6464	9.0188
Calcium (%)	0.7600	0.7600	0.7600	0.7600
Phosphorus available (%)	0.3500	0.3500	0.3500	0.3500
Lysine dig. for birds (%)	1.1300	1.1300	1.1300	1.1300
Methionine + Cystine dig. for birds (%)	0.8300	0.8300	0.8300	0.8300
Methionine dig. for birds (%)	0.5721	0.5727	0.5734	0.5741
Threonine dig. for birds (%)	0.7300	0.7300	0.7300	0.7300
Tryptophan dig. for birds (%)	0.2040	0.2040	0.2040	0.2040
Sodium (%)	0.2000	0.2000	0.2000	0.2000

<sup>1</sup>Guaranteed levels per kg of product (minimum): Folic acid 0.3 mg, Pantothenic acid 12 mg, Nicotinic acid 50 mg, Biotin 0.05 mg, Niacin 30 mg, Vitamin A 10,000.000 IU, Vitamin B1 1.5 mg, Vitamin B12 0.015 mg, Vitamin B2 6 mg, Vitamin B6 4 mg, Vitamin D3 2,000.000 IU, Vitamin E 28 IU, Vitamin K3 3 mg, Excipient q.s. 1,000 g.

<sup>2</sup>Guaranteed levels per kg of product (minimum): Cobalt 2 mg, Copper 10 mg, Iron 50 mg, Iodine 0.7 mg, Manganese 78 mg, Selenium 0.18 mg, Zinc 55 mg, Excipient q.s. 1,000 g.

The rations were provided in order to keep feeders always full (*ad libitum*), following the criteria used in the field. The water was also supplied *ad libitum* being replaced three times a day, thus avoiding the heating of the water in the drinkers. The feeders used were trough type and the drinkers were nipple type.

Data on productive performance were collected: weight gain (kg); feed intake (kg); feed conversion.

The birds were weighed at 21, 28, 35 and 42 days of age to evaluate body weight and weight gain (GP). The feed consumption was calculated on the basis of the difference between the amount of feed supplied and surplus in these periods, following a methodology by Sakomura & Rostagno (2007).

Mortality was recorded on a daily basis, for later conversion of the viability data (Vb).

The productive efficiency index (PEI) was calculated using the following equation (Stringhini et al., 2006).

$$PEI = \frac{\text{weight gain} \times \text{viability of creation}}{\text{days until the end of the experiment} \times \text{food conversion}} \times 100$$

### Statistical analysis

In order to evaluate the effect of the ambient temperature and metabolizable energy levels on the average values of the analysed variables, representative of the productive performance and the productive efficiency index, a statistical analysis was performed using the subdivided plots scheme for T1 and T2 with four subplots (3,050, 3,125, 3,200 and 3,275 kcal kg<sup>-1</sup>), in a completely randomized design, with five replications. The data were interpreted by means of analysis of variance and, for the quantitative factor, by regression, in which the models were chosen based on the significance of the regression coefficients using Student's t test, at the 5% probability level. The averages were compared using the Tukey test, at the 5% level of significance. For such analyses, the software SAEG, System for Statistical Analysis, version 9.1 (2007) was used.

All procedures used in this experiment were approved by the Committee on Ethics in the Use of Animals (CEUA) of the Federal University of Viçosa, Minas Gerais, Brazil, Protocol No. 75/2014.

## RESULTS AND DISCUSSION

Table 3 shows the ANOVA summary for the variables viability (Vb) and productive efficiency index (PEI) corresponding to climatic chambers temperatures (TEMP) and metabolizable energy levels (EL).

As it can be observed in Table 3, temperatures (TEMP) significantly influenced the variables of the average weight gain ( $P < 0.01$ ) and the results of the productive efficiency index (PEI) of the chickens ( $P < 0.05$ ). The levels of metabolizable energy in the diet did not significantly influence the productive efficiency index. Similar results were found by Leandro et al. (2003), who verified that the different treatments did not influence PEI, for the total period of rearing (1 to 46 days), although nutritional plans 1 and 2 obtained numerically better indexes for males and females.



**Table 3.** Summary of variance analysis of the variables of average weight gain (GP), feed conversion (CA) in kg, viability (Vb) and productive efficiency index (PEI) in %, for broilers aged between 21 and 42 days, submitted to different temperatures (TEMP) and different levels of metabolizable energy (EL) (DF: Degree of Freedom)

Factor	DF	GP	CA	Vb	PEI
TEMP	1	0.327**	0.034 <sup>NS</sup>	2,040.816 <sup>NS</sup>	12,380.400*
Residue (a)	8	0.011	0.025	647.959	1,582.858
EL	3	0.041 <sup>NS</sup>	0.070 <sup>NS</sup>	34.013 <sup>NS</sup>	846.730 <sup>NS</sup>
TEMP x EL	3	0.005	0.028 <sup>NS</sup>	40.816 <sup>NS</sup>	117.017 <sup>NS</sup>
Residue (b)	24	0.015	0.026	386.054	1,255.247

\*\* – significant 1%, \* – significant 5%, <sup>NS</sup> – not significant.

Table 4 presents the results of the test of averages applied to the mean weight gain variable (GP) of the birds, influenced by the temperatures.

It is observed in Table 4 that the average weight gain (GP) was 13.6% higher for the birds maintained at the thermoneutral temperature (T1, 25°C), when compared to those maintained at the temperature of cyclic stress by temperature (T2, 25–31 °C). This is due to the fact that when kept under heat stress, broilers reduce their growth in a higher proportion than feed consumption, which results in a worse feed conversion ratio, as cited.

Table 5 shows the results of the test of averages applied to the index of productive efficiency index (PEI) of broilers, influenced by temperatures.

**Table 4.** Mean values of the average weight gain index (GP) in kg, for chickens at the temperatures T1 (25 °C) and T2 (25–31 °C)

Temperature	GP
T1	1.494a
T2	1.315b

The averages followed by at least one letter in the column do not differ at the 1% level of significance by the Tukey test.

**Table 5.** Average values of the productive efficiency index (PEI) in %, for chickens at the temperatures T1 (25 °C) and T2 (25–31 °C)

Temperature	PEI
T1	143.449a
T2	108.263b

The averages followed by at least one letter in the column do not differ at the 1% level of significance by the Tukey test.

In Table 5 it can be observed that the productive efficiency index (PEI) was 32.5% higher for the birds maintained at the thermoneutral temperature (T1, 25 °C), when compared to those maintained at the temperature of the cyclic stress by temperature (T2, 25–31 °C). This difference is due to the fact that birds exposed to high temperatures reduce feed intake to decrease metabolic heat production and thereby maintain homeothermia, which also results in a decrease in growth. This aspect is well documented in literature (Hurwitz et al., 1980; Dozier III et al., 2007).

The stress generated to the animal by the variation of the thermal environment directly influences the productivity, in relation to variations in the heat exchanges with the environment, the amount of feed consumed, the body weight gain and, consequently, the nutritional requirements, as well documented in several works (Souza & Batista, 2012; Mohammed et al., 2018).

## CONCLUSIONS

As conclusion, the weight gain was 13.6% higher for the birds maintained in the thermoneutrality situation (T1). This is due to the fact that when kept under thermal stress, broilers reduce their growth in a higher proportion than food consumption, which results in a worse feed conversion ratio.

The productive efficiency index was 32.5% higher for birds kept in condition T1. This difference is due to the fact that birds exposed to high temperatures reduce feed intake to decrease the production of metabolic heat and thus maintain homeothermia, which also results in a decrease in growth.

However, both in thermoneutral and in heat stress conditions, the increase in the level of metabolizable energy in the diet did not influence the performance and the productive efficiency index of broiler chickens aged between 21 and 42 days.

ACKNOWLEDGEMENTS. AmbiAgro, CNPq, CAPES and FAPEMIG.

## REFERENCES

- Aarnink, A.J.A., Van Harn, J., Winkel, A., De Buissonje, F.E., Van Hattum, T.G. & Ogink, N.W.M. 2009. Spraying rapeseed oil reduces dust in poultry houses. In: *Precision Livestock Farming 2009, 4<sup>th</sup> European Conference on Precision Livestock Farming*. Elsevier. Wageningen, 73–79.
- Bertechini, A.G. 2012. *Nutrition of monogastric*. Lavras: Ed. UFLA. 2012, 373 pp. (in Portuguese).
- Borges, S.A., Da Silva, A.V.F. & Maiorka, A. 2007. Acid-base balance in broilers. *World's Poult. Sci. J.* **63**, 73–81.
- Bou, R., Guardiola, F., Barroeta, A.C. & Codony, R. 2005. Effect of dietary fat sources and zinc and selenium supplements on the composition and consumer acceptability of chicken meat. *Poult. Sci.* **84**, 1129–1140.
- Dozier III, W.A., Purswell, J.L., Kidd, M.T., Corzo, A. & Branton, S.L. 2007. Apparent metabolizable energy needs of broilers from two to four kilograms as influenced by ambient temperature. *J. Appl. Poult. Res.* **16**, 206–218.
- Hurwitz, S., Weiselburg, M., Eisner, U., Bartov, I., Riesenfeld, G., Sharvit, M., Niv, A. & Bornstein, S. 1980. The energy requirements and performance of growing chickens and turkeys as affected by environmental temperature. *Poult. Sci.* **59**, 2290–2299.
- Leandro, N.S.M., Café, M.B., Stringhini, J.H., Jardim Filho, R.M., Moura, K.A. & Silva Júnior, R.P. 2003. Feeding program with different dietary crude protein and metabolizable energy levels, for broilers. *Magazine Brazilian of Animal Husbandry* **32**, 620–631 (in Portuguese).
- Leeson, S., Caston, L. & Summers, J.D. 1996. Broiler response to diet energy. *Poult. Sci.* **75**, 529–535.
- Lopez, G. & Leeson, S. 2005. Utilization of metabolizable energy by young broilers and birds of intermediate growth rate. *Poult. Sci.* **84**, 1069–1076.
- Mello, H.H.C., Gomes, P.C., Rostagno, H.S., Albino, L.F.T., Souza, R.M. & Calderano, A.A. 2009. Metabolizable energy values of feedstuffs obtained from poultry at different ages. *Magazine Brazilian of Animal Husbandry* **38**, 863–868 (in Portuguese).
- Mohammed, A.A., Jacobs, J.A., Murugesan, G.R. & Cheng, H.W. 2018. Effect of dietary synbiotic supplement on behavioral patterns and growth performance of broiler chickens reared under heat stress. *Poult. Sci.* pex421.

- Morris, T.R. 2004. Nutrition of chicks and layers. *World's Poult. Sci. J.* **60**, 5–18.
- Kic, P. & Růžek, L. 2014. Microbiological environment in special rooms of University campus. *Agronomy Research* **12**(1), 837–842.
- Kic, P. 2016. Microclimatic conditions in the poultry houses. *Agronomy Research* **14**(1), 82–90.
- Pareja, J.C.A. 2014. *Zootechnical performance and physiological of broilers, in the final stage of growth, subjected to different levels of heat stress*. Viçosa: UFV, 2014. Master thesis. Universidade Federal de Viçosa.
- Reiter, K. & Bessei, W. 2000. Effect of stocking density of broilers on temperature in the litter and at bird level. *Arch. Geflügelkd.* **64**, 204–206.
- Rostagno, H.S., Albino, L.F.T., Donzele, J.L., Gomes, P.C., Oliveira, R.F., Lopes, D.C., Ferreira, A.S., Barreto, S.L.T. & Euclides, R.F. 2011. *Brazilian tables for poultry and pigs: food composition and nutritional requirements*. 3<sup>rd</sup> Ed. Viçosa: UFV. 252 pp. (in Portuguese).
- SAEG. 2007. *System for Statistical Analyzes*. Versão 9.1. Fundação Arthur Bernardes - UFV - Viçosa (in Portuguese).
- Sakomura, N.K. & Rostagno, H.S. 2007. *Methods of research on nutrition of monogastrics*. Jaboticabal: Funep, 283 pp. (in Portuguese).
- Sandercok, D.A., Hunter, R.R., Nute, G.R., Mitchell, M.A. & Hocking, P.M. 2001. Acute heat stress-induced alterations in blood acid-base status and skeletal muscle membrane integrity in broiler chickens at two ages: implications for meat quality. *Poult. Sci.* **80**, 418–425.
- Souza, B.B. & Batista, N.L. 2012. The effects of heat stress on animal physiology. *Agropecuária Científica no Semi-Árido* **8**, 6–10 (in Portuguese).
- Stringhini, J.H., Andrade, M.L., Andrade, L., Xavier, S.A., Gomes, C.M. & Leandro, N.S.M. 2006. Performance, nutrient balance and retention and biometrical measures of digestive organs of broilers fed different dietary protein levels in the prestarter period. *Magazine Brazilian of Animal Husbandry* **35**, 2350–2358 (in Portuguese).
- Syafwan, S., Kwakkel, R.P. & Verstegen, M.W.A. 2011. Heat stress and feeding strategies in meat-type chickens. *World. Poult. Sci. J.* **67**, 653–673.

## **Implementation of practical solutions to improve buffalo breeding development in rural areas of South Iraq**

G. Rossi<sup>1</sup>, L. Conti<sup>1</sup>, K. Al-Fartosi<sup>2</sup> and M. Barbari<sup>1,\*</sup>

<sup>1</sup>University of Firenze, Department of Agricultural, Food and Forestry Systems (GESAAF), Via San Bonaventura 13, IT50145 Firenze, Italy

<sup>2</sup>Dhi-Qar University, College of Science, University Campus, IQ0096442 Nassiriyah, Iraq

\*Correspondence: [matteo.barbari@unifi.it](mailto:matteo.barbari@unifi.it)

**Abstract.** Buffalo breeding is widely spread in rural areas of southern Iraq, especially in marshlands. In the Provinces of Basrah, Dhi Qar and Maysan the buffalo represents the main source for survival of local people in marshlands. The paper shows the situation in these areas, remarking the main critical points for buffalo breeding development. A survey in 24 buffalo farms was carried out by the Department of University of Firenze to verify the situation of buffalo farming in southern Iraq (years 2014–2016). In particular the study shows problems related to the shortage of food for animals, to the lack of suitable structures and infrastructures, to the poor herd management by farmers. In the paper the main critical points, which compromise the success of the breeding, are pointed out, with particular reference to animal welfare, animal health and labour (care of animals and risks for workers). Solutions to mitigate the scarce results are indicated. In particular some practical interventions carried out during a project funded by Italian Agency for Cooperation and Development are presented and discussed. Such interventions refer mainly to the application of feeding racks, systems for a safe capture of the animals, plants and equipment for heat protection. As conclusion, also in difficult and poor areas like Iraqi marshes, a correct design and a right application of simple constructive and plant solutions, with little employment of technological resources, coupled with a good training of farmers, can give a contribution to solve problems in buffalo breeding.

**Key words:** buffalo, livestock housing, marshlands, Iraq.

### **INTRODUCTION**

The marshlands in southern of Iraq historically comprised the largest wetland ecosystem of Western Eurasia. The Mesopotamian marshes of southern Iraq (30° to 33° N, 45° to 48° E) are considered the ‘cradle of western civilization’ and are often referred as the ‘Garden of Eden’.

Livelihood of inhabitants of marshes, the marsh Arabs or Ma'adan which are the descendants of ancient Sumerians, is entirely dependent, with a symbiotic relationship, on domestic Asian water buffalo (*Bubalus bubalis*) (Al-Saedy & Al-Fartosi, 2013). The buffaloes provide to people dairy products, meat, skin, dung for fuel, and labour. There is no house in the marshes area without buffaloes (Al-Saedy & Al-Fartosi, 2011).

Buffalos have high capacity to face adverse environmental conditions and a remarkable longevity (up to 10 years production period) (Pawar et al., 2012). The ideal habitats for water buffalo are floodplain environments with a mixture of abundant grasses and available water bodies (Petty et al., 2007). Access to water is important also for thermoregulation of buffalos which go to the marshes in the hot season to cool their body temperature (Fazaa, 2007). The water buffalo feeds on common reeds by grazing as well as on reeds cut by the farmers. Buffalos can be also fed with protein-rich concentrated food when available (Abid & Fazaa, 2007).

Many factors led to decline of buffalo population, such as the Gulf wars, the economic blockade, the removal of subsidies on feed for farmers (FAO, 2005; Al-Saedy, 2007). However, the systematic marsh drainage can be considered the main cause for the reduction of buffalo population. During the period of 1991–2003, wide areas of reed beds and lakes of southern Iraqi marshes were ditched and drained by the previous Iraqi regime for political reasons. Marsh desiccation was an environmental disaster that severely affected the wildlife of southern Iraq (Richardson & Hussain, 2006).

The number of buffalos decreased from 141,450 heads in 1986 to 98,700 heads in 1993 (Ligda, 1996). Another study reported that in the early 1990s total buffalo population in Iraq was about 200,000, but by 2003 it dropped to 130,000 heads, with a 35% decrease (Al-Marsomy, 2005).

The re-flooding of the marshland areas after the second Gulf war of 2003 allowed to many breeders to return to the marshes and buffalo breeding resumed developing. Buffalo population increased in the study area after re-flooding, reaching numbers higher than after desiccation and potentially close to the numbers recorded before desiccation.

In 2007 a report on Maysan and Dhi Qar Provinces showed a steady increase in the number of buffalos, estimated in 40,008 head. About 83% were female, and approximately 72% of these females were more than two years old, the age of fertility and milk productivity (Abid & Fazaa, 2007).

Al-Saedy & Al-Fartosi (2013), reporting data from Ministry of Agriculture Census (2008) about water buffalos distribution in Iraq, indicated a total number of 285,537. In Southern Mesopotamian marshes about 45% of buffalos of the entire Iraq were concentrated, and in Dhi Qar Province 49,283 heads (29,270 female) were counted.

The management pattern of buffalo herd is based on small holders, which own between 5 and 50 head. Only few herds can arrive to more than 150 head.

The main objective of buffalo breeding is the production of dairy products, in particular fresh milk and thick cream. Males, used to produce meat, are slaughtered after weaning.

The performance of adult buffalo female is considered in the range of 3–7 litres of milk per day, with 7–8% of fat. Lactation lasts 250 days on average (Al-Saedy & Al-Fartosi, 2011). The highest milk production is reached when the cow can be fed with concentrated high protein food, but even in the best cases, the amount of milk produced per cow is still low (Abid & Fazaa, 2007). Such relatively low milk yield cannot be attributed definitely to the genetic potential of the Iraqi buffalo, since much of the genetic potential is not fully performed for several environmental influences, such as inadequate nutrition and poor management (Speedy & Sansoucy, 1989). Milk production can be much higher, reaching up to 12 litres/cow per day, when good nutrition is available on a constant basis (Abid & Fazaa, 2007). Researchers underline also the importance of veterinary services, which have to be improved (Tabeekeh et al., 2017b).

About the restored wetlands, they need to be hydraulically designed to allow sufficient flow of non-contaminated water and flushing of salts through the ecosystems (Richardson et al., 2005).

The potential for developing new economic opportunities based on water buffalo husbandry could have a great impact on marshland communities. The local population needs assistance in developing this potential in a variety of ways. Tabeekh et al. (2017b) suggest to improve housing conditions of the animals and to provide good nutrition with government-supported feedstuffs, as well as to improve the living conditions of the farmers (drinking water plants, supply electricity to the villages, realization of schools, health clinics and veterinary clinics).

A cooperative, multi-disciplinary approach is needed to achieve such a goal and additional studies will be required to support better planning (Tabeeekh et al., 2017a).

