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Change in the carbon footprint of Iranians' food consumption from 1961 to 2019: A decomposition analysis of drivers

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Abstract. The study investigates the role of three drivers: population, energy intake per capita, and dietary change on the carbon footprint of food consumption in Iran from 1961 to 2019. Iran was chosen for this analysis because the country has experienced a noteworthy population increase in the past century, and the imposed international sanctions have changed the economic welfare of the nation. Logarithmic Mean Division Index, along with data of FAOSTAT Food balance sheets and carbon footprint per item, were utilized to decompose the impacts of the drivers. The results demonstrated that the carbon footprint of food consumption in Iran increased by 1.6 during this period. We also found that population increase, and energy intake per capita were the main drivers of the carbon footprint of food consumption in Iran while diet change contributed negatively.

Key words: carbon footprint, food consumption, diet change, Iran.

INTRODUCTION

Production of foods consumed by humans in their daily lives generate considerable amounts of greenhouse gases (GHG) (Bruno et al., 2019; Lenerts, Popluga and Naglis-Liepa, 2019; Kovacs et al., 2021; Zhang et al., 2022). Estimates show that the food system is responsible for up to 30% of global GHG emissions (Aleksandrowicz et al., 2016; Esteve-Llorens et al., 2019; Crippa et al., 2021; Lignicka et al., 2022). The food production emissions are expressed in most studies by an environmental indicator called carbon footprint (CFP). CFP captures the GHG emissions during all stages of production (Cao et al., 2020; Crippa et al., 2021). Hence there is an urgent need to reduce the CFP of food consumption (Zhang et al., 2022).

The national CFP associated with food consumption depends on population numbers, energy intake per capita, change of diet, and CFP per unit of food items (Gerbens-Leenes & Nonhebel, 2005; Kastner et al., 2012; Cao et al., 2020; Lignicka et al., 2022). These factors change over time and show spatial variation. For example, studies show that with economic development, growth rate of population decreases. Besides that, diet undergoes changes such as higher energy intake and consumption of more affluent foods for instance animal-based products, vegetable oils, and fruits. Therefore cereals and starchy roots become less important in food baskets (Garvey et al., 2021; Paris et al., 2022). Determining how these drivers place a burden on the environment is crucial to provide policymakers in designing food supply chains and people's diets to accomplish a sustainable food system (Cao et al., 2020).

Scientific literature is rich in studies that have investigated the environmental impacts of food consumption for a period in the past. For example, Cao et al. (2020) estimated the CFP of Chinese diet during 1961 to 2019 and indicated that it has been growing. Land required for food in the Philippines from 1910 to 2003 was evaluated by Kastner & Nonhebel, (2010). In a similar approach de Ruiter et al. (2017) estimated land footprint associated with the food supply in the United Kingdom during 1986–2011. There are many studies that have estimated environmental impacts of food consumption at global level as well. Crippa et al.(2021) estimated the global GHG emission from food systems for the years 1990–2015. In another study, researchers evaluated the global CFP of diet for the year 2018 and proposed dietary shifts to mitigate the climate change crisis (Kim et al., 2020).

The goal of the research was to analyze the change in carbon footprint (CFP) associated with food consumption in Iran from 1961 to 2019, and to decompose this change into three drivers: population, energy intake per capita, and diet pattern (Kastner & Nonhebel, 2010; Kastner et al., 2012; Cao et al., 2020) using the Logarithmic Mean Division Index (LMDI) method.

Iran was chosen as a case study because the country has undergone significant demographic and economic changes. Firstly, Iran experienced a noteworthy population increase in the past century, especially after the revolution of 1979 and it almost increased by two folds in the first two decades of 20th century (Madani, 2021). Secondly, Iran was targeted by international economic sanctions over the past four decades that changed the economic welfare of the nation (Madani, 2020, 2021). These characteristics could cause shift in food consumption of nation therefore make Iran a magnificent case to investigate the long-term change in the CFP of food consumption.

The research aims to provide insights into the historical transition in dietary CFP and the contribution of driving factors. This information will be valuable for experts in designing less impactful diets and making informed decisions in the future.

MATERIALS AND METHODOLOGY

Economic welfare of Iranians

The economic welfare of the nation has been fluctuating since 1961 (*World Bank Group*): increases in gross domestic product (GDP) per capita were achieved until 2012, however, after 2012 GDP per capita started to decline sharply until 2019 (Fig. 1). It is believed that intensified international sanctions and the increase in population were the main reasons for the decrease in the level of income in Iran that led the country to a sophisticated condition that drove significant changes in the people's living habits (Fu et al., 2020; Madani, 2020, 2021). Diet (energy intake per capita and diet pattern) is one of the habits that get affected by change in economic prosperity of people (Eini-Zinab, Sobhani et al., 2021; Garvey et al., 2021; Paris et al., 2022).

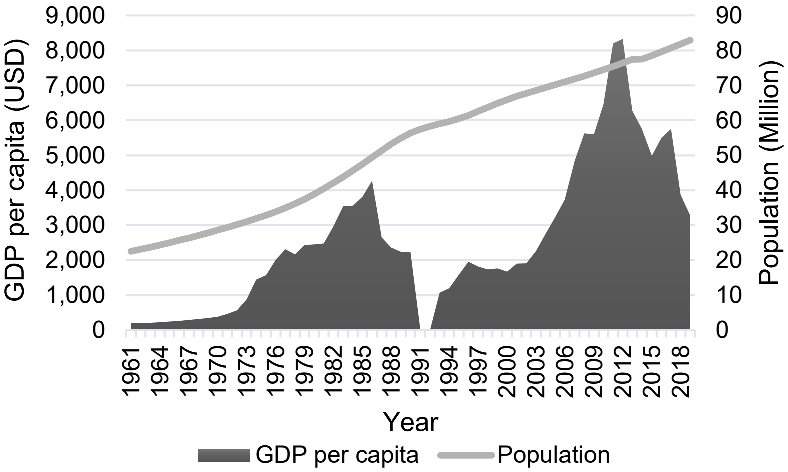

Figure 1. GDP per capita and population of Iran between 1961–2019 (based on the data from World Bank; GDP for years 1991 and 1992 were missing from database).

Food consumption data

Data on Iran's food consumption of period 1961–2019 was supplied by the Food Balance Sheets (FBSs) published by FAOSTAT (Food and Agricultural Organization of the United Nations, 2022). The FBS is claimed as one of the most detailed datasets currently available for retrieving the food supply patterns at the country level (Kastner et al., 2012; Bruno et al., 2019; Cao et al., 2020; Kim et al., 2020). The FBSs provide data on per capita food supply in a nation after accounting for import, export, losses, animal feed, and other non-food uses and the data are widely used to assess the average nutritional situation of a country. The food supply reported in the FBSs was assumed to equal consumption, not accounting for the losses at the household level. Data on the consumption of about 80 terrestrial food items were available on FBSs (Food and Agricultural Organization of the United Nations, 2022) and we categorized them into 9 categories, namely: 1 – Cereals, roots, and tubers; 2 – Pulses seeds, and nuts; 3 – Vegetables and fruits; 4 – Oils and sugar; 5 – Red meat; 6 – White meat; 7 – Eggs; 8 – Dairy products; 9 – Spices and stimulants (The food items under each category are provided in Appendix 1).

Carbon footprint of food consumption and decomposition analysis

LMDI is a method that enables us to decompose CFP of food consumption into its driving determinants to illustrate the contribution of drivers separately (Kastner et al., 2012; Tu et al., 2019; Cao et al., 2020). The CFP of food consumption was estimated based on four driving determinants using equation 1:

$$F = \sum(P \times E \times D_i \times I_i) \tag{1}$$

where F is the total CFP of food consumption at the national level (kg CO_2 equivalent per year, kg CO_2-eq.yr^{-1}), P is population number, E is energy intake per capita (kilocalories per capita per day, kcal.cap^{-1}.day^{-1}), D_i is proportion of food item i to total calorie intake and I_i is CFP of food item i. The list of CFP of food items is provided in Appendix A. The CFPs of food items were derived from the literature (Bruno et al., 2019; Cao et al., 2020) in units of (kg CO_2-eq/kg) and were converted to (kg CO_2-eq/kcal) using mass-to-calories conversion factors. The latter were obtained by dividing energy-based consumption (kcal.cap^{-1}.d^{-1}) to mass based consumption (kg.cap^{-1}.d^{-1}) values, which were provided in the FBSs. As the CFP of food items (I_i) were assumed to be constant over the whole period, three driving determinants (P, E, D_i) showed changes over time. This implied that CFP have no contribution to the change in total CFP of food consumption (Cao et al., 2020). The change in the CFP ($F^{'}$- F) was decomposed into three driving determinants expressed by equations 2–5 (Kastner et al., 2012; Cao et al., 2020):

$$F' - F = \Delta F = g(\Delta P) + g(\Delta E) + g(\Delta D) \tag{2}$$

$$g(\Delta P) = \sum \frac{F_i' - F_i}{\ln(F_i') - \ln(F_i)} \ln(\frac{P'}{P}) \tag{3}$$

$$g(\Delta E) = \sum \frac{F_i' - F_i}{\ln(F_i') - \ln(F_i)} \ln(\frac{E'}{E}) \tag{4}$$

$$g(\Delta D) = \sum \frac{F_i' - F_i}{\ln(F_i') - \ln(F_i)} \ln(\frac{D'}{D}) \tag{5}$$

RESULTS AND DISCUSSION

The food consumption in Iran from 1961 to 2019

Calorie intake per capita per day of Iranian residents increased from 1,724^{-}kcal.cap^{-1}.day^{-1} in 1961 to 2,904 kcal.cap^{-1}.day^{-1} in 2019 (Fig. 2). It is worth mentioning that per capita calorie intake peaked to 3,063 kcal.cap^{-1}.day^{-1} in 1993 and after that decreased marginally. Dietary pattern also underwent the changes during this period (Fig. 3). In 1961, Iranian residents received about 62% of their calories from starchy foods such as cereals and tubers, while in 2019 the contribution of these food items to daily calorific intake decreased to 55%. There was a decrease in the consumption of red meat (i.e., beef): from 81 kcal.cap^{-1}.day^{-1} in 1961 to 65 kcal.cap$_{-}^{1}$.day^{-1} in 2019. The contribution of red meat to total calorie intake had a similar trend and decreased from 4.7% to 2.2%. It is widely indicated that the consumption of red meat has a direct correlation with the purchasing power of people (Gerbens-Leenes & Nonhebel, 2005; Cao et al., 2020; Garvey et al., 2021). Hence, the collapse in the economical welfare of nation after 2010 due to the international sanctions could be the reason for less consumption of red meat. Moreover, it is expected that more sanctions would intensify such changes in food consumption generally (Madani, 2020, 2021) given the fact that food has a relatively large share in Iranian household budget (almost 24%) (Sobhani et al., 2021). There had been an appropriate increase in the consumption of white meat (i.e., poultry meat): namely, by 2019 the per capita calorie intake from white meat increased by 16 times and its contribution to total daily intake grown by 10 times. Although the calorie intake from dairy products increased from 116 kcal.cap^{-1}.day^{-1} in 1961 to 125 kcal.cap^{-1}.day^{-1} in 2019, the share of this category in total calorie intake dropped: from 6.7% to 4.3%, respectively. The calorie intake per capita from categories of 'vegetables & fruits', 'oil & sugar' and 'pulses, seed & nuts' experienced a light increase by 2 folds. Overall, the contribution of animal products in the total per capita calorie intake went down from 12.4% in 1961 to 11.6% in 2019 and noticeably cereals, roots and tubers remained as an important source of calories for Iranians and its proportion of daily calories intake still ranked first. Total energy intake by nation (labelled as Tera-calories per day, Tcal day^{-1}; 1 Tcal = 10^{12} cal) is also depicted on Fig. 2 (continuous black line) which incorporates the role of population increase in the consumption of food.