The present study aims at assessing the situation of buffalo farms, pointing out the main critical points, in order to propose simple solutions to improve the management and, consequently, obtain better performance, taking into account animal welfare and health conditions of the animals, and safety of the workers.

## MATERIALS AND METHODS

A survey on 24 buffalo farms was carried out in 2014–2016 in marshes area. The buffalo farms were located in Dhi Qar Province, in the area between the villages of Suk-Al-Suyuyk and Al-Chubaish (30°48' to 31°01' N, 46°49' to 47°08' E), as represented in Fig. 1. The survey was conducted by researchers of University of Firenze, guided by colleagues of Dhi Qar University.



**Figure 1.** Position of buffalo farms examined during the survey in marshlands area (Dhi Qar Province).

A questionnaire containing different items focused on buffalo farming was fulfilled during the visits of the farms. The questionnaire collected information by local buffalo breeders concerning housing solutions, plant and equipment for feeding, plant and equipment for heat protection, milking systems, manure management, milk storing and processing. Information on food, health problems and management of the herd were also collected.

The farms were divided first of all into two groups, 12 intensive farms and 12 extensive farms. This classification is mainly based on the kind of keeping of the animals.

In intensive farms the buffalos are housed in a barn (closed or open), tied or free in pens with fences. The farms with animals tied by neck belt collar or leg chain are indicated as a); the farms with animals free within fences/walls are indicated as b). In both these cases the feed is brought to the animals by a worker and distributed on the ground or inside a trough or a sack, but, generally, functional areas are not specifically provided.

In extensive farms the buffalos are free to move outdoor during the day, generally in marshlands, and to get food during grazing. This kind of farms is indicated as c). The animals come back to the village for the night and kept close to the house of the farmer without specific structures.

The survey, carried out in the farms, aimed at finding critical points for the breeding of buffalos. The main critical points were divided into four groups: 1) animal welfare; 2) animal health; 3) labour; 4) farm management. Taking into account the main critical points for the two kinds of breeding, solutions to improve the management of the herd were identified and proposed.

## RESULTS AND DISCUSSION

Due to the lack in data collection by the institutions present on the territory and the poor propensity of farmers to provide information, it was impossible to acquire exact information on the number of heads in the farms. The number of female buffalos per farm is included between 20 to 150 cows, with an average number of cows per farm around 50. The daily milk yield per cow is about 5 l, but the right amount is not collected in an official way, also because the milk is often used once a day to feed the calves. For the exposed reasons the performance of the herds was not evaluated during the survey.













Table 1 summarizes the main critical points related to the different keeping systems of the buffalos. The information are divided into three groups, in relation to the keeping system: a) animals kept in a barn, tied by neck collar or leg chain; b) animals kept free in a barn within fences or walls; c) animals free to go outdoor during the day. This last situation is referred to buffalos going to the marshlands for grazing during the day.

### **a) Animals kept in a barn - Tied by neck belt collar or leg chain**

The animals tied in the barn have problems related to poor animal welfare conditions. The buffalos are blocked in the same place during the day and cannot walk. This situation hampers a functional exercise and forces the animals to be subjected to the thermal environment of the barn. These conditions can have negative effects on productive and reproductive life of buffalos.

The constraint of the animals has negative repercussions also on animal health. Body condition is influenced by the scarce physical exercise. Furthermore the system to tie the animals can cause body lesions to the neck or the legs. Another aspect remarked during the visits concern the state of claws, feet and legs. In particular animals do not consume the claws, causing negative effects on the health of the feet and legs. This situation was found in all the farms included in the survey. Furthermore it has to be remarked that regular interventions by farrier are not provided.

**Table 1.** Main critical points related to different keeping systems of buffalos

Animal welfare	Animal health	Labour	Farm management
<i>a) Animals kept in a barn - Tied by neck belt or leg chain</i>			
			
<ul style="list-style-type: none"> <li>- Functional exercise</li> <li>- Thermal comfort</li> </ul>	<ul style="list-style-type: none"> <li>- Body condition</li> <li>- Body lesions</li> <li>- Claws, feet, legs</li> <li>- Hygienic conditions during milking</li> </ul>	<ul style="list-style-type: none"> <li>- Care of animals (<i>cleaning, feeding, milking</i>)</li> </ul>	<ul style="list-style-type: none"> <li>- Collection of data (<i>individual head</i>)</li> </ul>
<i>b) Animals kept in a barn – Free within fences/walls</i>			
			
<ul style="list-style-type: none"> <li>- Design of the barn (<i>not suitable areas</i>)</li> <li>- Thermal comfort</li> </ul>	<ul style="list-style-type: none"> <li>- Competition among animals</li> <li>- Hygienic conditions during milking</li> </ul>	<ul style="list-style-type: none"> <li>- Lack of rational solutions (<i>cleaning, feeding, milking</i>)</li> <li>- Risks for workers</li> <li>- Capture systems</li> </ul>	<ul style="list-style-type: none"> <li>- Collection of data (<i>individual head</i>)</li> </ul>
<i>c) Animals free to go outdoor during the day</i>			
			
<ul style="list-style-type: none"> <li>- Control of animals</li> </ul>	<ul style="list-style-type: none"> <li>- Control of animals</li> <li>- Competition among animals</li> <li>- Hygienic conditions during milking</li> </ul>	<ul style="list-style-type: none"> <li>- Lack of rational solutions (<i>cleaning, feeding, milking</i>)</li> <li>- Risks for workers</li> <li>- Unavailable capture systems</li> </ul>	<ul style="list-style-type: none"> <li>- Collection of data (<i>individual head</i>)</li> </ul>

Also the labour is negatively influenced by this kind of housing for several reasons. The care of animals requires time because each animal has to be managed individually: the feed has to be brought directly to the single animals, the milking has to be made by hand moving from head to head, the cleaning has to be made in each place. For these



reasons the amount of labour is higher than in other keeping systems, and the quality of work is worse, mainly due to the uncomfortable position of the workers during the milking.

One of problems underlined during the survey concerns the absence of milk plants to receive farmers' milk that leads to loss of the products especially in hot season.

#### **b) Animals kept in a barn – Free within fences/walls**

Concerning the welfare of buffalos kept in an area within fences, problems have to be remarked related to the poor design of the barn. The functional needs of the animals are not kept into account, as the absence of functional areas proves. Usually mangers are included in the resting area without a specific feeding alley. Furthermore no specific solutions for heat protection are applied in the barn. This situation is common for the totality of the farms.

Also animal health can be compromised by the poor housing solutions adopted in the farms. For example, a specific feeding area is not realized, and for this reason competition among the animals of the group is particularly strong during feed distribution.

The lack of acceptable hygienic conditions during milking, mainly due to the absence of a specific milking area, cause problems of quality of the milk and produce risks for the health of people consuming the dairy products.

About the labour in this kind of farms, the lack of rational housing solutions gives problems in terms both of amount and of quality: no manure evacuation systems are adopted; feed has to be brought directly to the animals in a high trough; milking has to be made inside the fence without specific areas designed. These conditions cause an increase in times of work, and, for the uncomfortable positions of the workers, a worsening in quality of labour. The total absence of systems for the capture of the animals compromises the safety of the men (farmers, technicians, veterinarians), which work in conditions of serious risks.

#### **c) Animals free to go outdoor during the day**

In these extensive farms the buffalos, which are free to move outdoor during the day, have to be checked at the return to barn or fence in the evening. From the animal welfare point of view, it is required an accurate daily check of all the animals when they come back from the marshlands, in order to assess the health conditions.

Competition among the heads of the herd can be relevant at the moment of collection in the barn or in the fence for feeding.

A specific milking area is never realized and the buffaloes are milked by hand in the same way of ones kept tied, causing the same problems.

About labour, the absence of rational solutions gives problems both in terms of amount and of quality. Feeding and milking are important working activities that in extensive farms are executed without specific safety procedures. Furthermore the risks for the workers are notable due to movements of the animals coming back to the barn. Also in this case capture systems of animals are unavailable in all the farms of the survey.

On the basis of the results of the survey, taking into account the main problems summarized in Table 2, solutions to improve the management of the herd were studied and proposed. The proposed solutions have to be suitable for the farmers living in poor conditions in the marshlands, where lacks in the supply of means of production

(including the electricity) represent the reality. The main objective taken into consideration defining the solutions for the farmers is to favour the management both for the welfare of animals and for the safety of farm workers. The solutions have to assure easy interventions on the animals, minimizing stress conditions. Furthermore it is necessary to guarantee the control of all the heads, including animals living far from the villages.

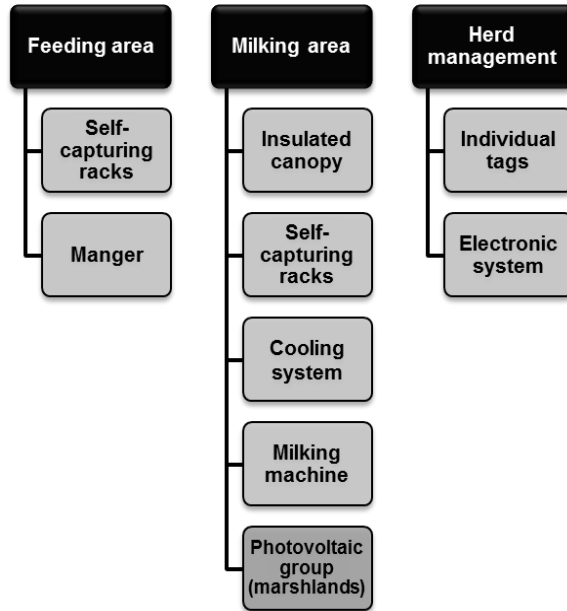
**Table 2.** Main mistakes found in the 24 buffalo farms of survey

Feeding	Feeding alley enough wide, but manger with high border Rack too thin, not suitable for cows or buffalos Rack does not allow to capture animals Flooring of cows higher than the bottom of the manger Kerb too wide and cutting Manger with high border, not accessible from outside Spaces for single animals not well calculated Manger without rack, allowing animals to go inside Manger too narrow, with high border, not accessible from outside (problems of feed wastes) Feed distribution without any control of feed intake by single animals
Drinking	Water distributed in tanks too big Water not continuously renewed Water distributed in a unusual water tank (e.g. a boat)
Heat protection	Barn too low for hot climate areas Shading area too small to cover all the heads Materials for covering not suitable (metallic sheets)
Milking	No specific milking areas Buffalos milked without the respect of hygienic rules Specific areas for milk processing not available
Manure collection	Manure not collected, remaining inside the barn/fence Floor wet, not hygienic Developing of flies for poor conditions
Calves	Situations with young animals not kept in an appropriate way
Safety-health of workers	Relevant risks for safety and health of workers (biological risks, chemical risks) Loads handling (materials, animals, equipment, etc.)
Information system	No collection of data on single head, no specific management procedures for the herd

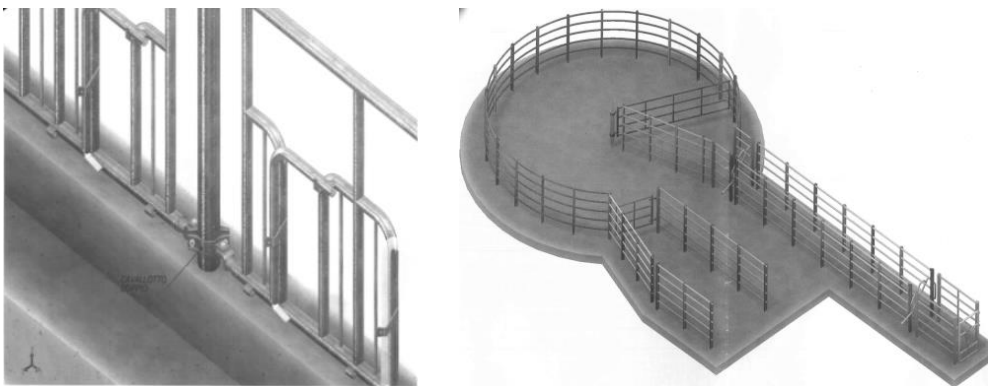
Another important need is related to the safety of the workers. For this reason solutions to capture the animals are proposed, based on fences or racks. The first solution is given by a system of fences (corral) able to block a single animal in a cage. The second solution is constituted by the self-capturing racks placed in a specific feeding alley. The realization of well-designed functional area can improve the comfort of the animals and simplify the labour of the farmers.

For milking, in relation to the low number of heads to milk each day and to the uncertainty of availability of electricity, a specific milking parlour is not suggested. The simple intervention, suitable also for marshlands area, is based on the realization of two stalls provided with a self-capturing rack to guarantee the safety of workers, concrete

floor to drain urines and cleaning water, and a cooling system to create a comfortable area both for animals and for men. Fig. 1 shows the main interventions designed for the farms located in marshlands area and realized in some pilot farms to train the farmers involved in the project. Fig. 2 reports the two solutions proposed and realized to improve the safety of the workers.



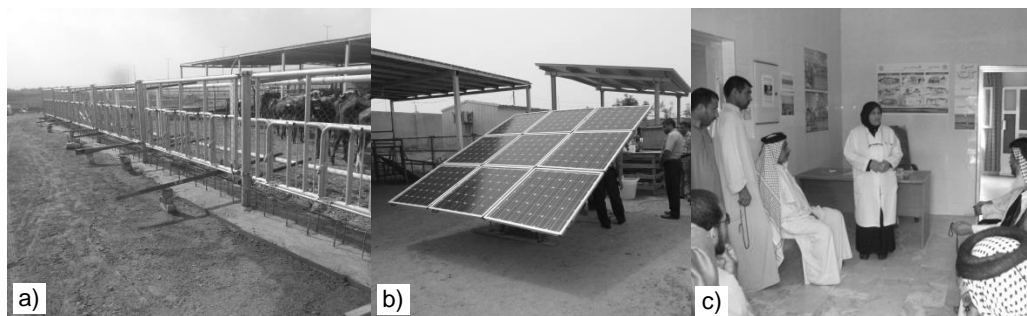
**Figure 1.** Main interventions proposed to the farmers and realized in some pilot farms to carry out FFS program.



**Figure 2.** Proposed interventions for capturing of the buffaloes: a self-capturing rack (left); a corral (right).

The selected pilot farms were used as Farmer Field Schools (FFS). Therefore an intensive program of training was carried out in these farms to involve a large number of persons: farmers with the whole family, technicians, and veterinarians.

Fig. 3 shows some phases of activities: realization of a self-capturing rack in the Training Centre of Dhi Qar University (a); training concerning the use of mobile photovoltaic panels provided for farms of marshlands (b); training of the farmers living in marshlands area (c). It is important to remark that this last activity of training was carried out by local technicians trained during the main course kept by the staff of University of Firenze. The training in FFS involved about 600 persons.



**Figure 3.** Examples of activities in the FFS: a self-capturing rack (a); photovoltaic panels (b); training of farmers of marshlands (c).

## CONCLUSIONS

As main conclusion, a correct design and a right application of simple constructive and plant solutions, with little employment of technological resources, coupled with a good training of farmers, can give a contribution to solve problems in buffalo breeding also in difficult and poor areas like Iraqi marshes. The solutions have to be applied taking into account the different requirements of the animals which can be kept in intensive or extensive system, and, at the same time, the need to create safety conditions for the workers.

A strong and long-term work is required to improve the buffalo sector in the marshlands area of South Iraq. The activities carried out during the project represent a first step in the development of livestock production. The information brought by the staff of University of Firenze in collaboration with Dhi Qar University has reached a high number of farmers in the villages of the marshlands. This goal was reached thanks to the involvement of several technicians of the various institutions of the Province, which carried out the following important phase of training of farmers based on the pilot interventions realized in the Farmer Field Schools.

To obtain a significant improvement in the productivity and to favour the development of a suitable chain linked to buffalo breeding, in the next years the services provided to the farmers have to be enhanced. The farmers of the small communities distributed along the southern marshes demonstrated to appreciate the assistance given by skilled technicians and are open to the introduction of innovations in the farm management.

Urgent priorities for Iraqi buffalo breeders are related to the provision of suitable foods for the animals, in particular concentrated feed, to the processing of the dairy products in healthy conditions, to the valorisation of dairy products on the market.

The next step that could be carried out in a further project is the realization of modern infrastructures, mainly dairy factories, in carefully selected areas. A cooperative approach, directly involving all the components of the dairy chain, is needed to achieve such a goal.

ACKNOWLEDGEMENTS. Project funded by Italian Agency for Cooperation and Development (AICS). The authors express gratitude to local people taking part to the activities of the project, to Dhi Qar Governorate and Dhi Qar Agriculture Directorate, and to all the other Iraqi Institutions involved in the project. A particular thank is addressed to Mr. Imad Ali Hayif for the important support given during all the phases of the project.

## REFERENCES

- Abid, H.S. & Fazaa, N.A. 2007. *Water Buffalo in the Iraqi Marshes*. Thi Qar and Missan Governorates. Nature Iraq Status Report, 29 pp.
- Al-Marsomy, J. 2005. *The Marshes: Neglected Economic Fortunes*. The Iraqi Society for marsh. Restoration and Development, 15 pp.
- Al-Saedy, J.K. 2007. Iraqi buffalo now. Proceedings 8<sup>th</sup> World Buffalo Congress. *Italian Journal of Animal Science* **6**, 1234–1236.
- Al-Saedy, J.K. & Al-Fartosi, K. 2011. Iraqi buffalo status and perspectives. *Buffalo Newsletter* **26**, 17–19.
- Al-Saedy, J.K. & Al-Fartosi, K. 2013. Mesopotamian buffaloes (the origin). *Buffalo Newsletter* **28**, 25–30.
- FAO. 2005. <http://www.fao.org/faostat/en>. Accessed 15.1.2015.
- Fazaa, N.A. 2007. Interim Draft Report on Water Buffalo in Chubayish, 15 pp.
- Ligda, D.J. 1996. The water buffalo population and production in Iraq. Cited by Nature Iraq Status Report 2007. <http://www.natureiraq.org/literature.html>. Accessed 02/10/2017.
- Pawar, HN, Ravi Kumar, G.V.P.P.S. & Raman, N. 2012. Effect of Year, Season and Parity on Milk Production Traits. *Journal of Buffalo Science* **1**, 122–125.
- Petty, A.M., Werner, P.A., Lehmann, C.E.R., Riley, J.E., Banfai, D.S. & Elliott, L.P. 2007. Savanna responses to feral buffalo in Kakadu National Park, Australia. *Ecological Monographs* **77**(3), 441–463.
- Richardson, C.J., Reiss, P., Hussain, N.A., Alwash, A.J. & Pool, D.J. 2005. The restoration potential of the Mesopotamian marshes of Iraq. *Science* **307**, 1307–1311.
- Richardson, C.J. & Hussain, N.A. 2006. Restoring the Garden of Eden: an ecological assessment of the marshes of Iraq. *Bioscience* **56**(6), 477–489.
- Speedy, A. & Sansoucy, R. 1989. *Feeding dairy cows in the tropics*. Proceedings of the FAO Expert Consultation, Bangkok, Thailand, July 7–11, 244 pp.
- Tabeeh, M.A.S.A., Mohsen, H.A.M.A. & Al Jaber, A. 2017a. The reality of buffalo breeding in Basra Governorate. *Journal of Buffalo Science* **6**, 8–18.
- Tabeeh, M.A.S.A., Maktoof, A.R. & Mohsen, H.A.M.A. 2017b. Solutions or alternatives to protect livestock, especially buffalo from the risk of drought in Basra Governorate. *Canadian Journal of Agriculture and Crops* **2**, 84–89.