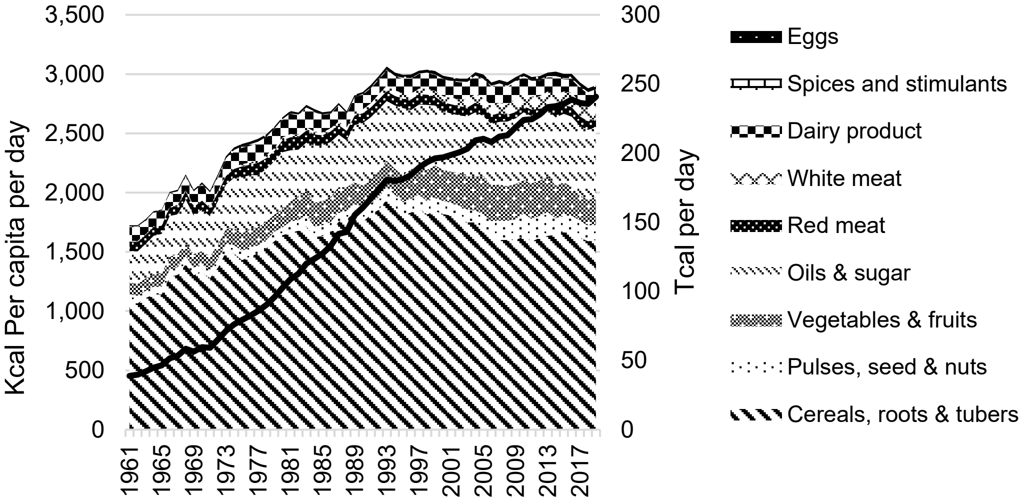

Figure 2. Energy intake per capita per day (stacked area, left axis) and total energy intake per day by nation (continuous black line, right axis) in Iran from 1961 to 2019.

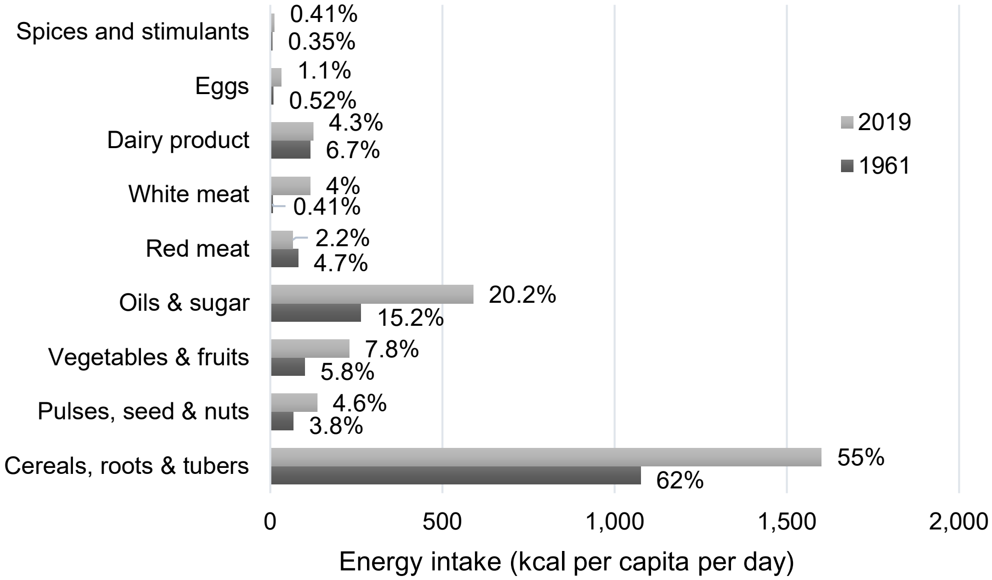

Figure 3. Absolute energy intake from food groups and their contribution in energy intake per capita in 1961 and 2019, %.

The CFP of food consumption in Iran from 1961 to 2019

The trend of the CFP of food consumption increased from 1.95 million tones (Mt) of CO_2-eq in 1961 to 2.83 Mt CO_2-eq in 2019 (Fig. 4). In 1961 the consumption of red meat had the highest contribution to CFP of food consumption (40% of the total) with 0.77 Mt CO_2-eq and category of dairy products ranked the second (24%) with 0.47 Mt CO_2-eq. In the end of period, due to the decrease in the consumption of red meat, category of dairy products became the most significant contributor to the CFP although the share of that remained almost constant (22%). The reason for this transition was that due to the decline in purchasing power, households switched from more expensive food products (e.g. red meat) to cheaper ones (e.g. dairy)(Garvey et al., 2021; Paris et al., 2022). However, from sustainability point of view less consumption of red meat is a favorable transition because in general animal products are well-known for their environmental impacts. For instance, the CFP of meats ranges from 2.43 kg CO_2eq kg^{-1} of poultry to 14.3 kg CO_2eq kg^{-1} of beef while that of cereals ranges from 0.54 kg CO_2eq kg^{-1} for barley to 1.66 kg CO_2eq kg^{-1} for rice (Treu et al., 2017; Bruno et al., 2019). Thus, although cereals were the main sources of energy intake, they were no longer the main contributors to the CFP. Emissions from the consumption of white meat increased from 0.01 to 0.24 Mt CO_2-eq over 1961–2019, whereas its contribution raised from 1% in the beginning to 9% at the end of period respectively. The CFP of vegetables and fruits went up from 0.26 in 1961 to 0.52 Mt CO_2-eq in 2019 and similarly the CFP of oils and sugar increased from 0.04 to 0.20 Mt CO_2-eq between 1961 and 2019. Although the CFP of consumption of 'cereals, roots, and tubers' and 'pulses, seed and nuts' increased during this period but their contribution to total CFP of food consumption only changed marginally.

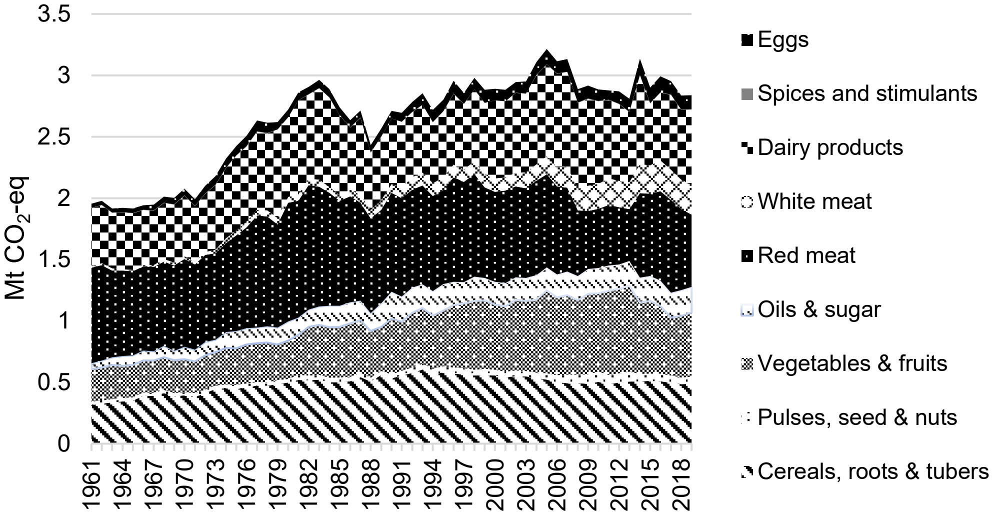

Figure 4. Carbon footprint of Iran's food consumption from 1961 to 2019 by food categories, Mt CO_2eq.

The contribution of three drivers of CFP

The contribution of three drivers (Population, Energy intake, Dietary change) to carbon footprint of food consumption in Iran during the period of 1961–2019 is illustrated in Fig. 5. The increase in population and energy intake per capita contributed positively to the CFP while the transition of the dietary pattern decelerated this trend and contributed negatively over the entire period, mainly because of the reduction in consumption of red meat. In the beginning of the period (1961–1967), the population and energy intake had almost equal contribution to the CFP. After the year 1973, the role of population became more significant, and the trend was increasing until the end of the period. The increasing rate of population growth was the main driver of CFP of food consumption during the entire period. Iran's population passed 35 million by the time of Islamic revolution in 1979, after which the population increased dramatically to about 86 million (annual growth rate = 4%) (Madani, 2021) due to socioeconomic, cultural and ideological changes among society. Iran's population is projected to reach 94–112 million by 2050 (Sobhani et al., 2022). Hence, feeding this population would impose more burden on limited resources of the country and would intensify the life cycle GHG emission by agriculture and food system.

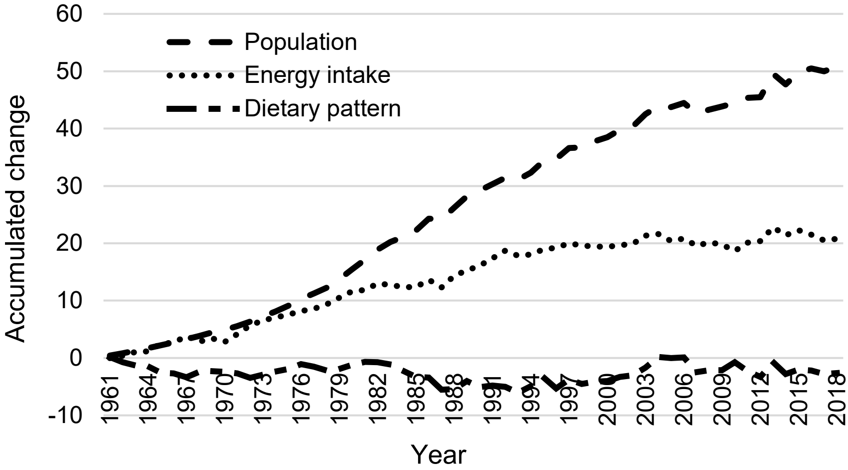

Figure 5. Drivers of carbon footprint of food consumption in Iran for the period 1961-2019 using LDMI decomposition method.