## **Poultry farming solutions for a sustainable development of marshlands areas of South Iraq**

G. Rossi, L. Conti, G. Bambi, M. Monti and M. Barbari\*

University of Firenze, Department of Agricultural, Food and Forestry Systems (GESAAF), Via San Bonaventura 13, IT50145 Firenze, Italy

\*Correspondence: [matteo.barbari@unifi.it](mailto:matteo.barbari@unifi.it)

**Abstract.** The Department of Agricultural, Food and Forestry Systems of University of Florence carried out cooperation projects in the South Iraq, funded by Italian Ministry of Foreign Affairs and International Cooperation, focused on the development of rural areas. All the proposed interventions were designed taking into account some key points, such as the development of sustainable farming systems, the protection of the environment, the empowerment of women role. Among the main activities, a particular importance is given to the simple solutions for poultry breeding that represents an important economic resource for many farmers and a source of food for a large segment of the population. Therefore the main aim of the project was to define new building solutions to apply in the area of marshlands. A mobile poultry shelter was designed and built to meet the demand for meat and eggs from a typical rural family. This shelter was designed to be used for extensive breeding, with animals free to graze outside during the day. A suitable self-building methodology was applied to obtain results from farmers without specific skills for wood construction thanks to which they learn the appropriate way to build poultry shelters. As conclusion, poultry breeding can be spread in the area of marshlands using the simple self-building structures presented in the study.

**Key words:** marshlands, poultry shelter, rural development, self-building.

### **INTRODUCTION**

According to the statistics section of the Food and Agriculture Organization of United Nations (FAOstat, 2013), Iraq produced 85,630 Mt of chicken in 2013, with a great increase from 2009 (33,000 Mt). Total poultry meat consumption reached 770,000 Mt in 2013, giving an average annual per capita consumption of 24 kg (USDA, 2013). The Iraqi farmers in South of Iraq, whose main occupation may be the palm dates production or the animal husbandry such as buffalos breeding, keep few local breed chickens near their premises to be consumed by the family or to be sold at the market. The local poultry breed, whose origin is difficult to trace, appears to be extremely rustic and adapted, still with a fair attitude for meat production but less for laying eggs. This kind of chicken is really appreciated on the local market and generally fetches a higher price against selected breed or imported frozen chicken. Chickens are usually sold alive in the market and slaughtered upon request of the buyer or directly from the consumer. However the presence on the market of the chickens is scarce as these animals trickle

from the farms, from individual sellers and a few dealers. This trend confirms that poultry breeding is an important economic resource for many farmers and a source of food for a large segment of the population. However rural population does not have sufficient skills to create optimal breeding conditions. Furthermore the lack of specific facilities produces difficulties in the poultry farming. In order to solve this problem, the research focused on solution 'farm-made' for breeding structures.

The mobile poultry shelter was designed respecting the self-building criteria. For that reason the shelter can be built directly by the farmer in the place of utilization or, as it is made up of independent components easily transportable, built in a simple carpentry workshop and assembled on site by the farmer using simple tools. This solution is suitable for poultry breeding located in marshlands area with high concentration of rural families. Beneficiaries can be single families or small groups of them. In particular the women, which are traditionally dedicated to poultry breeding within the family, can benefit from the spreading of the solution proposed during the research.

## **MATERIALS AND METHODS**

The mobile poultry shelter was designed first and foremost taking into account the needs of outdoor poultry farming and the choice of building materials suitable and available in the intervention area. Afterwards a logical workflow was identified, sufficiently simple to be understood from farmers or people without specific skills in construction, in order to obtain a self-construction of the shelter.

Chickens quickly destroy the turf adjacent to their house, and over time the damage of the ground can prosecute for some distance in all directions, leading to a yard that is alternately muddy and dusty. The concentration of manure in this area also leads to a build-up of manure-borne pathogens and health problems can occur (mainly coccidiosis and roundworms). The method chosen to deal with this problem has a profound effect on the housing design. With portable houses, the chickens are moved to a new spot before the damage becomes too great (Plamondon, 2003).

A simple and proper management of animal health requires the rotation of the areas over which the breeding is carried out. For this reason, poultry shelters have to be movable with simple means available in the farms, and, therefore, the shelters must be very light. It is important to guarantee a free access to an open-air area, mainly covered with vegetation and provided with protective fences. Rearing systems, with animals kept in a free-range environment or with outdoor access, are perceived as natural, environmentally friendly and animal welfare friendly (Husak et al., 2008).

Free movement systems are fundamental in the marshlands area in order to respect environment and allow rural people to develop sustainable agriculture. This feature determines the choice of the building materials, mainly based on solid wood, steel, aluminium, synthetic materials.

For the realization of the shelter timber of small size and low quality, like building lumber, was selected and used. Besides, the solid wood has, in general, some crucial advantages. Under the same load-bearing capacity, the energy required to produce a massive wooden structure is far less than that required by other materials. Indeed, the wood is a natural storage of solar energy. Furthermore, the construction of the shelters requires extremely simple and economic tools and equipment, and easy and quick learning techniques.

In the assessment of the size of the shelter, the rules of organic production in compliance of EC Regulation 1804/99 and the EC Regulations 834/2007 and 889/2008 were considered (Table 1).

**Table 1.** EC Regulation 1804/99 – Minimum surface areas indoors and outdoors, and other characteristics of housing in the different species and types of production

	Indoors area (net available to animals)			Outdoors area (m <sup>2</sup> of area available in rotation/head)
	N <sup>o</sup> animals m <sup>-2</sup>	Perch/head (cm)	Nest	
Laying hens	6	18	7 laying hens/nest or 120 cm <sup>2</sup> /bird (common nest)	4 (if limit 170 kg N ha <sup>-1</sup> per year not exceeded)
Fattening poultry in fixed housing	10, with maximum of 21 kg live weight m <sup>-2</sup>	20 (guinea fowls only)		4 broilers and guinea fowls 4,5 ducks – 10 turkeys 15 geese (if limit 170 kg N ha <sup>-1</sup> per year not exceeded)
Fattening poultry in mobile housing	16 <sup>(1)</sup> , in mobile poultry houses with a maximum of 30 kg live weight m <sup>-2</sup>			2,5 (if limit 170 kg N ha <sup>-1</sup> per year not exceeded)

<sup>(1)</sup> Only in the case of mobile houses not exceeding 150 m<sup>2</sup> floor space.

The designed shelter has an internal useful surface of about 8 m<sup>2</sup>. The installations can be fitted with perches for a total length of 24 m, and can be equipped with 8 individual nests. The capacity of the shelter in compliance of EC Regulation 1804/99 can be summarized in the following way:

- Laying hens: 6 heads m<sup>-2</sup> x 8 m<sup>2</sup> = 48 heads; perches required 48 x 0.18 = 8.64 m
- Broilers (fixed shelter): 10 heads m<sup>-2</sup> x 8 m<sup>2</sup> = 80 heads
- Broilers (mobile shelter): 16 heads m<sup>-2</sup> x 8 m<sup>2</sup> = 128 heads.

In Fig. 1 the exploded isometric drawing of the shelter shows 8 main frames: base and roof (Frame01 and SC01), 6 external surfaces (Frame02, Frame03a, Frame03b). The figure shows also the four nets (Nest01).

Fig. 2 shows the drawing tables of the base of the shelter (Frame01). In the corners of the base steel braces (R01) that allow to move the shelter are installed.

Fig. 3 shows the drawing tables for lateral frame. It is important to stress the concept of workflow. In fact, thanks to the execution of individual actions such as cutting, drilling, and assembling, it is possible to build all the frames. Each construction procedure is not arbitrary of the farmer, but is a part of specific sequences which as a whole give rise to the workflow.

The supporting structure is completely braced on all 6 external surfaces, so that it can be stressed by even asymmetric actions without deforming. The main structure consists of flat frames incapable of being deformed, which are assembled together using only screws or nails. All frames of components have been designed with such weight and dimensions that they can be easily handled by two persons and transported by means of limited capacity. Assembling the shelter requires simple wood workshop equipment,



but it is not possible to leave out all relevant safety measures, including the use of personal protective equipment.

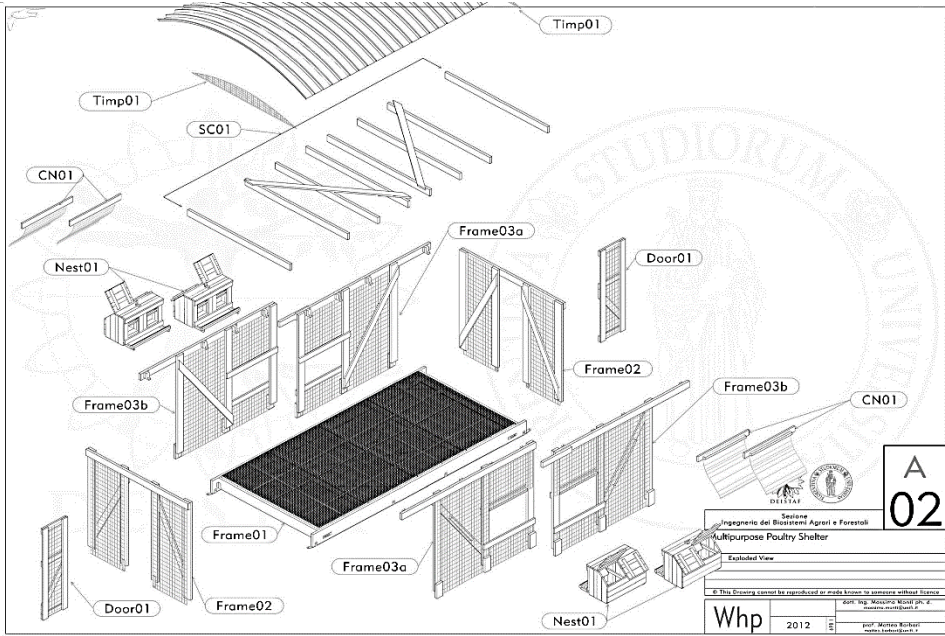


Figure 1. Technical drawing showing the assembly of frames.

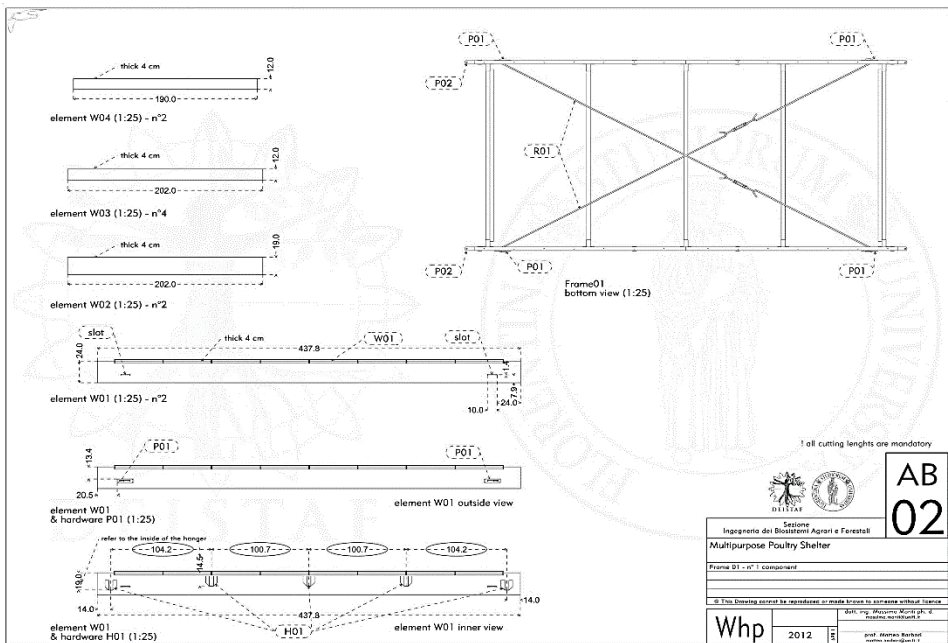


Figure 2. Technical drawing table for Frame01.



South Iraq’, funded by Italian Ministry of Foreign Affairs and International Cooperation (years 2012–2014). The specific activity presented in this study was executed in 2013.

For these pilot interventions Iraqi trainees with different educational levels were selected: farmers, technicians and students.

Workflow was applied during training carried out in the cooperation projects with the support of Italian tutor that instructed trainees to the correct procedures of work. At the end of the training the poultry shelter was built and all trainees were able to repeat with success the activities of the project building new shelters. In Fig. 4 a brief photo report shows some phases of the training related to the pilot intervention near Nasiriya.



**Figure 4.** Phases of construction with Iraqi trainees during pilot intervention in the project: ‘Rational management of water resources for agricultural development of rural areas in South Iraq’.

After the training the capacity for two Iraqi trainees to rebuild the shelter without the Italian tutor was tested. The two Iraqi trainees, following the workflow learned during the training and using the same wood workshop of the courses, were able to replicate the shelter approximately in 8 working days. In the Table 2 the results of the building time are reported.

This test confirmed that trainees have gained technical knowledge and expertise to self-building the shelter.

One of the shelters built in Iraq was donated to a women's NGO located in the marshlands. Thanks to this facility the NGO started the production of broilers. According to the interviews conducted during an inspection in 2015 (Fig. 5), the NGO manager confirmed that with the new shelter an improvement in farm management was obtained, increasing the income due to selling alive broilers in the local market.

**Table 2.** Times of execution phases

Phases	Hours
Lumber selection and workshop organization	1
Cutting wood boards, thickness 40 mm	6
Cutting wood boards, thickness 25 mm	4
Cutting wood boards, thickness 40 mm	4
Preparation 6 wood boards with brackets	2
Assembly 4 lateral frame with net	4
Assembly 2 frontal frame with net	2
Assembly 2 roof frame with net	3
Assembly 2 door frame with net	1
Preparation iron sleigh	3
Welding	1
Cutting wood boards for nest	5
Assembly nest	3
Assembly base	2
Final assembly	2
Treatment with linseed oil	4
<b>Total</b>	<b>47</b>



**Figure 5.** Poultry shelter donated to Iraqi NGO.

## CONCLUSIONS

The present work demonstrated that farmers can build the poultry shelter directly within their farm, also because specific skills are not requested. However, in order to reach this result it is important to follow correct procedures that in our research were inside a workflow resumed in tables of drawings and specific actions.

This approach conducts to several benefits, for example allows to focus on individual parts of the work restricting mistakes and waste of time. Furthermore, with

this methodology, the farmer familiarizes immediately with the processes that bring to the construction of the shelter.

As conclusion, the construction of poultry shelters could be spread in the villages of Iraqi marshlands bringing advantages in agricultural sector, favouring a sustainable development with benefits also from the social point of view.

ACKNOWLEDGEMENTS. Project funded by Italian Agency for Cooperation and Development (AICS). The authors express gratitude to Dhi Qar Governorate and Dhi Qar Agriculture Directorate, and to all the other Iraqi Institutions involved in the project.

## REFERENCES

- Commission Regulation EC 2008. *No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control.* Official Journal L 250, 18/09/2008, 1–23.
- Council Regulation EC 1999. *No 1804/1999 of 19 July 1999 supplementing Regulation (EEC) No 2092/91 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs to include livestock production* Official Journal L 222 , 24/08/1999, 1–28.
- Council Regulation EC 2007. *No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91.* Official Journal L 189, 20/07/2007, 1–84.
- EN 1995-1-1 2004. *Eurocode 5: Design of timber structures - Part 1-1: General - Common rules and rules for buildings.* Authority: The European Union Per Regulation 305/2011, Directive 98/34/EC, Directive 2004/18/EC.
- FAO 2013. *FAOSTAT Database.* <http://www.fao.org/faostat/en/#data/QL>. Accessed 21.9.17.
- Husak, R.L., Sebranek, J.G. & Bregendahl, K. 2008. A survey of commercially available broilers marketed as organic, free-range, and conventional broilers for cooked meat yields, meat composition, and relative value. *Poultry Science* **87**(11), 2367–2376.
- Plamondon, R. 2003. *Range Poultry Housing.* NCAT Agriculture Specialists, Fayetteville, 16 pp.
- PIMC Primaries Industries Ministerial Council 2002. *Model Code of Practice for the Welfare of Animals – Domestic Poultry*, 4<sup>th</sup>, Collingwood, Australia, Csiro Publishing, 45 pp.
- USDA 2013. Report June 2013. <https://www.fas.usda.gov/commodities/poultry-and-eggs>. Accessed 18.9.17.



## **Diagnosis of air quality in broilers production facilities in hot climates**

F.C. Sousa<sup>1,\*</sup>, I.F.F. Tinôco<sup>1</sup>, M. Barbari<sup>2,\*</sup>, F. Baptista<sup>3</sup>, C.F. Souza<sup>1</sup>,  
A.O. Saraz<sup>4</sup>, D.J.R. Coelho<sup>1</sup> and A.L. Silva<sup>5</sup>

<sup>1</sup>Universidade Federal de Viçosa, Department of Agricultural Engineering, Av. Peter Henry Rolfs, Campus University of Viçosa, BR30570-000 Viçosa, MG, Brazil

<sup>2</sup>Università degli Studi di Firenze, Department of Agricultural, Food and Forestry Systems (GESAAF), Via San Bonaventura 13, IT50145 Firenze, Italy

<sup>3</sup>Universidade de Évora, Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Apartado 94, PT7006-554 Évora, Portugal

<sup>4</sup>Universidad Nacional de Colombia, Faculty of Agricultural Sciences, Calle 59 A N 63, CO400-03 Medellín, Colombia

<sup>5</sup>Universidade Federal de Viçosa, Department of Animal Science, Av. Peter Henry Rolfs, Campus University of Viçosa, BR30570-000 Viçosa, MG, Brazil

\*Correspondence: [matteo.barbari@unifi.it](mailto:matteo.barbari@unifi.it); [fernanda.sousa@ufv.br](mailto:fernanda.sousa@ufv.br)

**Abstract.** The objective of this study was to evaluate air quality of industrial farms of broilers production, located at Zona da Mata region, Minas Gerais, Brazil. The environmental air quality was evaluated during the last rearing week, between 35 and 42 days of life of broilers. Facilities with beds constituted by two types of substrates were evaluated: coffee husks (6 reuse cycles) and shavings (4 reuse cycles). A total of 30 facilities (3 per each of the 10 reuse cycles) were investigated. Air quality was diagnosed by determining air temperature and relative humidity and by ammonia and carbon dioxide concentrations. Air temperature and relative humidity were not affected by reuse cycles in coffee husks bed, but these variables were affected by reuse cycles in shavings bed. Ammonia and carbon dioxide concentrations increased linearly according to the reuse cycles for both types of bed. The maximum concentrations of ammonia and carbon dioxide were 25 ppm and 1,348 ppm in facilities with bedding of coffee husks and 10 ppm and 1,075 ppm in facilities with bedding of shavings, respectively. Air quality of facilities using coffee husk bed tends to be worse when compared to facilities using shavings bed due to the higher values of ammonia and carbon dioxide concentrations, as observed in this study. In conclusion, regardless bedding type, increases in reuse cycles tend to decrease air quality inside the facility, since a linear increasing in ammonia and carbon dioxide concentrations can be observed in relation to the number of bed reuse cycles.

**Key words:** air pollutants, air quality, gas concentration, livestock, poultry.

### **INTRODUCTION**

Brazil is the second largest producer and the world's largest exporter of chicken meat, with 12.9 million tons produced in 2016 (ABPA, 2017). This intensive production

combined with increased production of waste in the facility can cause significant impacts on soil, water and air (Calvet et al., 2011).

Air quality is one of the most important factors in poultry production (Menegali et al., 2009), since air pollutants, possibly present in aviaries, can alter the ideal characteristics of the air. Therefore, respiratory diseases may occur in animals and people, besides damages in the production system by negative effects on animal performance (Alencar et al., 2004; Nääs et al., 2007).

Ammonia is an air pollutant that is frequently found in high concentrations in aviaries (Owada et al., 2007). In broilers production facilities, ammonia is generated during the microbial decomposition processes of wastes that are deposited in the avian bedding. This process is influenced by air temperature, air humidity and pH of waste (Manno et al., 2011; Marín et al., 2015; Cemek et al., 2016). When the ammonium ion ( $\text{NH}_4^+$ ) present in waste is converted to ammonia ( $\text{NH}_3$ ), volatilization of ammonia occurs to the environment (Oliveira & Monteiro, 2013). On the other hand, the ammonium ion ( $\text{NH}_4^+$ ) by nitrification and denitrification processes can be converted to nitrous oxide ( $\text{N}_2\text{O}$ ), an important greenhouse gas (Felix & Cardoso, 2004; Marques, 1992).

The carbon dioxide production in animal production facilities is related to animal metabolism (CIGR, 1994), which, in turn, is affected by air temperature and relative humidity (Calvet et al., 2011). Under normal conditions carbon dioxide presents concentration from 500 to 3,000 ppm in animal production facilities, which may represent a health risk and affect animal production performance (CIGR, 1994).

Considering its impacts on the environment, more than a decade ago, the emissions of polluting gases were the focus of studies of researchers in several countries of Europe and North America (Scholtens et al., 2004; Mosquera et al., 2005; Faulkner & Shaw, 2008). In these regions, there are inventories that allow the establishment of protocols for gases emission reduction. For these countries, the determination of emissions in the structures is relatively simple, since most of the facilities are closed and, therefore, have an adequate control on the volume of air.

However, for regions of tropical and subtropical climates, such as Brazil, the determination of these emissions are much more complex (Mendes et al., 2014). In fact in Brazil almost all animal production facilities are kept open for most part of time (Tinôco, 2001). Therefore, in such situations it is difficult to determine gases emissions (Saraz et al., 2013). The same condition is observed for hybrid systems, where installations can remain open or closed, according to environmental thermal variables. This is the case of positive ventilation systems which are open-side facilities that rely on fans to control the internal thermal environment (Manno et al., 2011).

The climatic conditions of warm climate countries allow animal production in open facilities, and thus, provide ideal conditions for the practice of reusing the avian bed (Marín et al., 2015). This practice has become a reality in Brazil mainly due to the impossibility of using avian bed in ruminant feeding (Brasil, 2001) and also due to the difficulty of acquiring new substrates. The avian bed reuse allows the reduction of waste generated, and thus, contributes to minimize the environmental impacts (Vieira et al., 2015). For hot climate regions there are few studies and methods to determine the emission of polluting gases (Mendes et al., 2014). In addition, studies related to air quality in livestock production are still limited to some initiatives related to animal and worker health or odours problems close to the facilities.

Knowledge of gas emission levels is a major guiding factor for national and international regulatory agencies to exercise environmental control and eliminate barriers in the commercialization of products in the poultry production (Osorio-Saraz et al., 2014). As well as maintaining the position of the largest exporter of chicken meat, Brazil must comply with international standards and requirements, taking into account the required quality standards, animal welfare requirements and environmental issues related mainly to air quality.

Consequently, research is needed in the area with the aim to identify and quantify pollutants in facilities, and then to adopt systems for the mitigation of environmental impacts. It will allow to improve the quality of the production environment due to lower emission of gases with greenhouse potential for the planet, aiming sustainability of production by preserving the environment for future generations (Marín et al., 2015).

Therefore, given the need of air quality monitoring in animal production environments in hot weather conditions, that is the reality of Brazil, this study was carried out aiming to make the diagnosis of air quality. The diagnosis was based on air temperature and relative humidity, and ammonia and carbon dioxide concentrations in industrial broiler production facilities commonly adopted in hot climate countries, i.e. predominantly open facilities.

## MATERIALS AND METHODS

This study was conducted in farms for broilers production, located in the region of Zona da Mata of Minas Gerais, Brazil (Fig. 1). The poultry facilities were subjected to similar climatic conditions. The climate of this region according to the Köppen classification, is the type Cwb - tropical climate of altitude, with rainy summer and mild temperatures.



**Figure 1.** Location of the Zona da Mata, Minas Gerais, Brazil.



All facilities had the same construction typologies, representative of the poultry industry of Minas Gerais state and Brazil, typical of the patterns adopted in hot countries. The constructional features were the same; orientation East-West, width between 12 and 14 m, height in the eaves from 2.8 to 3.2 m, length greater than 100 m, walls of 20 cm on the sides and closure with screens and curtains. The facilities had automatic feeders and drinkers. They remained predominantly open and had a positive pressure ventilation system with axial fans arranged on the sides, activated according to the necessity of internal temperature control, especially in the final phase of breeding and in very hot days (Fig. 2, A and B).

A layer of bedding was kept on the floor (approximately 10 cm depth) and the stocking density was maintained between 14 and 18 birds per m<sup>2</sup>. The animals were Cobb males. The birds were fed with the same feed, made using the ideal protein concept, which is widely used for the broiler production industry (Campos et al., 2012).



**Figure 2.** External (A) and internal (B) view of one of the evaluated facilities.

The air quality was evaluated in facilities that had different types of substrate in the bed (coffee husks and shavings) with different numbers of reuse cycles (up to 6 cycles for coffee husks and up to 4 cycles for shavings). These materials are commonly used in the mentioned regions, due to the great availability and low cost. A total of 10 different avian beds were analysed: 4 shavings beds (from 1<sup>st</sup> to 4<sup>th</sup> reuse cycle) and 6 coffee husks bed (1<sup>st</sup> to 6<sup>th</sup> reuse cycle). For each of these 10 types of avian beds, 3 facilities containing the same type of bed in the same reuse cycle were analysed, totalling 30 investigated aviaries.

The diagnosis of air quality was performed by determining the instantaneous air temperature and relative humidity, and ammonia and carbon dioxide concentrations. The data were measured during the last week of rearing, between 35 and 42 days of life of the chickens, in order to obtain homogeneous samples, representative of each case and to ensure the presence of the largest possible waste load in bedding, i.e. the maximum potential situation of greenhouse gas emissions.

The data of air temperature and humidity were obtained with use of data loggers HOBO U14-002 (Onset Computer Corp.) with a resolution of 0.02 °C and 0.05%, accuracy  $\pm 0.21$  °C  $\pm 2.5\%$  and range measurement from - 20 to + 50 °C and 0 to 100%. The carbon dioxide concentration was measured by a CO<sub>2</sub> sensor with a resolution of

1 ppm, accuracy of  $\pm 30$  ppm ( $\pm 5\%$  of reading) and measurement range from 0 to 5,000 ppm. The ammonia concentration was determined using an electrochemical ammonia detector 'Gas Alert Extreme NH<sub>3</sub>' (Honeywell / BW Technologies) with 1 ppm resolution, measuring range from 0 to 100 ppm, properly calibrated before data collection using a calibration gas standard (White Martins®).

The experiment was conducted in a completely randomized design, in which the effects of different reuse cycles of coffee husks and shavings beds were evaluated. All analyses were performed by analysis of variance (ANOVA), using the MIXED procedure of SAS (SAS Institute Inc., 2008), according to the model presented in Eq. 1:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij} \quad (1)$$

where  $Y_{ij}$  – dependent variable;  $\mu$  – overall constant;  $T_i$  – treatment effect (reuse cycles);  $\varepsilon_{ij}$  – random error.

Due to the different number of reuse cycles obtained for coffee husks (6 cycles) and shavings (4 cycles), the ANOVA was conducted separately for each type of bed. The effect of bed cycles was evaluated by the orthogonal decomposition of the fixed effect of treatment into linear, quadratic and cubic effects. The denominator of degree of freedom was estimated using the Kenward-Roger approximation, and significant differences were declared when  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Coffee husks bed

From the descriptive data referring to the facilities using coffee husks bed, it is evident that the experiment was carried out under different climatic conditions, due to the difference between maximum and minimum values of air temperature and relative humidity (Table 1). It was observed that there was more than 10 °C of difference between minimum (18.4 °C) and maximum (28.8 °C) temperatures and 35% of difference between the extreme values of relative humidity, ranging from 43 to 78%.

The maximum ammonia concentration was 25 ppm. This value exceeds the maximum recommended for both people and animals. The health tolerance limit set by the Brazilian regulatory standard NR-15 (Brasil, 1978) for operations and unhealthy activities of workers exposed to these contaminants is 20 ppm. On the other hand, the recommended limit for ammonia that does not affect animal performance in the facility is 10 ppm (CIGR, 1994).

**Table 1.** Descriptive data for bed of coffee husks

Variable	<i>n</i>	Avg.	Std.	Min.	Max
Air temperature (°C)	18	24.8	27.6	18.4	28.8
Air relative humidity (%)	18	58.83	8.27	43.00	78.00
Ammonia (ppm)	18	12.44	6.71	4.00	25.00
Carbon dioxide (ppm)	18	974.94	194.01	662.00	1,348.00

The carbon dioxide concentration ranged from 662 to 1,348 ppm. The maximum value observed in this study is lower than the limits established for animal and human exposure. Carbon dioxide concentration up to 3,000 ppm does not affect the health and performance of animals (CIGR, 1994) and the concentration of 3,900 ppm is defined as

the limit of tolerance for health exposure of workers, established by the NR-15 (Brasil, 1978).

As expected, air temperature and relative humidity were not affected by the number of reuse cycles of coffee husks bed (Table 2). These values were related to external local climatic conditions at the time of data collection, since the facilities remained open during the experimental period that was carried out in the last week of rearing of broilers, when the temperature of thermal comfort is in the range of 21 and 23 °C (Macari, 1996). According to the averages in the different reuse cycles it is observed that despite the use of positive pressure ventilation systems as a way of lowering the air temperature, almost all the installations were in a condition of thermal discomfort by heat.

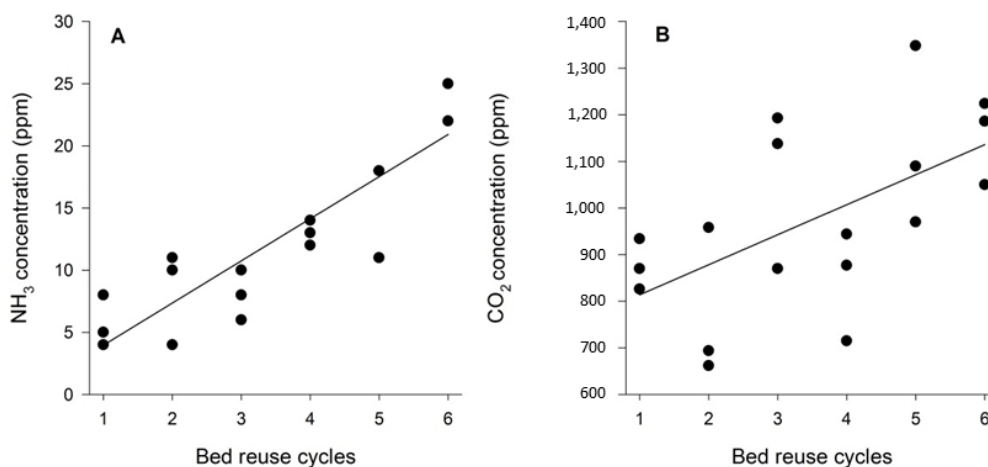
The average values of ammonia and carbon dioxide concentrations increased linearly ( $P < 0.05$ ) as bed reuse cycle increased (Table 2). The highest averages occur in the largest numbers of reuse cycles.

**Table 2.** Average values of air temperature (T), air relative humidity (RH), ammonia (NH<sub>3</sub>) and carbon dioxide (CO<sub>2</sub>) concentrations in facilities using coffee husks bed in the different reuse cycles

Variable	Reuse cycles						<i>P</i> -valor		
	1	2	3	4	5	6	Lin.	Quad.	Cub.
T (°C)	24.3	23.8	26.7	26.0	21.7	26.1	0.895	0.803	0.247
RH (%)	58.00	59.33	54.33	54.33	68.00	59.00	0.455	0.619	0.421
NH <sub>3</sub> (ppm)	5.66	8.33	8.00	13.00	15.67	24.00	<b>&lt;0.001*</b>	0.111	0.337
CO <sub>2</sub> (ppm)	876.67	771.33	1,067.00	845.33	1,136.00	1,153.33	<b>0.011*</b>	0.507	0.833

\* Significant ( $P < 0.05$ ).

In general, the patterns of ammonia and carbon dioxide concentration were similar, increasing due to the increase in the number of reuse cycles (Fig. 3).



**Figure 3.** Pattern of ammonia (A) and carbon dioxide (B) concentrations as a function of reuse cycle of coffee husk bed.

The generation and emission of ammonia in wastes are influenced by air temperature and relative humidity (Popescu et al., 2010; Arcidiacono et al., 2015; Cemek et al., 2016), because these factors are able to directly influence the temperature and humidity of the bed, thus favouring the microbial activity in the waste (Manno et al., 2011). According to several studies (Webb & Misselbrook, 2004; Zhang et al., 2005; Furlan, 2006; Furtado et al., 2006; Fabbri et al., 2007; Van der Stelt et al., 2007; Ndegwa et al., 2008; Carvalho et al., 2009; Osório et al., 2009; Rong et al., 2009; Vitorasso & Pereira, 2009; França et al., 2014; Mendes et al., 2014) there is a relationship between air temperature and ammonia concentration in animal production environments, in the presence of waste. In situations of high temperatures (around 35 °C) the microbial activity is intensified, allowing a higher rate of uric acid mineralization, which induces the increase of the potential of both generation and emission of ammonia (França & Tinôco, 2014).

### Shavings bed

By the descriptive data of facilities that used shavings bed (Table 3), it can be observed that the data collections were carried out under similar climatic conditions, since there was a difference of only 3.5 °C between minimum and maximum air temperature. However, the relative humidity ranged from 37 to 76%. The maximum ammonia concentration was 10 ppm and the carbon dioxide concentration varied between 726 and 1,075 ppm.

The maximum values of ammonia (10 ppm) and carbon dioxide (1,075 ppm) were within the exposure limits recommended for animals and people. As previously mentioned, in animal production facilities, the limit for ammonia concentration is 10 ppm (for animal) and 20 ppm (for people) and carbon dioxide concentration for animals and humans is 3,000 and 3,900 ppm, respectively (Brasil, 1978; CIGR, 1994).

**Table 3.** Descriptive data for shavings bed

Variable	<i>n</i>	Avg.	Std.	Min.	Max
Air temperature (°C)	12	27.6	1.37	25.8	29.3
Air relative humidity (%)	12	52.58	12.25	37.00	76.00
Ammonia (ppm)	12	5.63	2.65	2.00	10.00
Carbon dioxide (ppm)	12	891.83	99.61	726.00	1,075.00

The air temperature was linearly affected by reuse cycles, while air relative humidity presented a cubic effect of reuse cycles (Table 4). However, as the facilities were predominantly open during the data collection period, the effects observed for temperature and relative humidity appear to be more related to environmental variations than to the effect of the reuse cycles. From the average values of temperatures, it was observed that the facilities with bed of shavings were in condition of thermal discomfort by heat, with temperatures above 25 °C while the thermal comfort range for broilers in the last week of breeding is in the range between 21 and 23 °C (Macari, 1996).

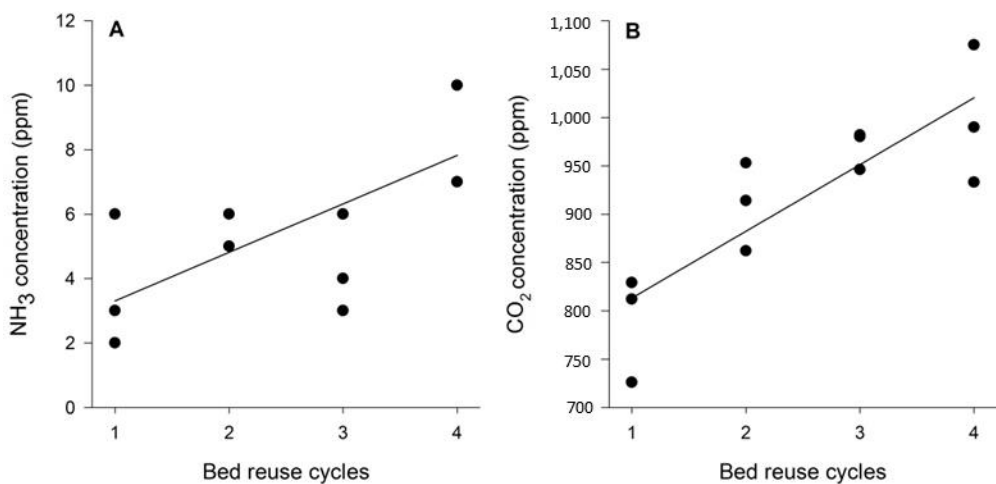
The average values of ammonia and carbon dioxide concentrations of shavings bed increased linearly ( $P < 0.05$ ) as the reuse cycles increased (Table 4). The highest average values of ammonia and carbon dioxide were observed for the highest number of reuse cycles.

**Table 4.** Average values of air temperature (T), air relative humidity (RH), ammonia (NH<sub>3</sub>) and carbon dioxide (CO<sub>2</sub>) concentrations in facilities using shavings bed in the different reuse cycles

Variable	Reuse cycles				<i>P</i> -valor		
	1	2	3	4	Lin.	Quad.	Cub.
T (°C)	25.9	28.7	28.0	28.5	<b>0.046*</b>	0.125	0.105
RH (%)	57.67	41.00	66.00	45.67	0.746	0.818	<b>0.002*</b>
NH <sub>3</sub> (ppm)	3.67	5.50	4.33	9.00	<b>0.019*</b>	0.184	0.108
CO <sub>2</sub> (ppm)	789.00	909.67	969.33	999.33	<b>0.044*</b>	0.071	0.763

\*Significant (*P* < 0.05).

In general, ammonia and carbon dioxide concentrations increased as the reuse cycle increased (Fig. 4), presenting similar pattern as observed for coffee husks bed and previously discussed.



**Figure 4.** Pattern of ammonia (A) and carbon dioxide (B) concentrations as a function of reuse cycle of shavings bed.

Comparing the two types of bedding (coffee husks and shavings), from the first to the fourth reuse cycle, it is observed that in general the values of ammonia concentration in the facilities with coffee husks bed were higher than the values found in the facilities with shavings bed. The same pattern is observed for the carbon dioxide concentration values. By the general pattern of the carbon dioxide concentration values, for both the shavings and coffee husks beds, an increasing linear pattern of the carbon dioxide concentration with the increase in the number of bed reuse cycles is remarked.

The environmental air conditions influence the characteristics of the avian bed, as the air temperature increases the beds become dryer (Tasistro et al., 2008; Oliveira & Monteiro, 2013). The moisture content of the bed influences the ammonia volatilization, which increases with the increase of moisture. According to data obtained in this study, before the first use the shavings have a moisture content of 7%, while the coffee husks have a moisture content of 15%. This higher moisture content of coffee husks may give greater ammonia volatilization and consequently provides an environment with higher ammonia concentration. On the other hand, the pH values obtained in this study for the

shavings were around 4.0 and for the coffee husks were around 5.0. As the pH values of both materials before the first use are quite similar, the factor that has greater influence on the final bed pH is manure that is deposited in the avian bed over the reuse cycles. Therefore, the increase in the number of bed reuse cycles causes increases in the ammonia concentration in the environmental air of the aviaries. As conclusion, in facilities with coffee husks bed the air quality tends to be worse when compared to the facilities with shavings bed.