The role of energy intake had been increasing from the beginning of the period until the year 1997 and stayed almost unchanged until the end of period. Negative contribution of dietary pattern means that it has regulated the trend of the CFP by consuming more environmentally friendly food items such as cereals, vegetables, fruits, and white meat and decrease in the consumption of red meat mainly due to the collapse in purchasing power of households (decrease in GDP per capita)

In Iran, shifting towards more sustainable diets is inevitable, given the fact that there might be a continuous growth of population in the next decades and emergence of severe environmental problems such as water shortage, GHG emission and warmer climate (Madani, 2021; Sobhani et al., 2021). However, our decomposition analysis shows that there was a smooth change in the diet since 1961, and studies show that there is still scope for shifting the diet towards more sustainable eating habits.

CONCLUSION

This paper investigates the change in the CFP of food consumption in Iran in the period 1961 to 2019. The results of this study imply the role of population, energy intake and dietary pattern in the CFP of food consumption. The findings revealed that the growth in the population had the biggest contribution in the CFP of food consumption while a slight shift in the diet regulated the growing CFP of the food consumption in Iran. This happened mainly due to the decrease in the consumption of red meat which is believed to be the results of the growing price of the animal products and decrease in purchasing power of households. As the Iran's population is projected to grow in future, the demand for more food products will grow accordingly and GHG emission will be intensified consequently. This statement implies that shift in diet towards consumption of more environmentally friendly food product is a necessity in Iran and needs to be promoted by governmental bodies and requires policy and action.

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Appendix 1: Carbon footprints used in this analysis

Food groups	Food items	Carbon footprint per energy unit (kg CO_2eq.kcal^{-1})
Cereals, roots & tubers	Wheat and products	2.59E-4
	Rice and products	4.61E-4
	Barley and products	2.22E-4
	Maize and products	2.43E-4
	Rye and products	2.82E-4
	Oats	3.62E-4
	Cereals, Other	3.72E-4
	Potatoes and products	4.49E-4
Pulses, seeds & nuts	Honey	3.24E-4
	Beans	3.77E-4
	Peas	9.88E-5
	Pulses, Other and products	4.38E-4
	Nuts and products	3.83E-4
	Groundnuts	2.12E-4
Oils & sugar	Soyabean Oil	6.85E-4
	Groundnut Oil	3.24E-4
	Sunflower seed Oil	3.61E-4
	Rape and Mustard Oil	3.34E-4
	Cottonseed Oil	1.62E-4
	Palm kernel Oil	3.66E-4
	Coconut Oil	3.5E-4
	Sesame seed Oil	3.66E-4
	Olive Oil	4.31E-4
	Maize Germ Oil	3.25E-4
	Oil crops Oil, Other	4.06E-4
	Sugar (Raw Equivalent)	1.63E-4
	Sweeteners, Other	1.73E-05
Vegetables & fruits	Tomatoes and products	2.36E-3
	Coconuts - Incl Copra	3.01E-4
	Olives (including preserved)	7.65E-4
	Onions	1.46E-3
	Vegetables, other	3.1E-3
	Oranges, Mandarins	8.89E-4
	Lemons, Limes, and products	1.31E-3
	Grapefruit and products	1.08E-3
	Citrus, Other	1.04E-3
	Bananas	6.69E-4
	Apples and products	9.78E-4
	Pineapples and products	5.64E-4
	Dates	3.09E-3
	Grapes and products (excl wine)	7.05E-4
	Fruits, other	1.13E-3
Spices and stimulants	Coffee and products	8.6E-3
	Cocoa Beans and products	5.03E-05
	Tea (including mate)	6.99E-4
	Pepper	1.58E-4
	Pimento	1.64E-4
	Spices, Other	1.4E-4
	Wine	1.78E-3
	Beer	2.45E-4
	Beverages, Fermented	2.48E-3
	Beverages, Alcoholic	4.25E-4
Red meat	Bovine Meat	9.16E-3
	Mutton & Goat Meat	0.010215
	Pig meat	4.51E-3
	Meat, Other	5.75E-3
	Offal, Edible	8.62E-3

Dairy products	Butter, Ghee	7.30E-3
	Cream	7.11E-3
	Milk - Excluding Butter	2.89E-3
Eggs	Eggs	3.61E-3
White meat	Poultry Meat	1.58E-3
	Freshwater Fish	5.83E-3
	Demersal Fish	4.16E-3
	Pelagic Fish	1.9E-3
	Marine Fish, Other	5.83E-3
	Crustaceans	2.25E-2
	Cephalopods	4.22E-3
	Molluscs, Other	4.22E-3
	Aquatic Animals, Others	4.22E-3

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The role of energy management in the agricultural sector: key prerequisites and impacts

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Abstract. Agriculture is one of the most energy-consuming sectors in the EU's economy. Implementing sustainable agriculture to reduce GHG emissions and increase energy efficiency through energy management is a crucial strategy to tackle climate change. In this paper, the role of energy management in the agricultural sector is studied, and experiences from Europe and the world have been considered. Literature analysis regarding the chosen topic has been conducted, including the methodology of energy management plan development and its implementation in the case study of Latvia. Data from Latvia's agricultural and other sectors have been analysed and compared. Latvia's Inventory Report regarding GHG emissions in the agricultural sector was reviewed, and all emission sources in the agricultural sector were highlighted. The primary purpose of the study is to find out if energy management were introduced in an agricultural company, what would be the potential GHG emission, energy savings and additional advantages. Two companies working in Latvia were surveyed, and potential emission and energy consumption reduction measures in agriculture that would be applied to companies were developed. The research showed that by implementing the basic principles of energy management, it would be possible to reduce the average energy consumption by 17%. If measures are applied to reduce GHG emissions from agricultural companies, the average emissions would be reduced by 43%.