Regardless bedding type, it is observed that the increase in the number of reuse cycles tends to decrease air quality due to the linear increase in ammonia and carbon dioxide concentrations. Similar results were reported by Pereira & Mesquita (1992); Oliveira et al. (2003); Miles et al. (2011); Marín et al. (2015). They evaluated the beds of wood chips and coffee husks with up to four reuse cycles and concluded that the increase in the number of reuse resulted in higher ammonia emissions.

## CONCLUSIONS

The study allows to draw some conclusions. In particular, in relation to the type of bedding, it has been remarked that facilities using coffee husks bed tend to have poorer air quality when compared to facilities using shavings bed, due to the higher values of ammonia and carbon dioxide concentrations.

Regardless of the reuse of the bedding, the increase in the number of cycles tend to worsen air quality inside the facility, as shown by the linear patterns of ammonia and carbon dioxide concentrations values in relation to the number of bed reuse.

ACKNOWLEDGEMENTS. The authors thank the sponsors CAPES, CNPq and FAPEMIG.

## REFERENCES

- ABPA. 2017. ABPA 2017 Annual Report. *Associação Brasileira de Proteína Animal* São Paulo, 134 pp. (in Portuguese).
- Alencar, M.C.B., Nääs, I.A. & Gontijo, L.A. 2004. Respiratory risks in broiler production workers. *Revista Brasileira de Ciência Avícola* **6**, 23–29.
- Arcidiacono, C., Porto, S.M.C. & Cascone, G. 2015. On ammonia concentrations in naturally ventilated dairy houses located in Sicily. *Agricultural Engineering International: CIGR Journal* **2015**, 294–310.
- Brasil. 1978. Ordinance 3,214 of July 1978. Regulatory norms for safety and health at work - NR-15: Unhealthy activities and operations. *Ministério do Trabalho e Emprego* 85 pp. (in Portuguese).
- Brasil. 2001. Normative Instruction Nº 15 of July 17, 2001. *Ministério da Agricultura Pecuária e Abastecimento* 2 pp. (in Portuguese).
- Calvet, S., Cambra-López, M., Estellés, F. & Torres, A.G. 2011. Characterization of gas emissions from a Mediterranean broiler farm. *Poultry science* **90**, 534–542.
- Campos, A.M.A., Rostagno, H.S., Nogueira, E.T., Albino, L.F.T., Pereira, J.P.L. & Maia, R.C. 2012. Update of the ideal protein for broilers: arginine, isoleucine, valine and tryptophan. *Revista Brasileira de Zootecnia* **41**, 326–332 (in Portuguese).
- Carvalho, V.F., Yanagi Junior, T., Damasceno, F.A., Morais, S.R.P. & Tinôco, I.F.F. 2009. Development of software to predict the thermal environment and performance of broiler chickens in climatized sheds. *Revista Brasileira de Agroinformática* **9**, 1–16 (in Portuguese).

- Cemek, B., Kucuktopcu, E. & Demir, Y. 2016. Determination of spatial distribution of ammonia levels in broiler houses. *Agronomy Research* **14**, 359–66.
- CIGR. 1994. *Aerial Environment in Animal Housing: Concentrations in and Emissions from Farm Buildings*. Dublin, 116 pp.
- Fabbri, C., Vallia, L., Guarinob, M., Costab, A. & Mazzotta, V. 2007. Ammonia, methane, nitrous oxide and particulate matter emissions from two different buildings for laying hens. *Biosystems Engineering* **97**, 441–55.
- Faulkner, W.B. & Shaw, B.W. 2008. Review of ammonia emission factors for united states animal agriculture. *Atmospheric Environment* **42**, 6567–6574.
- Felix, E.P. & Cardoso, A.A. 2004. Atmospheric ammonia: sources, transformation, sinks, and methods of analysis. (Amônia (NH<sub>3</sub>) atmosférica: fontes, transformação, sorvedouros e métodos de análise). *Química Nova* **27**, 123–30 (in Portuguese).
- França, L.G.F. & Tinôco, I.F.F. 2014. Characterization of environmental factors and zoning of egg production in the state of Minas Gerais. In *XLIII Congresso Brasileiro de Engenharia Agrícola - CONBEA 2014*. Campo Grande, pp. 27–30 (in Portuguese).
- França, L.G.F., Tinôco, I.F.F., Mendes, M.A.S.A. & Coelho, D.J.R. 2014. Characterization of factors that influence the emission of ammonia from laying hens and proposing a score for maximum emission potential. In *XLIII Congresso Brasileiro de Engenharia Agrícola - CONBEA 2014*. Campo Grande, 30–35 (in Portuguese).
- Furlan, R.L. 2006. Influence of temperature on broiler production. In: *VII Simpósio Brasil Sul de Avicultura*. Chapecó, **2006**, pp. 104–35 (in Portuguese).
- Furtado, D.A., Dantas, R.T., Nascimento, J.W.B., Santos, J.T. & Costa, F.G.P. 2006. Effects of different environmental conditioning systems on the productive performance of broilers. *R. Bras. Eng. Agríc. Ambiental* **10**, 484–89 (in Portuguese).
- Macari, M. 1996. Environmental comfort for birds: physiologist's point of view. In: *Goiano Symposium on Poultry UFG/AGA*, Goiânia, 57–69 (in Portuguese).
- Manno, M.C., Lima, K.R.S., Aguilar, C.A.L., Souza, N.S.S., Barata, Z.R.P. & Viana, M.A.O. 2011. Production of ammonia inside poultry houses with environmental modifications. *Revista de Ciências Agrárias* **54**, 159–64 (in Portuguese).
- Marín, O.L.Z., Tinôco, I.F.F., Saraz, J.A.O., Souza, C.F. & Vieira, M.F.A. 2015. Evaluation of the fertilizer and contamination potential of different broiler litter types subjected to various use cycles. *Revista Facultad Nacional de Agronomía* **68**, 7637–46.
- Marques, V.S. 1992. The greenhouse effect and global warming. *Anuario IGEO* **15**, 93–106 (in Portuguese).
- Mendes, L.B., Tinoco, I.F.F., Ogink, N.W.M., Rocha, K.S.O., Osorio Saraz, J.A. & Santos, M.S. 2014. Ammonia emissions from a naturally and a mechanically ventilated broiler house in Brazil. *Revista Brasileira de Engenharia Agrícola e Ambiental* **18**, 1179–85.
- Menegali, I., Tinôco, I.F.F., Baêta, F.C., Cecon, P.R., Guimarães, M.C.C. & Cordeiro, M.B. 2009. Thermal environment and concentration of gases in facilities for broiler chickens in the warm-up period. *Revista Brasileira de Engenharia Agrícola e Ambiental* **13**, 984–90 (in Portuguese).
- Miles, D.M., Rowe, D.E. & Cathcart, T.C. 2011. Litter ammonia generation: moisture content and organic versus inorganic bedding materials. *Poultry Science* **90**, 1162–1169.
- Mosquera, J., Monteny, G.J. & Erisman, J.W. 2005. Overview and assessment of techniques to measure ammonia emissions from animal houses: the case of the netherlands. *Environmental Pollution* **135**, 381–88.
- Nääs, I.A., Miragliotta, M.Y., Baracho, M.S. & Moura, D.J. 2007. Aerial environment in housing broilers: dust and gases. *Engenharia Agrícola* **27**, 326–35 (in Portuguese).
- Ndegwa, P.M., Hristov, A.N., Arogo, J. & Sheffield, R.E. 2008. A review of ammonia emission mitigation techniques for concentrated animal feeding operations. *Biosystems Engineering* **100**, 453–69.

- Oliveira, M.C., Almeida, C.V. & Andrade, D.O. 2003. Dry matter content, pH and volatilized ammonia of chicken bed treated or not with different additives. *Revista Brasileira de Zootecnia* **32**, 951–954.
- Oliveira, P.A.V. & Monteiro, A.N.T.R. 2013. Ammonia emission in the production of broilers. In *Conferência FACTA*, ed. FACTA. Campinas, 11 pp. (in Portuguese).
- Osorio-Saraz, J.A., Ferreira-Tinoco, I.F., Gates, R.S., Oliveira-Rocha, K.S., Combatt-Caballero, E.M. & Campos-de-Sousa, F. 2014. Adaptation and validation of a methodology for determining ammonia flux generated by litter in naturally ventilated poultry houses. *DYNA* **81**, 137–43.
- Osório, J.A., Tinôco, I.F.F. & Ciro, H.J. 2009. Ammonia : a review of concentration and emission models in livestock structures. *Dyna* **76**, 89–99.
- Owada, A.N., Nääs, I.A., Moura, D.J. & Baracho, M.S. 2007. Estimation of well-being of broiler chicken as a function of the ammonia concentration and degree of luminosity in the production shed. *Engenharia Agrícola* **27**, 611–18 (in Portuguese).
- Pereira, J.T. & Mesquita, M.M. 1992. *Composting of urban solid waste: theoretical, operational and epidemiological aspects*. Lisboa. 25p. (in Portuguese).
- Popescu, S., Stefan, R., Borda, C., Lazar, E.A., Sandru, C.D. & Spinu, M. 2010. The ammonia concentration in growing-finishing pig houses. *Lucrari Stiintifice Medicina Veterinara* **43**, 320–26.
- Rong, L., Nielsen, P. & Zhang, G. 2009. Effects of airflow and liquid temperature on ammonia mass transfer above an emission surface: experimental study on emission rate. *Bioresource technology* **100**, 4654–61.
- Saraz, J.A.O., Tinôco, I.F.F., Gates, R.S., Paula, M.O. & Mendes, L.B. 2013. Evaluation of different methods for determining ammonia emissions in poultry buildings and their applicability to open facilities. *Dyna* **80**, 51–60.
- SAS Institute Inc. 2008. *SAS/STAT(r) 9.2 User's Guide*. Cary, NC: SAS Institute Inc.
- Scholtens, R., Dore, C.J., Jones, B.M.R., Lee, D.S. & Phillips, V.R. 2004. Measuring ammonia emission rates from livestock buildings and manure stores - part 1: development and validation of external tracer ratio, internal tracer ratio and passive flux sampling methods. *Atmospheric Environment* **38**, 3003–15.
- Van der Stelt, B., Temminghoff, E.J.M., Van Vliet, P.C.J. & Van Riemsdijk, W.H. 2007. Volatilization of Ammonia from Manure as Affected by Manure Additives, Temperature and Mixing. *Bioresource technology* **98**, 3449–55.
- Tasistro, A.S., Cabrera, M.L., Ritz, C.W. & Kissel, D.E. 2008. Manipulating bedding materials and PLT to reduce NH<sub>3</sub> emissions from broiler manure. *Bioresource Technology* **99**, 1952–1960.
- Tinôco, I.F.F. 2001. Industrial poultry: new concepts of materials, concepts and constructive techniques available for Brazilian poultry houses. *Revista Brasileira de Ciência Avícola* **3**, 1–24 (in Portuguese).
- Vieira, M.F.A., Tinoco, I.F.F., Santos, B.M., Cassuce, D.C., Aquino, L.L. & Sousa, F.C. 2015. Sanitary quality of broiler litter reused. *Engenharia Agrícola* **35**, 800–807.
- Vitorasso, G. & Pereira, D.F. 2009. Comparative analysis of the poultry environment with different packing systems. *Revista Brasileira de Engenharia Agrícola e Ambiental* **13**, 788–94 (in Portuguese).
- Webb, J. & Misselbrook, T.H. 2004. A mass-flow model of ammonia emissions from uk livestock production. *Atmospheric Environment* **38**, 2163–76.
- Zhang, G., Strøm, J.S., Li, B., Rom, H.B., Morsing, S., Dahl, P. & Wang, C. 2005. Emission of ammonia and other contaminant gases from naturally ventilated dairy cattle buildings. *Biosystems Engineering* **92**, 355–64.



## Combustible in selected biofuels

I. Vitázek\*, J. Tulík and J. Klůčik

Slovak University of Agriculture in Nitra, Faculty of Engineering, Department of Transport and Handling, Tr.A. Hlinku 2, SK949 76 Nitra, Slovak Republic

\*Correspondence: [ivan.vitazek@uniag.sk](mailto:ivan.vitazek@uniag.sk)

**Abstract.** The aim of the research was to determine the moisture, combustible and ash content in selected biofuels, in dependence on temperature by the means of gravimetric method. For this purpose, the furnace Nabertherm L9/11/SW/P330 was used. Analyzed samples consisted of crushed biomass which is used in small heat sources (e.g. spruce wood, cherry wood, apple wood, black locust wood) with bark and without additives. Biomass for larger heat sources (woodchips cuttings from coniferous trees), sawdust mix (plum, cherry, walnut, apricot, apple) and pellets (90% spruce and 10% fir) were also analyzed. The results are processed graphically and enable to characterize the tested fuels. The highest content of combustible was found in spruce wood sample without bark (99.889%), the lowest content of combustible indicated cuttings from coniferous trees (88.402%). Gravimetric experiments were supplemented by DSC measurement of selected samples on calorimeter Toledo DSC822e. The graphic courses of reactions are provided up to 500 °C.

**Key words:** gravimetry, ash, biomass, combustion.

### INTRODUCTION

Constantly increasing prices of fossil fuels and efforts to limit the production of gaseous emissions facilitate rapid use of renewable energy sources (Jablonický et al., 2012). An environment protection, fossil fuels depletion in the near future, legislation and development of new technologies are the main reasons for using biofuels (Kosiba et al., 2016). It should be remarked that decrease in fuel consumption is an effective way how to eliminate the negative aspects of the fuel combustion (Szabó et al., 2013a; Szabó et al., 2013b). Renewable energy has become more important globally especially with the current fuel and economic crisis (Branca & Blasi, 2015). Especially solid biofuels will be increasingly used as a source of thermal energy. Biomass means all organic matter that arose through photosynthesis, or material of animal origin. This term often refers to plant biomass usable for energy purposes, as a renewable energy source (Maga et al., 2010). Biofuel is the fuel derived from biomass. According to the chemical composition, the biofuels can be divided into solid, liquid and gaseous. Quality of solid biofuels as an energy source depends on the moisture and ash content and volatile combustible matter (Mikulová et al., 2014; Holubčík et al., 2016). Research of the amount and course of combustible release in selected biofuels in dependence on temperature is described in the work (Mikulová & Vitázek, 2016). Chemical energy from

biofuels is released mainly from the combustion process. The combustion process is considered as oxidation process, wherein the combustible fuel components are oxidized by atmospheric oxygen, where the energy content of fuel is transformed into heat. The impact of the temperature affects the loss of moisture in the fuel as well. Biofuels as an energy source depend on the quality of combustible and the proportion of ballast – moisture and ash. Compared to solid fossil fuels, biomass contains significantly higher proportion of volatile combustible matter, which is essentially due to its origin. Biomass combustion does not pollute the environment by excessive production of CO<sub>2</sub>. Another advantage of the biomass combustion is that the ash as a by-product of combustion may be used as a high quality fertilizer. Biomass offers us a large variety of raw materials and gradually finds its universal use in the energetics. It is a suitable fuel to replace heating with natural gas or electricity. Moreover, it is used for heat and electricity generation in modern combustion plants (Mižáková, 2014). The design and testing of a cost-effective control system for medium-sized biomass boilers based on information regarding not only the oxygen concentration in the flue gases but also the carbon monoxide emission values is discussed in the work (Pitel' et al., 2013). Original composition of solid biofuels (wood, straw, corn) in terms of combustion is as follows (Piszczałka, 2010):

- Volatile combustible matter (wood gas) 60–70%,
- A non-volatile solid combustible (charcoal in the case of wood) to 20%,
- Ballast water (up to cca 14%), and ash from the charcoal combustion 0.5–4%.

This paper presents the method of determining the proportion of the biofuel components by the means of gravimetric method. We focused on the fuels that are used by the heat producer for residential houses in Vráble, and also for the boilers for family houses which use piece wood and pellets. Samples were collected from specific users. An exception is the boiler room in Vráble, which burns wood biomass with different moisture content and composition (waste from wood processing in forests and cuttings from orchards).

Analysis of the course of the release of combustible materials for different fuels is described. Thermal analysis of the samples was carried out using the DSC method (differential scanning calorimetry) by the means of calorimeter Mettler Toledo DSC822e.

## MATERIAL AND METHODS

Currently, biomass is widely used for heating of the drying medium as a substitute for natural gas. The use of solid biofuels in combustion process enhances the competitiveness of the modern agriculture. Biomass is used to heat family houses and is also used in boilers for central heat supply. An important factor in the combustion of biofuels is the release of combustible. The residue is substantially ash. Ash content is determined after the combustion of fuel. Analyzed samples consisted of crushed biomass which is used in small heat sources (e.g. spruce wood, cherry wood, apple wood, black locust wood) with bark and without additives. Biomass for larger heat sources (woodchips cuttings from coniferous trees), sawdust mix (plum, cherry, walnut, apricot, apple) and pellets (90% spruce and 10% fir) were also analyzed.

To measure the proportions of components in solid biofuels, gravimetric method was used. It was performed by the means of a furnace Nabertherm L9/11/SW/P330. Input of the heating element is 3.0 kW. The controller P330 enables to program the selected courses of heating and endurance by the computer. Heating of the tested samples is possible up to the temperature of 1,100 °C. To measure weight during the course of the experiment, digital scales were used. The device is connected to a computer and enables to record the temperature and weight curves for the selected time intervals. This device enables to determine the proportion of moisture, combustible and ash in the tested solid biofuels.

**Proportions of particular components are calculated according to the following relations**

*Moisture content w:*

$$w = \frac{m_1 - m_2}{m_1} 100 \quad (1)$$

*Ash content in original sample A':*

$$A' = \frac{m_3}{m_1} 100 \quad (2)$$

*Ash content in dry matter p<sub>ps</sub>:*

$$p_{ps} = \frac{m_3}{m_2} 100 \quad (3)$$

*Combustible content in original sample h':*

$$h' = \frac{m_4}{m_1} 100 \quad (4)$$

*Combustible content in dry matter p<sub>hs</sub>:*

$$p_{hs} = \frac{m_4}{m_2} 100 \quad (5)$$

where  $m_1$  – original weight of sample, g;  $m_2$  – weight of dry matter, g;  $m_3$  – weight of ash, g;  $m_4$  – weight of combustible, g.

For the determination of the volatile matter, the standard STN EN 15148 is applied. For the determination of ash content in solid biofuels, the standard STN EN 14775 applies together with STN ISO 1171. The experiments were carried out in accordance with standard STN ISO 1171. The required temperatures and operating times were programmed by the means of PC. Table 1 shows the parameters during gravimetric measurements. The given intervals allow determination of the moisture, combustible and ash content. In the last interval, the sample was annealed at 815 °C for 60 minutes. The same method is used by the Agricultural Technical and Testing Institute in Slovak Republic (Rusňák & Šmidová, 2010).

**Table 1.** Parameters for gravimetric measurement procedure

	Time interval					
	1	2	3	4	5	6
Impact period, minute	60	120	60	60	6	60
Temperature, °C	20–105	105	105–500	500	500–815	815

Source: EN 14775:2010, and ash content determination – STN ISO 1171:2003.

The analysed sample is first heated to  $105\text{ °C} \pm 2\text{ °C}$  and dried for 120 minutes. Weight loss in this interval is considered as the removed moisture. Weight loss in the fourth interval ( $500\text{ °C}$ ) is considered as volatile combustible matter, whereas mass residue at the end of the experiment consists of ashes.

For the thermal analysis of the samples by differential scanning calorimetry (DSC method), Mettler Toledo DSC822e device was used. Two instances of the reaction up to  $500\text{ °C}$  are presented – for samples of spruce wood and black locust wood.

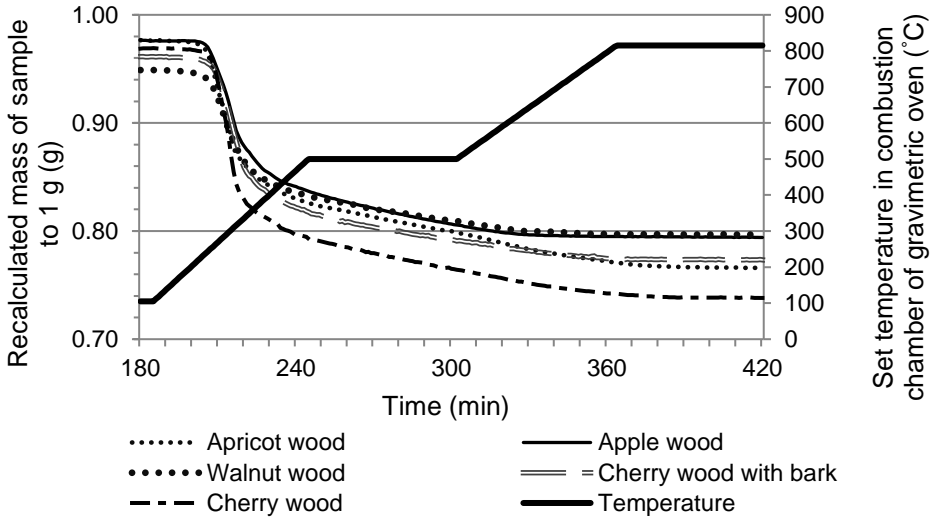
## RESULTS AND DISCUSSION

Processed results of gravimetric measurements are shown in Table 2. Proportions of moisture, ash and combustible are calculated according to the relations (1) to (5). Heating with biomass is increasingly used as an alternative to natural gas. The boiler manufacturers have begun to react to this trend. Even with the gradual arrival of new innovations of biomass boilers, high ash content and inadequate temperature can cause sintering of ash. This can cause temporary interruption of combustion and lead to partial or permanent damage to the boiler. Therefore it is necessary to take into account the information about release of the combustible substances in solid biofuels.

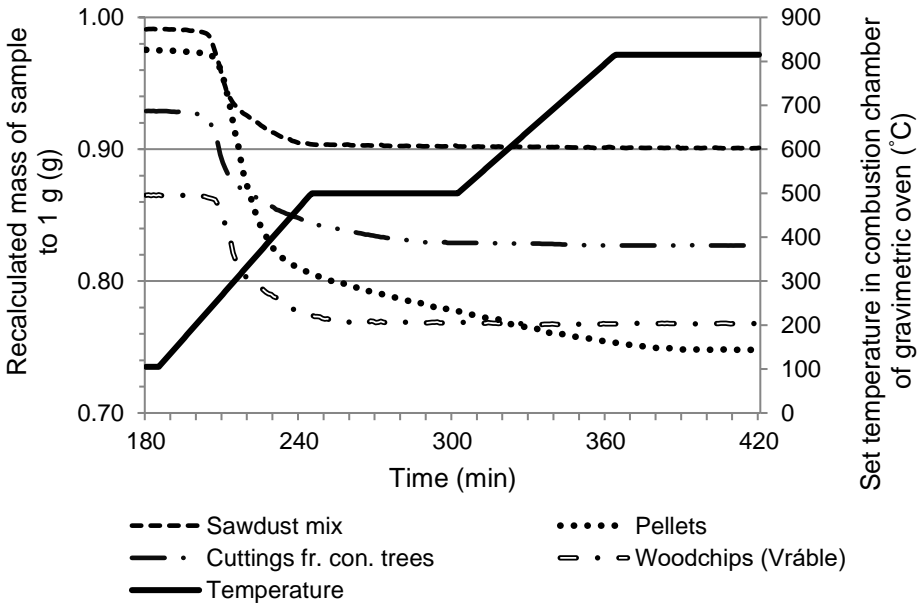
**Table 2.** Processed results of gravimetric measurements of analyzed samples

BIOFUELS	PARAMETER				
	$W$	$A'$	$h'$	$p_{ps}$	$p_{hs}$
Spruce wood without bark	8.742	0.101	91.157	0.111	99.889
Pellets	10.329	0.549	89.123	0.616	99.388
Black locust wood	6.204	0.239	93.557	0.255	99.745
Cherry wood without bark	13.551	0.425	86.024	0.492	99.508
Cherry wood with bark	17.197	1.198	81.605	1.447	98.553
Apple wood with bark	11.211	0.834	87.956	0.939	99.061
Woodchips from Vráble	58.782	0.657	40.562	1.593	98.407
Apricot wood with bark	7.320	1.628	91.052	1.757	98.243
Sawdust mix	9.206	2.399	88.394	2.643	97.357
Walnut wood with bark	24.869	2.352	72.779	3.131	96.869
Cuttings from coniferous trees	44.277	6.463	49.261	11.598	88.402

Figs 1 and 2 show weight loss rate of analysed samples of solid biofuels.



**Figure 1.** Weight loss rate of analyzed samples of solid biofuels.

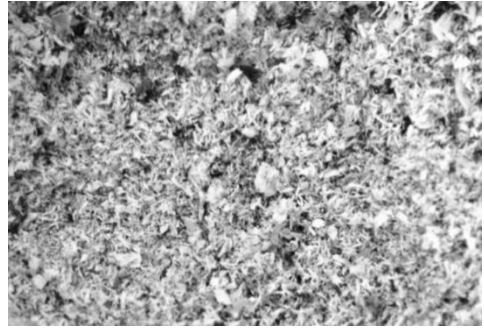


**Figure 2.** Weight loss rate of analyzed samples of solid biofuels.

Figures show samples of biofuels before the processing for the purpose of the experiment. Fig. 3 shows Apple wood, Fig. 4 shows sawdust mix, Fig. 5 shows cuttings from coniferous trees, Fig. 6 shows woodchips from Vrable.



**Figure 3.** Apple wood.



**Figure 4.** Sawdust mix.



**Figure 5.** Cuttings from coniferous trees.

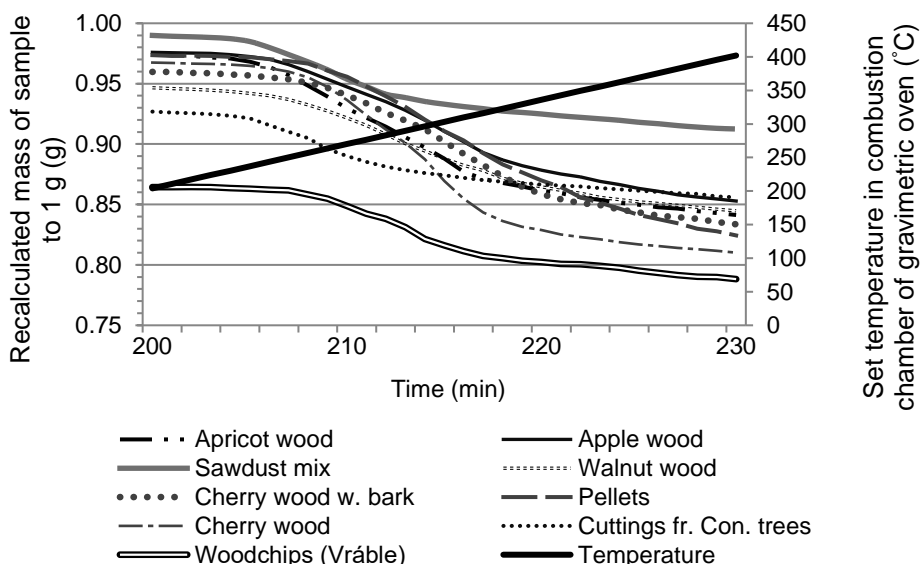


**Figure 6.** Woodchips from Vráble.

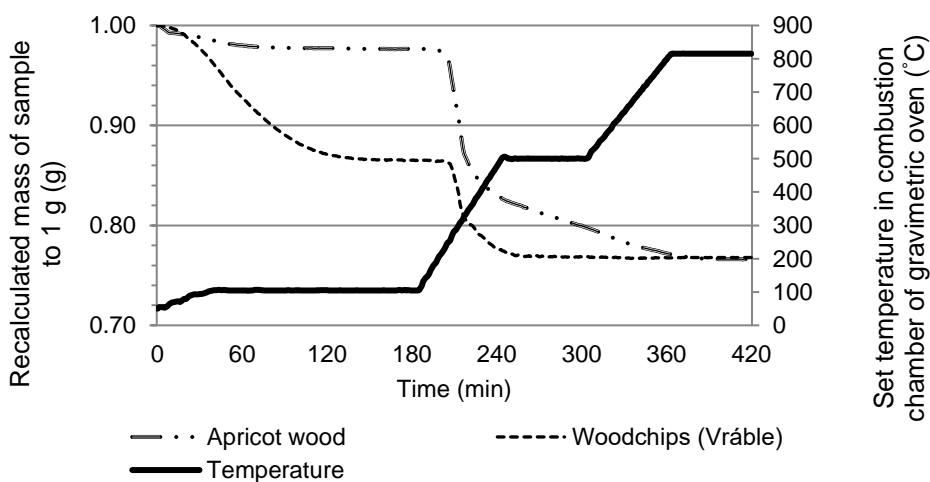
The amount of volatile matter in biomass depends on the type of fuel and is inversely proportional to water and ash content of the fuel. In terms of long-term storage, the water content of 14–15% is considered as the most suitable. According to (Maga et al, 2010), biomass combustion as a process does not always provide the maximum amount of energy contained in it. More preferably, the biomass may be converted to the kind of fuel that allows extracting the most out of its energy. As regards methods of biomass conversion, thermal conversion is of significant importance. This method enables to produce liquid, gaseous or solid fuel of higher quality from biomass, which allows achieving a high energy yield and increase of combustible content in solid biofuel. The results show a significant difference in the proportion of the combustible of solid biofuels. The highest content of combustible was found in spruce wood without bark (99.889%), the lowest content was present in cuttings from coniferous trees (88.402%). The results show content of the substances in dry matter. When comparing the content in original samples (moist), the highest content of combustible was present in black locust wood (93.557%), the lowest content in woodchips from Vráble (40.562%).

Figs 1 and 2 show weight loss rates of analysed biomaterial samples at a time interval from 180 minute (after evaporation of moisture) to 420 minute, when volatile combustible is being released and weight loss is apparent to the greatest extent. In addition, Fig. 7 depicts weight loss rate at an interval from 200 to 230 minute. Fig. 8

shows the course of the experiment for the fuels with the highest moisture content – woodchips from Vráble and apricot wood with bark, when rapid weight loss occurs after moisture removal. This weight loss is accounted for the release of volatile combustible.



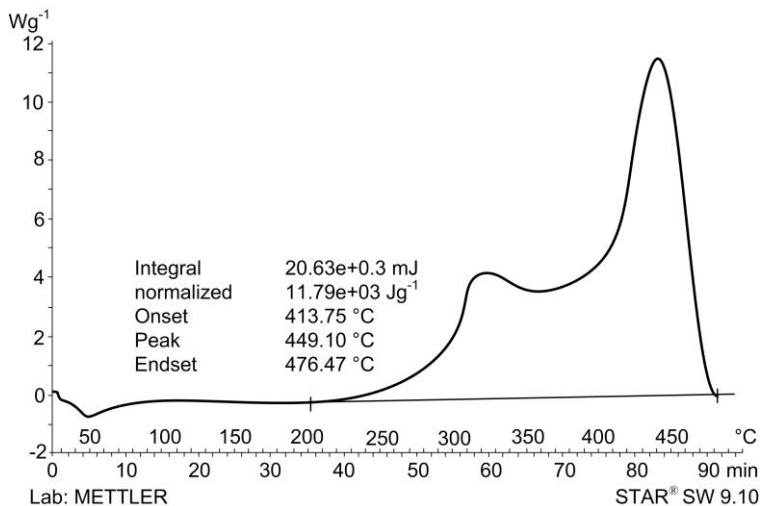
**Figure 7.** The fastest weight loss rate of analysed samples of solid biofuels at a selected time interval.



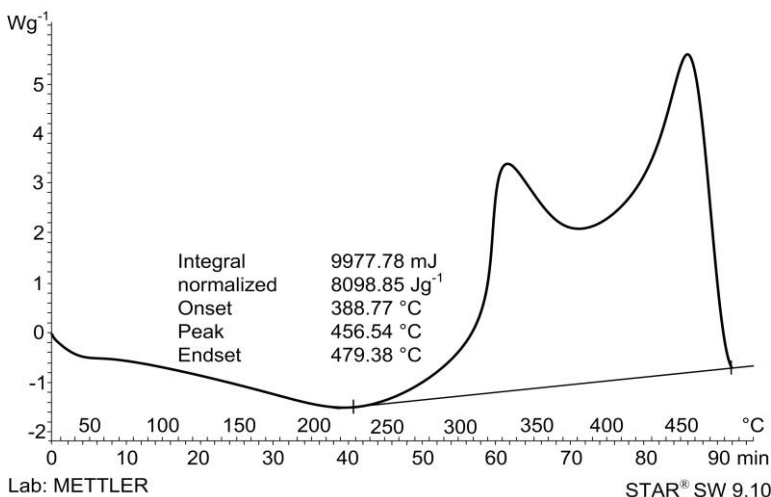
**Figure 8.** The fastest and slowest weight loss rates of analysed samples of solid biofuels.

Figs 9 and 10 show results of thermal analysis measurements of samples by differential scanning method using the calorimetric device Mettler Toledo DSC822e. Weight of the examined samples was 1.5 mg. The released energy is measured only in a certain time interval and at the temperature up to 500 °C. For the sample from black

locust wood, the amount of released energy equals  $11,790 \text{ J g}^{-1}$  and for spruce wood it is  $8,098 \text{ J g}^{-1}$ . In the case of spruce wood, the beginning of the reaction was significantly affected by the initial moisture, but the end of the reaction can be observed clearly. The initial moisture content of the sample affects beginning of the reaction – an exothermic reaction takes place continuously from endothermic.



**Figure 9.** Graphic representation of the results of measurements for the black locust wood sample – DSC method.



**Figure 10.** Graphic representation of the results of measurements for the spruce wood sample – DSC method.

In both cases, two peaks can be observed. The sample of woodchips from Vráble shows even three peaks (not shown). The results for the different materials will be processed after a special software is obtained. Selected samples will be examined by the means of the method of thermogravimetry (TG). The results of the non-isothermal



thermogravimetric analysis in the dynamic air atmosphere, which can be used for the optimization of combustion process, are presented in the article (Ondro et al., 2017).

Experiments were carried out with fuels, which are used in family houses with low-power heat sources as well as in the heat source for residential district heating in Vrábě. Samples were collected from specific users. An exception is the boiler room in Vrábě, which burns wood biomass with different moisture content and composition (waste from wood processing in forests and cuttings from orchards).

With gradual expansion of biomass combustion, the given conditions will decline (availability of suitable biomass). The results of wood show a high content of combustible from 99.889% to 96.869%. The impact of bark in case of cherry wood sample causes an increase in ash content of 0.955%. Softwood pellets (90% spruce, 10% fir) have the same content of combustible and can be considered as equivalent substitutes. The samples taken from boiler room (Vrábě) had a high initial moisture (woodchips up to 58.782%), but the combustible content in dry matter was 98.407%. Cuttings from coniferous trees contained various additives, the combustible content decreased to 88.402%. The heat source also enabled the combustion of these fuels.

We have carried out dozens of experimental measurements with various biofuels, even in combination with coal dust (Mikulová et al., 2014). The method according to STN ISO 1171 has been approved to evaluate moisture, combustible and ash content. The unavailability of the originally intended fuel leads to the use of substitutes, where it is necessary to know their properties and to set up the adequate process of combustion and ash production (cleaning of the boiler). In addition, it is necessary to know the proportion of volatile and solid combustible, which has an impact on the combustion (supply of secondary and tertiary air). Further research is carried out in this area.

Similar results obtained from samples of another fuels are presented in the work (Vitáček & Vitázková, 2012) which shows processed gravimetric measurements of selected types of biofuels. In terms of ash content, pellets from rape waste and cereals contain 7.988%, maize straw pellets contain 5.194%, corn flake pellets contain 4.708%, corncob pellets contain 4.378%, softwood pellets contain 1.029% and softwood pellets without bark contain 0.462%. The results obtained in the paper (Mikulova et al., 2014) show that the highest ash content was found in sample from cuttings from coniferous trees – 11.59% and the lowest ash content has spruce wood – 0.11% and therefore confirm the results presented in this work. Similar results are presented also in the work (Chrastina et al., 2015) – distillery refuse from corn distiller's dried grain has ash content of 4.64%.

Research of biofuels includes other works from this field. Impact of the physical properties of solid biofuels on the design of combustion devices is discussed in (Vítěz & Trávníček, 2010). Influence of the input raw material properties on the quality and energetic demand in briquette production is presented in the paper (Muntean et al., 2017). Usage of additives has significant impact on the properties of wood pellets, which include combustion and production of emissions (Jandačka et al., 2011; Jandačka et al., 2015). The article (Ivanova et al., 2015) provides comparison of selected types of solid biofuels for heating private houses. Efficiency of boilers and the production of gaseous emissions is also observed. The work (Kirsanova et al., 2016) deals with the thermodynamic model of biomass gasification and the impact on tar and char production.

In all cases, it is necessary to know the physical properties of the particular biofuels.

## CONCLUSION

Various types of biomass are used for the purpose of combustion. In small devices, piece wood is often used. In the case of bigger devices, different types of woodchips, cuttings or waste from wood processing are used. For this reason, the article is focused on selected types of wood (pure and with bark) and fuels collected from the boiler room in Vrábľe, Slovakia (cuttings from coniferous trees and woodchips). High moisture of fuel (woodchips 58.78%) was observed. The second fuel indicated the highest content of ash (cuttings from coniferous trees 11.598%). The heat source in question allows combustion of these fuels without operational problems. Pure wood indicates the highest content of combustible, bark additives increase the ash content. For small combustion devices, a lower content of combustible may have an impact on the operation, for example the increased need of cleaning. Apart from high combustible content, proportion of volatile combustible and its rate of release also affects the quality and properties of the fuel. The obtained results enable easy decision making when replacing the type of fuel.

The experiments continue with further examination of biofuels and their combinations with fossil fuels (pellets from sawdust and coal dust) and with analysis of the content of volatile and solid combustible and its rate of release. Results of thermal analysis of samples by DSC method will be subject to further examination and other fuels will be analyzed.

**ACKNOWLEDGEMENTS.** Supported by the Ministry of Education of the Slovak Republic, Project VEGA 1/0464/17 'Monitoring of the impact of ecological fuels obtained from the agricultural production and additives in hydrocarbon fuels to technical and environmental performance of internal combustion engines used in agricultural and transport technique'.