Key words: agriculture, benchmarking, indicators, energy efficiency, GHG emissions, sustainability.

INTRODUCTION

Energy production and consumption is the primary source of greenhouse gas (GHG) production not only in Latvia but also in Europe (Agency, n.d.), (Intergovernmental Panel on Climate Change (IPCC), n.d.). In 2020, the energy sector was the largest source of GHG emissions, generating 64.8% of total GHG emissions in Latvia, including indirect carbon dioxide (CO_2) emissions. Part of these emissions was created by the agricultural sector (Center of Environment, 2022). In addition to energy emissions, the agricultural sector generated 21.5% of total emissions in Latvia in 2020, including indirect CO_2 emissions (Center of Environment, 2022).

Energy consumption within the agriculture sector and its greenhouse gas emissions are essential topics to policymakers, as agricultural activities must meet food safety objectives and ensure proper economic, environmental, and social impacts (Streimikis et al., 2022).

The issues of energy management and the amount of produced emissions are also topical since the European Union (EU) has set the goal to reduce GHG emissions, including in the agricultural sector. Energy management and agriculture can be linked together since the agricultural sector uses energy and generates GHG emissions, which can be reduced by implementing resource management measures. Within the framework of the EU's Climate and Energy policy, the member states of the EU must achieve a reduction of greenhouse gases of at least 55% by 2030 (including agriculture, land use, and forestry). Additionally, the member states must achieve at least 27% in the share of renewable energy compared to 1990 ('The 2030 climate and energy framework - Consilium,' n.d.).

To reduce impact on the environment and economics, wise and practical resource management is necessary at all supply chain stages, as well as proper measures of impact reduction are advisable.

As surveys show, with an increase in manufacturing intensity, the amount of produced GHG emissions increases simultaneously (Bais-Moleman et al., 2019). GHG emissions will only increase as production increases if the company's management is not effective and sustainable, for instance, when in a livestock farm, no management system controls cattle, their feed, and manure, as well as energy and fuel consumption. Efficient livestock farms must have a resource management system designed and planned to reduce greenhouse gas emissions (Fiore et al., 2018). Thereby the agricultural sector should introduce low-emission practices and effective methods, for example:

Agricultural practices, which would preserve lands' fertility, increase organic matter content and release atmospheric carbon;

Better animal health and welfare management would reduce the cattle's infertility and increase their comfort level and health condition, which would also increase productivity (Fiore et al., 2018; Batlle-Bayer et al., 2019);

As agricultural product manufacturing and land-use change in land cultivation would significantly increase the amount of greenhouse gas emissions (Yan et al., 2017; Rose et al., 2019), shifting towards sustainable agriculture by introducing integrated farm management (Shen et al., 2022);

Reducing GHG emissions through the use of urease inhibitors (Adu-Poku et al., 2022);

Implementing common agricultural policy (Bradfield et al., 2022).

Carbon dioxide (CO_2) is claimed to be the most critical GHG emission in the energy sector and CH_4 and N_2O (Priedniece, Kirsanovs, Freimanis, Veidenbergs, & Blumberga, n.d.). Li et al. (2016) examined and analyzed the main drivers of energy-related CO_2 emissions in various European agricultural sectors. Two main directions have been studied in the mentioned research: 1) Index Division Analyse (IDA) that has been supplemented with Shapley Index and is used to identify significant CO_2 emission drivers; 2) Slack-based model (SBM) was applied to rate environmental performance of European agricultural sectors. Applying these technologies makes achieving environmental efficiency and shadow price measures possible, encouraging discussions regarding CO_2 emission reduction activities in the agricultural sector. Because of the importance of GHG emissions, an integrated approach to CO_2 analysis is developed based on advanced decomposition and efficiency analysis models. The research covers eighteen European countries, and the applied methodology divides installments into CO_2 emissions in regions and factors (Li et al., 2016). The results of IDA showed that the reduction of energy intensity is the leading factor in reducing CO_2 emissions. The lowest carbon shadow prices were observed in France, Finland, Sweden, Denmark, the Netherlands, Poland, and Belgium, thereby having the highest CO_2 emission reduction potential. Also, measures directed at increasing energy efficiency are the most profitable way to reduce the amount of CO_2 (Li et al., 2016).

To reduce GHG and NH_3 emissions, optimizing the new livestock spatial management system and using it as a basis for future policy success is necessary. Instructions for the policy and farmers should concentrate on properly managing manure and livestock feed and optimizing industrial production systems and pig and poultry sectors in suburban areas (Aan den Toorn et al., 2021; Jahangir et al., 2022; He et al., 2023). The United Kingdom has developed a national strategy that states that by 2030 greenhouse gas emissions need to be decreased by 50% compared to 1990 (Rose et al., 2019). It was evaluated that technological improvements in the agricultural sector are required to achieve this goal by reducing livestock farming production intensity by 30% (Rose et al., 2019).