## REFERENCES

- Branca, C. & Di Blasi, C. 2015. Thermogravimetric analysis of the combustion of dry distiller's grains with solubles (DDGS) and pyrolysis under kinetic control. *Fuel Processing Technology* **129**, 67–74.
- Holubčik, M., Jandačka, J. & Ďurčanský, P. 2016. Energy properties of wood pellets made from the unusual woody plants. In *The meeting of Departments of Fluid Mechanics and Thermomechanics*. 1st ed. online. New York, American Institute of Physics, 10 pp., online: <<http://aip.scitation.org/content/aip/proceeding/aipcp/10.1063/1.4963035>>.
- Chrastina, J., Staroňová, L., Vitázek, I. & Pšenka, M. 2015. Analysis of residual biomass of liquid biofuels using gravimetric method and combustion heat. *Research in agricultural engineering* **61**(Special Issue), 21–25.
- Ivanova, T., Kaválek, M., Havrland, B., Kolaříková, M. & Skopec, P. 2015. Comparison of technologic parameters of pellets and other solid fuels produced from various raw materials. *Agronomy Research* **13**(2), 303–310.
- Jablonický, J., Tkáč, Z., Majdan, R., Uhrinová D., Hujo E. & Vozárová, V. 2012. *Hodnotenie vlastností biopalív a biomazív (Properties evaluation of biodegradable lubricants and biofuels)*. Slovak University of Agriculture in Nitra, Nitra, 143 pp. (in Slovak).
- Jandačka, J., Malcho, M. & Nosek, R. 2011. Vplyv pridávania aditív do drevných peletiek na produkciu tuhých znečisťujúcich látok (Impact of additives in wood pellets on production of solid pollutants). In *Zborník z vedeckej konferencie Tepelná technika v teorii a praxi*. VŠB-TU, Ostrava, pp. 80–83 (in Slovak).

- Jandačka, J., Holubčík, M. & Malcho, M. 2015. Production of emissions during combustion of dendromass with different types of additives. *The holistic approach to the environment* **5**(2), 53–59.
- Kirsanova, V., Žandeckis, A. & Rochas, C. 2016. Biomass gasification thermodynamic model including tar and char. *Agronomy Research* **14**(4), 1321–1331.
- Kosiba, J., Uhrinová, D., Jablonický, J. & Majdan, R. 2016. *Alternatívne palivá a pohony motorových vozidiel. (Alternative fuels and power drives for vehicles)*. Slovak University of Agriculture in Nitra, Nitra, 145 pp. (in Slovak).
- Maga, J., Piszczalka, J. & Pepich, Š. 2010. *Využitie rastlinnej a drevnej biomasy na výrobu tepla (The use of plant and wood biomass for heat production)*. Slovak University of Agriculture in Nitra, Nitra, 171 pp. (in Slovak).
- Mikulová, Z., Vitázek, I. & Klůčik, J. 2014. Gravimetric analysis of selected types of biofuels. *Acta technologica agriculturae* **17**(2), 53–56.
- Mikulová, Z. & Vitázek, I. 2016. Proportion of volatile matter in selected biofuels. In *MendelNet 2016*. 1st ed. 1 CD-ROM (1048 s.). Mendel university, Brno, pp. 892–897.
- Mizáková, J. 2014. Monitorovanie systému výroby tepla na báze biomasy (Monitoring of biomass-based heat production system). In *Automatizácia a riadenie v teórii a praxi. ARTEP 2014*. Technická univerzita, Košice, pp. 00-1/00-8 (in Slovak).
- Muntean, A., Ivanova, T., Hutla, P. & Havrand, B. 2017. Influence of raw material properties on the quality of solid biofuel and energy consumption in briquetting process. *Agronomy Research* **15**(4), 1708–1715.
- Ondro, T., Húlan, T. & Vitázek, I. 2017. Non-isothermal kinetic analysis of the dehydroxylation of kaolinite in dynamic air atmosphere. *Acta technologica agriculturae* **20**(2), 52–56.
- Piteř, J., Mizáková, J. & Hořovský, A. 2013. Biomass combustion control and stabilization using low cost sensors. *Advances in Mechanical Engineering*, vol. 2013, Article ID 685157, 7 pp, <http://dx.doi.org/10.1155/2013/685157>.
- Piszczalka, J. 2010. Termická konverzia biomasy na palivo (Thermal conversion of biomass to biofuels). *Agrobioenergia* **5**(4), 20–22.
- Rusňák, P. & Šmidová, E. 2010. Laboratórne analýzy biomasy a biopalív v Technickom a skúšobnom ústave pôdohospodárskom (Laboratory analyzes of biomass and biofuels in Agricultural Technical and Testing Institute). *Agrobioenergia* **5**(3), 23–25 (in Slovak).
- Szabó, M., Majdan, R., Tkáč, Z., Čápora, R. & Hujo, Ľ. 2013a. Evaluation of fuel consumption in road freight transport. *Acta technologica agriculturae* **16**(1), 17–20.
- Szabó, M., Majdan, R., Angelovič, M. & Čápora, R. 2013b. Deviations in fuel consumption measurement of lorries. In *Acta technologica agriculturae* **16**(3), 72–75.
- STN EN ISO 18134-2. Solid biofuels. Determination of moisture content. Oven dry method Part 2: Total moisture. Simplified method.
- STN EN 14775. 2010. Solid biofuels. Determination of ash content.
- STN EN 15148. 2010. Solid biofuels. Determination of the content of volatile matter.
- STN ISO 1171. 2003. Solid mineral fuels. Determination of ash.
- Trenčiansky, M., Lieskovský, M. & Oravec, M. 2007. *Energetické zhodnotenie biomasy (Energetical evaluation of biomass)*. NLC, Zvolen, 147 pp. (in Slovak).
- Vitázek, I. & Vitázková, B. 2012. Selected physical properties of solid biofuels. *PTEP – Journal on processing and energy in agriculture* **16**(4), 165–168.
- Vítěz, T. & Trávníček, P. 2010. Particle size distribution of sawdust and wood shavings mixtures. *Research in Agriculture Engineering* **56**(4), 154–158.

## **Analysis of cost and performances of agricultural machinery: reference model for sprayers**

T. Yezekyan<sup>1,\*</sup>, F. Marinello<sup>1</sup>, G. Armentano<sup>2</sup> and L. Sartori<sup>1</sup>

<sup>1</sup>University of Padova, Department of Land, Environment, Agriculture and Forestry, Via dell'Università 16, IT35020, Legnaro, Italy

<sup>2</sup>Edizioni L'Informatore Agrario srl, Via Bencivenga - Biondani, 16, IT37133 Verona, Italy

\*Correspondence: [tatevik.yezekyan@phd.unipd.it](mailto:tatevik.yezekyan@phd.unipd.it)

**Abstract.** Management of agricultural operations is currently rapidly changing caused by increased attention to the concepts of sustainable development and sustainable intensification. Enhancement of productivity and efficiency of agricultural machinery are the leading factors in sustainable agriculture. The complete application and exploitation of engineering advances require the revision of traditional agricultural machinery management process. The definition of the farm fleet (tractors and implements), as well as machinery planning and management, must consider different parameters, including not only the cost of the machines but also their dimensions, weight, working width, needed power, etc. All of this information related to an agricultural machine is eventually influencing the impact on productivity, on the return on the investment, and also on the environment.

The present work is aimed at identifying the most relevant parameters which are influencing costs and performances of sprayers, including tank volume, maximum flow, needed power, weight and price. The different parameters are analysed in a correlation matrix, in order to allow identification of dependencies and to extract reference models.

The study is based on linear and multiple linear regression analysis carried out on technical specifications of about 700 models of sprayers. Relevant correlations were highlighted between price and weight, between weight and tank capacity and in some cases between power and weight. Following such correlations, models have been proposed, which can be implemented in order to support the decision making phases.

**Key words:** agricultural machinery, sprayer, optimization model, stepwise regression, correlation analysis.

### **INTRODUCTION**

Agriculture is moving toward sustainable intensification. Information technology developments applied in agriculture production are called to ensure sustainable development of the sphere and solve the main challenge of last decades – to produce more. According to the Strategic Research Report provided by the European Union for Agriculture and Rural Development nowadays agricultural production currently directs research towards increasing production per unit area, caused by population growth at the global level, the consequent increase in food requirements and limited resources (Zarco-

Tejada et al., 2014). Information-based and decision-making approaches of farm management are designed to improve the agricultural process by precisely managing each step, thus optimizing both agricultural production and profitability, leading to cost savings and also environmental benefits (Sopegno et al., 2016; Dubbini et al., 2017). However, the full exploitation of engineering advances requires the revision of traditional agricultural machinery management processes, such as:

- agricultural machinery planning methods (tractors and implements) (Bochtis et al., 2014);
- combination of different and appropriate technological operations (choice of machine or implement) (Fountas et al., 2015a);
- characterization of multiple parameters for ensuring the balance between economic, environmental and social issues (Fountas et al., 2015b).

Application of proper decision parameters in agricultural mechanization is not trivial since it supposes balanced choices and trade-off between proper machinery and technological operation based on the actual needs arising from farm size, crop or management approach, and other factors (Søgaard & Sørensen, 2004). The definition of the farm fleet (tractors and implements), as well as machinery planning and management, must consider different parameters, including not only the cost of the machines but also their dimensions, weight, working width, working speed (or efficiency), needed power, etc. All of this information related to an agricultural machine is eventually influencing the impact of that machine on productivity, on the return on the investment, and also on the environment.

One of the most critical agricultural operations is connected with plant protection: in particular, there is a wide variability of machines and implements devoted to the application of pesticides. The success of crop protection products (herbicides, pesticides and fungicides) depends on the effectiveness of their timely application and plays a vital role in ensuring better yields. Mechanization of application and correct selection of the appropriate equipment are the most important key issues of plant protection.

Sprayers play essential importance both for the cultivation of herbaceous crops, and for fruit tree crops or viticulture, as protection from adversity is, in many cases, a determining factor not only of quantity but also of production quality (Matthews & Thornhill, 1994; Matthews, 2000). The performance of crop protection machines and implements is estimated by relative balance between environmental contamination and biological efficacy (Duga et al., 2013). Which are caused by construction and operational parameters of the sprayers, treated plant features and environmental conditions.

The sprayers on the market are characterized by a considerable variety, both in terms of size and components, which makes them suitable for every type of crop and situation. Based on the cultivated crop, in general, the sprayers can be divided into two groups: ground sprayers and orchard sprayers. Representatives of the first group by themselves can be designed as attachments to tractors (mounted or trailed) and as individual machines (self-propelled) (Hunt, 1983), the second group include trailed or mounted mistblowers and tunnel sprayers.

The basic structure, however, proves to be substantially constant despite this variety and is characterized by some fundamental components that are always present regardless of the specific machine. The necessary components of any sprayer are a tank with agitator and strainer, a pump, a filter, a pressure regulator, valves, piping and nozzles

(Pimentel, 2002). Depending on the crop being treated, the applied protection and technology the main technical parameters are accordingly changed (weight, power, dimensions, etc.). Thereby contributing to a variation in productivity and performance, which are the fundamental factors for price formation.

The decision on the best solution for a farm is not easy since many variables are influencing costs and performances. Therefore the aim of the present work is to identify the most relevant functional parameters determining the performances and costs of sprayers. In particular, the study pays specific attention to tank volume, maximum flow, needed power, weight, working width and price. Regression analysis was then applied in order to determine the dependencies of the variables and to extract reference equation models, which can be implemented in order to support the decision making phases and optimize the choice of the machine.

## MATERIALS AND METHODS

### Reference database

The reference database was populated with the support of Edizioni l'Informatore Agrario: it reports the main technical characteristics and list prices of all the categories of machines on the Italian market and is updated annually thanks to the close collaboration with the main companies of the agricultural sector. The categories of agricultural machinery include tractors, harvesting machines, implements for tillage and sowing, spraying and crop protection, mineral and organic fertilization, hay-making, trailers, etc. Data (relating to over 6,000 machines in total) concern the technical characteristics in numerical or category format.

The work here presented is based on main technical specifications of 729 commercial sprayers of 26 different machine producers from Italy, Germany, Netherlands, Norway and Denmark. Considered data were as follows: constructing company, type of machine (field or orchard), model/series name, type of attachment to the tractor (self-propelled, mounted or trailed), weight, tank capacity and materials, maximum pump flow rate, working width, number of sections and spray nozzles, standard tires, list price, type of fan and diameter for atomizers, minimum power required and nominal engine power, or engine characteristics for self-propelled machines. Data provided were sorted, edited from errors, completed and a single database for the study was created. The range for minimum and maximum values of the considered variables in accordance with available dataset is presented in Table 1, which additionally helps drawing a brief picture of the machinery considered within the current work.

**Table 1.** The range for minimum and maximum values of the considered variables according to dataset

	Power, kW	Tank capacity, L	Pump flow, L min <sup>-1</sup>	Working width, m	Weight, kg	Price VAT excl., k€
Mistblower	10–70	300–4,000	50–250	-	100–1,700	2–38
Tunnel	5–40	300–3,200	60–250	-	900–2,800	23–73
Field sprayer	5–100	120–6,000	40–520	9–32	200–5,000	1–100
Self-propelled	45–180	1,200–5,000	120–400	16–40	3,000–10,000	80–420

The dataset includes 453 implements for orchard crop protection and vineyards, 19 of which are tunnel machines. The designed and homogenised dataset for orchards is constituted by 143 mounted, 5 semi-mounted, and 305 trailed implements. The remaining 276 sprayers in the dataset are intended for field crop protection and are available in all possible varieties (35 self-propelled, 156 mounted, 8 semi-mounted, 77 trailed). Available data represent a clear figure of the present plant protection machine market and allow to produce comparisons and models for different groups of commercial sprayers.

### Data analysis

This study investigates the correlation between functional parameters of commercial sprayers and their influence on the performance of the machines and price. To this purpose, data were classified into four main groups according to the type of machine (mistblowers, tunnel sprayers, self-propelled field sprayers, mounted/trailed field sprayers) and analysed separately. Main parameters were investigated: power, working width, weight, tank capacity, pump flow rate, price.

Statistical analyses were applied in order to design models describing the relationship between technical characteristics of sprayers. The relevance of the models was quantified by means of correlation studies and dependences modelled according to linear (1) and multiple linear (2) characteristic equations:

$$y = m_1 \cdot x + q \tag{1}$$

$$y = \sum_i m_i \cdot x_i + q \tag{2}$$

where  $x$  – and  $y$  – represent respectively independent and dependent variables;  $m_i$  – is the slope (or linear coefficient) related to the  $i$ -th independent variables;  $q$  - is the intercept between  $y$  and  $x$  variables.

For model development and analysis, Microsoft Excel program and tools were used. Excel spreadsheet was implemented to analyse data, to create the correlation matrices between abovementioned six parameters, and to carry out and interpret regression analysis. On the first step, simple linear regression was applied in order to understand correlations and dependencies between selected variables. Data are reported in simplified tables in terms of Pearson correlation coefficient  $r$ , slope  $m$  and intercept  $q$  of the linear models. As a second step, one of the methods of multiple linear regression (specifically stepwise regression analysis) was applied for estimating the relationships between the dependent and independent variables as an alternative to linear regression. Multiple linear equations are more complex and their implementation might be more difficult in simulated scenarios where optimization or definition of break even points are needed, however, they can be useful whenever a deeper description is needed for different parameters. The applied stepwise regression analysis allows to identify and avoid misleading regression of variables and overfitting of data. The regression model explains the relationship between response and explanatory variables. In our study case, the response variables were represented by power, weight and price; explanatory variables converged time by time to tank capacity, working width, weight, power and pump flow rate. Price was not included between independent variables, being itself a

function of machines performances. Multiple linear regression output was evaluated in terms of adjusted multiple coefficient of determination (adjusted  $R^2$ ).

## RESULTS AND DISCUSSION

### Linear modelling

#### Mistblowers

For the group of orchard sprayers, a linear regression analysis performed comparatively high Pearson coefficients in particular between weight and tank capacity ( $r = 0.88$ ) and also between price and weight ( $r = 0.82$ ), as reported in Table 2. Slightly lower correlation was also found between price and tank capacity ( $r = 0.67$ ), as well as between price and pump flow ( $r = 0.55$ ).

**Table 2.** Correlation matrix of Pearson coefficient for functional parameters of orchard atomizers

<i>r</i>	Required power, kW	Tank capacity, L	Pump flow, L min <sup>-1</sup>	Weight, kg	Price VAT excluded, €
Required power	1				
Tank capacity	0.458	1			
Pump flow	0.467	0.41	1		
Weight	0.448	0.884	0.465	1	
Price VAT excl.	0.427	0.672	0.558	0.818	1

As a result of the linear regression, linear coefficient and intercept were estimated for all of the variables combinations. Results are summarized in Table 3. It can be noticed how a power supply of about 9.5 kW is needed for each additional cubic meter of tank capacity, while for the same volume, a weight of about 0.34 tonnes has to be taken into account. With regard to needed investment, a starting price of at least 4.7 k€ has to be considered, increasing by 4.8 k€ for each cubic meter of tank capacity.

**Table 3.** Linear coefficient and intercept matrix for functional parameters of orchard atomizers

	Required power, kW	Tank capacity, L	Pump flow, L min <sup>-1</sup>	Weight, kg	Price VAT excl., €
<i>m</i> Required power		0.0095	0.209	0.024	0.0012
Tank capacity	22.04		8.819	2.275	0.094
Pump flow	1.046	0.019		0.056	0.0036
Weight	8.367	0.343	3.884		0.044
Price VAT excl.	147.1	4.812	85.91	15.08	
<i>q</i> Required power		30.46	16.79	28.25	28.53
Tank capacity	208.3		88.84	-102.8	164.4
Pump flow	73.19	94.91		86.39	79.51
Weight	191.1	152.4	83.49		85.96
Price VAT excl.	4,082	4,764	142.8	2,061	



### Tunnel machines

Linear regression analysis on orchard tunnel implements gave lower evidence of a high correlation with respect to other implements analysed here (Table 4). This is most probably ascribable to the reduced number of models available in the market and a progress stage still under development. However, a relatively high Pearson coefficient was found between weight and tank capacity ( $r = 0.88$ ) and also between price and tank capacity ( $r = 0.70$ ). Slightly lower correlations were also found between pump flow and power needed by the machine to operate in standard conditions ( $r = 0.54$ ), and also between price and weight ( $r = 0.55$ ).

**Table 4.** Correlation matrix of Pearson coefficient for functional parameters of orchard tunnel sprayers

<i>r</i>	Required power, kW	Tank capacity, L	Pump flow, L min <sup>-1</sup>	Weight, kg	Price VAT excluded, €
Required power	1				
Tank capacity	0.492	1			
Pump flow	0.538	0.309	1		
Weight	0.323	0.884	0.234	1	
Price VAT excl.	0.367	0.703	0.114	0.547	1

Estimated linear coefficients and intercepts are summarized in Table 5. It can be noticed how a power supply of about 8.4 kW is needed for each additional cubic meter of tank capacity, while for the same volume, a weight of about 0.7 tonnes has to be taken into account. With regard to the price, a starting investment of at least 27 k€ has to be considered, increasing by 13.6 k€ for each cubic meter of tank capacity.