Sufficient animal feed and manure management can reduce methane and nitrogen oxide emissions in the agricultural sector (Escribano et al., 2022; Hossain et al., 2023). All agricultural segments have management possibilities to reduce the negative environmental impact (Bumbiere et al., 2022). Lovendahl et al. wrote that GHG emission reduction is possible if different types of cattle are chosen for cultivation - the type whose genetics have been modified and improved, making the nutrient digestion process faster and who, during their metabolic processes, produce less methane (CH_4) (Lovendahll et al., 2018).

Agriculture is Latvia's second most significant source of GHG emissions ('Ministry of Agriculture of the Republic of Latvia LATVIAN AGRICULTURE 2020,' n.d.). The agricultural sector emitted 21.5% of Latvia's total greenhouse gases in 2020 ('Ministry of Agriculture of the Republic of Latvia LATVIAN AGRICULTURE 2020,' n.d.). Latvia has developed a national-level strategy to increase energy efficiency and decrease GHG emissions ('National Energy and Climate Plan for 2021–2030 | Ekonomikas ministrija,' n.d.). In Latvia, 9.1% of all agricultural lands are biologically or organically cultivated, and the product market is still growing. It is one of the good examples of effective land cultivation and low GHG emission levels. The Rodale Institute states that regenerative organic agriculture and its managing practice is a potentially important tool for distributing more than the current global annual emissions and for changing the greenhouse effect ('Regenerative Organic Agriculture and Climate Change A Down-to-Earth Solution to Global Warming,' n.d.). The current diversion of soils and pastures to regenerative organic farming is expected to lead to 111% of annual carbon emissions, leading to annual negative emissions ('Regenerative Organic Agriculture and Climate Change A Down-to-Earth Solution to Global Warming,' n.d.). Scientific research is devoted to traditional farming methods by introducing crop and many plant species rotation to preserve land fertility and natural growth conditions and supply residents with local food in an innovative area (Niu et al., 2019). There are many recommendations for controlling weeds and other pests, ensuring plant nutrients, and reducing energy consumption (Saldukaitė et al., 2022). Plant rotation, correctly and well-defined soil purity, respected ecosystems, and natural plant growth conditions are the main principles of successful plant cultivation in an organic agricultural system (Morugán-Coronado et al., 2022; Saldukaitė et al., 2022). Farm experience shows that suitable results may be achieved in the long term and strictly follow organic farming principles (Verburg et al., 2022).

This study is carried out to develop knowledge on achieving a higher reduction of GHG emissions by looking at two levels - sector and company. The study results in a decrease in GHG emissions, therefore helping to achieve EU targets to reduce GHG emissions in the agricultural sector. This research aims to measure the potential energy and emission savings from the implementation of energy management actions and to propose the framework for an energy management system in the agricultural sector on a company level. All segments of agricultural activity have management options that can reduce their environmental impact. Therefore, awareness of the basic principles of energy management in agricultural companies should be promoted, and informative measures on energy management and reduction of GHG potential should be implemented.

METHODOLOGY

The methodology was based on the IPCC guidelines, written in 2017–2018. The year 2005 was compared to 2015 to see the increase in emissions in the agricultural sector. In analysing the agricultural sector, the bottom-up approach for evaluating impacts can be helpful; for example, Adewale et al. (2019) used an agricultural carbon footprint to examine the impact of two farms. Blancard and Marti (Blancard & Martin, 2014) used Data Envelopment Analysis to analyze farm energy efficiency, and Hosseinzadeh-Bandbafha et al. (2017) to evaluate fattening farms. Alonso and Guzman (Alonso & Guzmán, 2010) used the energy balance method to analyze energy efficiency in producing energy crops. Meul et al. (2007) used process analysis methodology for the calculation of energy balance in farms.

Thus, the following methods, guidelines, and manuals will be used in this publication: IPCC Guidelines, Latvian Inventory Report on GHG Emissions, and manual 'Guide for Farmers to calculate GHG at farm level and measures to reduce it'. Analysis of indicators and comparison of agricultural enterprises will be carried out, and a methodology that can be applied at a certain level will be developed.

Two specific companies were chosen because they are relevant to the research's needs, and it is appropriate to compare them. One of these companies did not apply energy management principles, which increased annual emissions, while the other involved half of these principles, and the emissions were reduced. The study demonstrated that if the basic principles of energy management in agriculture are used, emissions will be reduced several times.

To achieve the goal of this research, an algorithm of methodology has been developed (Fig. 1). It is divided into eight stages, showing the advisable actions on each level – (1) evaluation of data on GHG emissions, (2) analysis of data on the national, (3) sectoral, or (4) company level, (5) analysis of the data on energy consumption, (6) comparison of the companies, (7) improvement measures are proposed, and (8) energy efficiency measures are defined. The algorithm's first part is oriented toward identifying and analyzing the current situation. Still, the second part is identifying future perspectives, searching for possibilities, and implementing practical solutions to promote development.

As it is seen on the scheme, the methodology includes eight modules, of which three are the main ones: state level (2), sectoral level (3), and company level (4). From stages 1 to 5, data collection and publicly available data are analyzed using data analysis methods. Data are compared in stages 6 to 8, and GHG emissions and energy reduction measures are proposed. These measures are also called energy efficiency measures.

Each year, every country in the European Union must submit an inventory report on GHG emissions developed by the IPCC guidelines related to the UN Framework Convention on Climate Change.