**Table 5.** Linear coefficient and intercept matrix for functional parameters of orchard tunnel sprayers

	Required power, kW	Tank capacity, L	Pump flow, L min <sup>-1</sup>	Weight, kg	Price VAT excl., €
<i>m</i> Required power		0.0084	0.171	0.007	0.0003
Tank capacity	28.65		5.731	1.130	0.0363
Pump flow	1.694	0.017		0.016	0.0003
Weight	14.73	0.691	3.385		0.0221
Price VAT excl.	414.6	13.62	40.99	13.55	
<i>q</i> Required power		8.11	-5.370	6.132	4.301
Tank capacity	714.4		444.3	-745.5	-351.2
Pump flow	108.7	119.5		111.8	126.3
Weight	1,490	901.7	1,290		791.2
Price VAT excl.	36,309	2,7030	38,290	20,120	

### Field sprayers (trailed/mounted)

Relatively higher correlations between the considered parameters have been recognised after linear analysis in the case of trailed and mounted implements. As can be seen from the Table 6, there high correlations arise between weight and tank capacity ( $r = 0.95$ ), price and weight ( $r = 0.91$ ), working width and pump flow ( $r = 0.87$ ); slightly

lower values can be recognised between price and working width ( $r = 0.85$ ), price and tank capacity ( $r = 0.83$ ), weight and pump flow ( $r = 0.83$ ), weight and working width ( $r = 0.83$ ), working width and tank capacity ( $r = 0.82$ ), pump flow and tank capacity ( $r = 0.82$ ). The robustness of these results is supported by the numerosity of the related starting data set.

**Table 6.** Correlation matrix of Pearson coefficient for functional parameters of trailed/mounted field sprayers

<i>r</i>	Required power, kW	Tank capacity, L	Pump flow, L min <sup>-1</sup>	Working width, m	Weight, kg	Price VAT excluded, €
Required power	1					
Tank capacity	0.505	1				
Pump flow	0.543	0.822	1			
Working width	0.569	0.822	0.870	1		
Weight	0.480	0.948	0.833	0.833	1	
Price VAT excl.	0.579	0.830	0.791	0.854	0.910	1

Estimated linear coefficients and intercepts are reported in the Table 7. With regard to the weight, about 0.94 t is needed for each additional cubic meter of tank capacity, or 0.15 t for each additional meter in working width. With regard to the price, a starting investment of about 1.2 k€ is needed, increasing by 21.2 € per kilogram. Alternatively, the price can be expressed also through the tank dimension, with about 18 € per litre of capacity. For working width  $L$  larger than 9 m, the price  $Pr$  can be expressed also as  $Pr = 3,671 \cdot L - 29,552$ .

**Table 7.** Linear coefficient and intercept matrix for functional parameters of trailed/mounted field sprayers

	Required power, kW	Tank capacity, L	Pump flow, L min <sup>-1</sup>	Working width, m	Weight, kg	Price VAT excl., €
<i>m</i> Required power		0.012	0.159	2.486	0.010	0.0006
Tank capacity	21.88		10.01	152.8	0.951	0.038
Pump flow	1.850	0.067		12.11	0.066	0.003
Working width	0.130	0.004	0.062		0.004	0.0002
Weight	22.29	0.945	10.56	154.2		0.039
Price VAT excl.	589.3	18.03	209.4	3,671	21.17	
<i>q</i> Required power		34.83	24.06	14.06	40.51	37.84
Tank capacity	356.5		-308.2	-840.5	374.7	551.1
Pump flow	80.41	79.37		-4,690	99.24	106.2
Working width	8.977	8.904	4.181		10.21	10.23
Weight	54.70	-235.2	-687.7	-1,145		156.0
Price VAT excl.	-4,935	-2,115	-12,839	-29,552	1,207	

### Self-propelled

In comparison with above mentioned three groups, for self-propelled sprayers, the high correlation is mainly associated with the power of the machines (Table 8). High Pearson coefficients were found between tank capacity and power ( $r = 0.93$ ), price and weight ( $r = 0.85$ ), as well as between weight and power ( $r = 0.83$ ) or weight and tank

capacity ( $r = 0.83$ ). Slightly lower correlations performed between price and working width ( $r = 0.79$ ), working width and tank capacity ( $r = 0.75$ ), price and tank capacity ( $r = 0.75$ ) or weight and working width ( $r = 0.75$ ).

**Table 8.** Correlation matrix of Pearson coefficient for functional parameters of self-propelled field sprayers

<i>r</i>	Nominal power, kW	Tank capacity, L	Pump flow, L min <sup>-1</sup>	Working width, m	Weight, kg	Price VAT excluded, €
Required power	1					
Tank capacity	0.932	1				
Pump flow	0.412	0.382	1			
Working width	0.733	0.750	0.208	1		
Weight	0.834	0.835	0.508	0.747	1	
Price VAT excl.	0.728	0.748	0.206	0.790	0.853	1

Estimated linear coefficients and intercepts are summarized in Table 9. Self-propelled machines exhibit a nominal which is typically higher than 42 kW and increases by 26 W per litre of tank capacity. The weight  $M$  ranges between 4 t and 10 t, and is about 54.5 kg per kilowatt of nominal engine power; related price can be expressed as  $Pr = 37.6 \cdot M - 60,020$ .

**Table 9.** Linear coefficient and intercept matrix for functional parameters of self-propelled field sprayers

	Nominal power, kW	Tank capacity, L	Pump flow, L min <sup>-1</sup>	Working width, m	Weight, kg	Price VAT excl., €
<i>m</i> Nominal power		0.026	0.258	4.951	0.013	0.0002
Tank capacity	33.05		8.457	179.8	0.453	0.009
Pump flow	0.660	0.017		2.255	0.012	0.0001
Working width	0.108	0.003	0.019		0.002	0.00004
Weight	54.55	1.539	20.73	330.2		0.019
Price VAT excl.	2,095	60.75	369.5	15379	37.55	
<i>q</i> Nominal power		42.58	52.20	8.803	37.37	74.47
Tank capacity	-1,003		729.7	-1,085	21.98	1,291
Pump flow	194.9	223.2		224.1	192.4	254.1
Working width	9,725	13.49	17.77		11.71	15.26
Weight	6,705	2,005	997.7	-900.1		2,994
Price VAT excl.	-65,548	6,189	90,337	-162,680	-60,020	

### Multiple linear modelling

In the simulation of different scenarios for optimization of agricultural machinery management (Boscaro et al., 2015; Pezzuolo et al., 2014), some variables play a particularly important role.

The needed power is relevant to understand the suitability in relation to the available tractors fleet and other available agricultural machinery. Also needed or nominal power have a major influence on fuel consumption, which is eventually determining the environmental impact in terms of gas emissions. Carbon footprint related to implemented machinery has to take into account also the mass of the machines, which is indeed another relevant characteristic. The latter is additionally important to foresee and possibly prevent soil stress, which is of major concern in the case of tank equipped machinery. Finally, the price is needed in many applications (Sopegno, 2016), where optimization of costs is needed to schedule most profitable management conditions. Thus, power, weight and price underwent multiple linear modelling. The table below (Table 10) reports the results of stepwise regression analyses, considering power, weight and price as response variables. In some cases, the relevant correlations revealed after the linear regression were sufficiently high to properly describe model equations, as was in the case of power for tunnel, self-propelled and field sprayer machines and in the case of weight and price for tunnel machines. The most frequent independent variables are tank capacity and pump flow, which appear in over 50% of the equations; in the case of the price, a relevant influence is determined by the weight of the sprayers, while the working width can be relevant for field and self-propelled machines. In most of the cases the models demonstrate reasonably high predictive ability, in particular in the case of weight and price estimation.

**Table 10.** Equation models for response variables (power, weight, price) determined by stepwise regression analyses

	Power	Adjusted R <sup>2</sup>	Standard error
Mistblower	$P = 16.2 + C \cdot 0.007 + N \cdot 0.15$	0.300	11.8
Tunnel	$P = -5.37 + N \cdot 0.17$	0.248	10.7
Field sprayer	$P = 14 + L \cdot 2.49$	0.320	19.9
Self-propelled	$P = 42.58 + C \cdot 0.026$	0.864	12.4
	Weight	Adjusted R <sup>2</sup>	Standard error
Mistblower	$M = 54.7 + C \cdot 0.32 + N \cdot 1.03$	0.792	120
Tunnel	$M = 901.7 + C \cdot 0.69$	0.768	271
Field sprayer	$M = -493 + C \cdot 0.77 + N \cdot 1.4 + L \cdot 29.47$	0.918	294
Self-propelled	$M = -11.23 + C \cdot 1.382 + N \cdot 9.03$	0.722	1,156
	Price	Adjusted R <sup>2</sup>	Standard error
Mistblower	$Pr = -1121 - C \cdot 1.65 + N \cdot 34.9 + M \cdot 16.9$	0.718	2,586
Tunnel	$Pr = 27030 + C \cdot 13.6$	0.465	10,200
Field sprayer	$Pr = -13407 + P \cdot 217 - C \cdot 6 + L \cdot 895 + M \cdot 20.1$	0.850	9,127
Self-propelled	$Pr = -31142 - N \cdot 446.9 + L \cdot 4877 + M \cdot 34.87$	0.806	42,600

where  $C$  – tank capacity, L;  $M$  – weight, kg;  $P$  – power, kW;  $N$  – pump flow, L min<sup>-1</sup>;  $L$  – working width, m;  $Pr$  – estimated price for different groups of machines, €.

Proposed equation models can constitute a reference for practical implementation of simplified forecasting and optimization approaches. Specifically, an application of the proposed price models is now ongoing in connection with estimation of costs connected with agricultural machinery. The models are being used by some regional Italian

authorities to estimate supports for investments in modernization of agricultural fleets, in connection with the European Rural Development Programmes 2014–2020 (Zarco-Tejada et al., 2014).

## CONCLUSIONS

One of the most important and at the same time vulnerable aspects of agricultural fleet management organization is the decision-making process, due to the wide range of machinery types and performances. The current study was carried out in order to investigate the correlation between functional parameters of commercial sprayers and their influence on the performance and price of the machines. Based on the output of two approaches (linear and multiple linear regression) it can be noted that the examined parameters revealed especially in some cases relevant correlations, which enable to model the most relevant characteristics of sprayers and the corresponding prices based on weight, power and tank capacity as explanatory variables.

Based on the proposed analysis, it can be highlighted how power has relevant influence only in the case of self-propelled machines where it highly correlates with tank capacity, weight and price. For other implements, power plays only a secondary role: this is mainly ascribable to the fact that needed power is not easily measured but is just estimated by manufacturers. Conversely, the weight has a rather relevant influence and is clearly correlated with tank capacity, in a different way according to the type of the studied machines. For the price, which is a target parameter for our study, all the considered parameters have quite an essential influence depending on the type of sprayer. From the Pearson correlation coefficient it can also be noticed some expected high correlation between weight and tank capacity, which eventually play a key role and impact on the price formation and on the performance of the machines.

The applied stepwise regression analysis proposed as an alternative option to linear regression, show a slight increase in correlations (quantified through adjusted  $R^2$ ) thus providing more detailed prediction models. The developed multiple linear models are comparatively more complex (mostly not linear), however are intended for deep analyses and more accurate forecasting of required variables. In both cases the equation models resulting from the study can be implemented by agricultural machinery and farm management applications, programs or software as an initial reference for optimization of simulated scenarios.

## REFERENCES

- Bochtis, D.D., Sørensen, C.G. & Busato, P. 2014. Advances in Agricultural Machinery Management: A Review. *Biosystems Engineering* **126**, 69–81.
- Boscaro, D., Pezzuolo, A., Grigolato, S., Cavalli, R., Marinello, F. & Sartori, L. 2015. Preliminary analysis on mowing and harvesting grass along riverbanks for the supply of anaerobic digestion plants in North-Eastern Italy. *Journal of Agricultural Engineering* **46**(3), 100–104.
- Dubbini, M., Pezzuolo, A., De Giglio, M., Gattelli, M., Curzio, L., Covi D., Yezekyan, T. & Marinello, F. 2017. Last generation instrument for agriculture multispectral data collection. *CIGR Journal*, vol. **19**, 158-163.

- Duga, A.T., Endalew, A.M., Hendrickx, N., Goossens, T., Dekeyser, D., Nuyttens, D., Nicolai, B. & Verboven, P. 2013. Computational Fluid Dynamics Modelling of Orchard Sprayer Performance: Machine Type and Operational Parameters Characterization. *Acta Horticulturae* **1008**(100848), 251–257.
- Fountas, S., Sorensen, C.G., Tsiropoulos, Z., Cavalaris, C., Liakos, V. & Gemtos, T. 2015a. Farm Machinery Management Information System. *Computers and Electronics in Agriculture* **110**, 131–138.
- Fountas, S., Carli, G., Sørensen, C.G., Tsiropoulos, Z., Cavalaris, C., Vatsanidou, A., Liakos, B., Canavari, M., Wiebensohn, J. & Tisserye, B. 2015b. Farm Management Information Systems: Current Situation and Future Perspectives. *Computers and Electronics in Agriculture* **115**, 40–50.
- Hunt, Donnell. 1983. *Farm Power and Machinery Management*. Eight edition. Iowa State University Press Ames, Iowa, 352 pp.
- Matthews, G. A. & Thornhill, E. W. 1994. *Pesticide Application Equipment for Use in Agriculture. Mechanically powered equipment*. FAO Agricultural Services Bulletin 112/2, 145 pp.
- Matthews, G.A. 2000. *Pesticide Application Methods*. Third Edition. Blackwell Science, London, 426 pp.
- Pezzuolo, A., Basso, B., Marinello, F. & Sartori, L. 2014. Using SALUS model for medium and long term simulations of energy efficiency in different tillage systems. *Applied Mathematical Sciences* **8**, 129–132.
- Pimentel, D. 2002. *Encyclopedia of Pest Management*. Marcel Dekker, New York, 451 pp.
- Søgaard, H.T. & Sørensen, C.G. 2004. A Model for Optimal Selection of Machinery Sizes within the Farm Machinery System. *Biosystems Engineering* **89**, 13–28.
- Sopegno, A., Calvo, A., Berruto, R., Busato, P. & Bocthis, D. 2016. A Web Mobile Application for Agricultural Machinery Cost Analysis. *Computers and Electronics in Agriculture* **130**, 158–168.
- Zarco-Tejada, P.J., Hubbard, N. & Loudjani, P. 2014. *Precision Agriculture: An Opportunity for EU Farmers - Potential Support with the CAP 2014-2020*. Study. European Union, 52 pp.

## INSTRUCTIONS TO AUTHORS

Papers must be in English (British spelling). English will be revised by a proofreader, but authors are strongly urged to have their manuscripts reviewed linguistically prior to submission. Contributions should be sent electronically. Papers are considered by referees before acceptance. The manuscript should follow the instructions below.

**Structure:** Title, Authors (initials & surname; an asterisk indicates the corresponding author), Authors' affiliation with postal address (each on a separate line) and e-mail of the corresponding author, Abstract (up to 250 words), Key words (not repeating words in the title), Introduction, Materials and methods, Results and discussion, Conclusions, Acknowledgements (optional), References.

### Layout, page size and font

- Use preferably the latest version of **Microsoft Word**, doc., docx. format.
- Set page size to **B5 Envelope or ISO B5 (17.6 x 25 cm)**, all margins at 2 cm.
- Use single line spacing and justify the text. Do not use page numbering. Use indent 0.8 cm (do not use tab or spaces instead).
- Use font Times New Roman, point size for the title of article **14 (Bold)**, author's names 12, core text 11; Abstract, Key words, Acknowledgements, References, tables and figure captions 10.
- Use *italics* for Latin biological names, mathematical variables and statistical terms.
- Use single ('...') instead of double quotation marks ("...").

### Tables

- All tables must be referred to in the text (Table 1; Tables 1, 3; Tables 2–3).
- Use font Times New Roman, regular, 10 pt. Insert tables by Word's 'Insert' menu.
- Do not use vertical lines as dividers; only horizontal lines (1/2 pt) are allowed. Primary column and row headings should start with an initial capital.

### Figures

- All figures must be referred to in the text (Fig. 1; Fig. 1 A; Figs 1, 3; Figs 1–3). Use only black and white or greyscale for figures. Avoid 3D charts, background shading, gridlines and excessive symbols. Use font **Arial** within the figures. Make sure that thickness of the lines is greater than 0.3 pt.
- Do not put caption in the frame of the figure.
- The preferred graphic format is EPS; for half-tones please use TIFF. MS Office files are also acceptable. Please include these files in your submission.
- Check and double-check spelling in figures and graphs. Proof-readers may not be able to change mistakes in a different program.

### References

- **Within the text**

In case of two authors, use '&', if more than two authors, provide first author 'et al.':

Smith & Jones (1996); (Smith & Jones, 1996);  
Brown et al. (1997); (Brown et al., 1997)

When referring to more than one publication, arrange them by following keys: 1. year of publication (ascending), 2. alphabetical order for the same year of publication:  
(Smith & Jones, 1996; Brown et al., 1997; Adams, 1998; Smith, 1998)

- **For whole books**

Name(s) and initials of the author(s). Year of publication. *Title of the book (in italics)*. Publisher, place of publication, number of pages.

Shiyatov, S.G. 1986. *Dendrochronology of the upper timberline in the Urals*. Nauka, Moscow, 350 pp. (in Russian).

- **For articles in a journal**

Name(s) and initials of the author(s). Year of publication. Title of the article. *Abbreviated journal title (in italic)* volume (in bold), page numbers.

Titles of papers published in languages other than English, German, French, Italian, Spanish, and Portuguese should be replaced by an English translation, with an explanatory note at the end, e.g., (in Russian, English abstr.).

Karube, I. & Tamiyra, M.Y. 1987. Biosensors for environmental control. *Pure Appl. Chem.* **59**, 545–554.

Frey, R. 1958. Zur Kenntnis der Diptera brachycera p.p. der Kapverdischen Inseln. *Commentat.Biol.* **18**(4), 1–61.

Danielyan, S.G. & Nabaldiyan, K.M. 1971. The causal agents of meloids in bees. *Veterinariya* **8**, 64–65 (in Russian).

- **For articles in collections:**

Name(s) and initials of the author(s). Year of publication. Title of the article. Name(s) and initials of the editor(s) (preceded by In:) *Title of the collection (in italics)*, publisher, place of publication, page numbers.

Yurtsev, B.A., Tolmachev, A.I. & Rebristaya, O.V. 1978. The floristic delimitation and subdivisions of the Arctic. In: Yurtsev, B. A. (ed.) *The Arctic Floristic Region*. Nauka, Leningrad, pp. 9–104 (in Russian).

- **For conference proceedings:**

Name(s) and initials of the author(s). Year of publication. Name(s) and initials of the editor(s) (preceded by In:) *Proceedings name (in italics)*, publisher, place of publishing, page numbers.

Ritchie, M.E. & Olf, H. 1999. Herbivore diversity and plant dynamics: compensatory and additive effects. In: Olf, H., Brown, V.K. & Drent R.H. (eds) *Herbivores between plants and predators. Proc. Int. Conf. The 38<sup>th</sup> Symposium of the British Ecological Society*, Blackwell Science, Oxford, UK, pp. 175–204.

**Please note**

- Use ‘.’ (not ‘,’) for decimal point: 0.6 ± 0.2; Use ‘,’ for thousands – 1,230.4;
- Use ‘-’ (not ‘-’) and without space: pp. 27–36, 1998–2000, 4–6 min, 3–5 kg
- With spaces: 5 h, 5 kg, 5 m, 5°C, C : D = 0.6 ± 0.2;  $p < 0.001$
- Without space: 55°, 5% (not 55 °, 5 %)
- Use ‘kg ha<sup>-1</sup>’ (not ‘kg/ha’);
- Use degree sign ‘°’ : 5 °C (not 5 ° C).