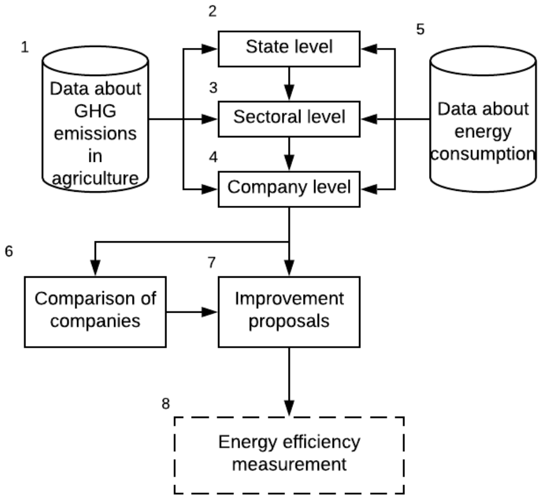

Figure 1. Scheme of the methodology.

The inventory report includes direct and indirect GHG emissions from all sectors in the country, which are expressed in CO_2 equivalent. In the report submitted in 2017, GHG emissions were calculated for the timeframe starting with 1990 until 2015, considering the global warming potential coefficients for a one-hundred-year period.

In the Convention reporting guidelines, GHG emissions were compiled for such areas or sectors as energetics, industry and product manufacture, agriculture, land cultivation, land-use change method and forestry, and waste management.

The following subsection compares GHG emissions in CO_2 equivalent for 2005 and 2015. In the case study, data were taken from Latvia's inventory report about GHG emissions in the agricultural sector.

As the Inventory report divides the agricultural sector into several areas, this division will be further explained. On the bottom of the energy sector stands the category 'Other', in which emissions from fuel (both - for heating and transport purposes) combustion are located. These emissions are produced in all sectors - agriculture, forestry, and fishery. Unfortunately, there were no data available regarding fuel consumption in the agricultural sector, and because of that, the total amount was used and analyzed.

In agriculture, forestry and fishery usually utilize:

Stationary combustion appliances – liquid, solid-type fuel, and biomass;

District transport and other mechanic systems – gasoline and diesel fuel;

Fishery – gas and diesel fuel.

The agricultural sector is analyzed as a separate sector, and emissions are calculated in the following categories:

Agricultural lands;

Intestinal fermentation;

Manure;

Land liming;

Urea utilisation ('National Inventory Submissions 2022 | UNFCCC,' n.d.).

In Fig. 2, the division of emissions in the agricultural sector, the type of produced emissions and in what area of the sector is explicitly shown.

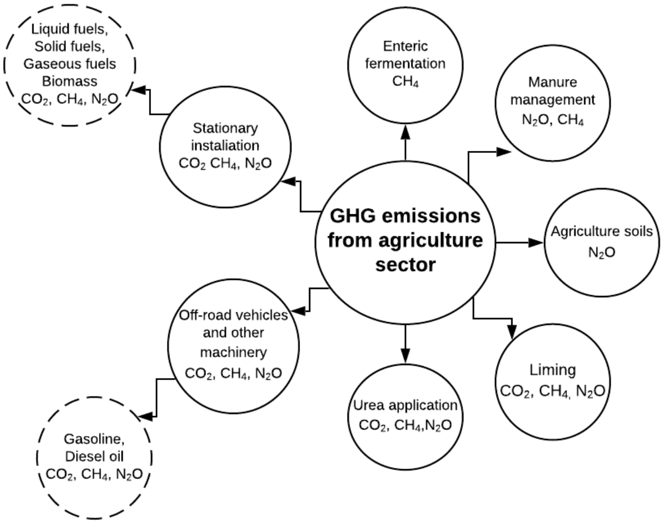

Figure 2. Breakdown of emissions from the agricultural sector.

This research aimed to measure the potential energy and emission savings from implementing energy management actions and propose a framework for the energy management system in the agricultural sector on a company level.

RESULTS AND DISCUSSION

A significant part of GHG emissions in Latvia comes from agricultural lands and cattle's intestinal fermentation, which is why, in this work, measures of GHG reduction are explicitly proposed in these areas. GHG reduction measures are described in the 'Guide for Farmers to calculate GHG at farm level and measures to reduce it.' This guidebook is based on the IPCC guidelines, and this advice can be implemented in the case of Latvia. Some of the measures are introduced in the surveyed companies.

As the literature survey shows, a significant amount of emissions comes from land cultivation. The division of produced GHG emissions in both areas is as follows:

Agricultural land:

Implementation of precise fertilization system - plan development and required technique purchase - perform soil analysis;

Use of practical techniques and technologies - combined field processing machines, zero or minimal tillage technique implementation;

Land reclamation or improvement;

Trenches around the cultivated land to avoid water pollution by fertilizers.

Intestinal fermentation:

Nutrient dosage management (plan developed and introduced);

Nutrient additive utilization to improve digestion;

Purchasing cattle that produce less methane (CH_4) in their metabolic processes.

It is worth noting that the emission division in the agricultural sector emissions does not include the emissions from transport utilization and maintenance. In the Latvian agricultural sector's emissions, fuel produces only 11% of the total GHG emissions (Center of Environment, 2022). This percentage would decrease if the proposed agricultural land and intestinal fermentation management measures were implemented.

In the case study, comparing two agricultural companies, where the main working areas are connected to livestock, has been performed and evaluated as to how much electricity each consumes and what GHG emissions are produced. Besides, for both these criteria – electricity and GHG emissions, individual reduction measures have been developed for each company.

Company 'A' acquires 1,120 ha of agricultural land, on which a biogas plant, cattle sheds, cow milking carousel machine, refrigerator premises, personnel rooms, offices, and warehouses are located. The company's 'B' inventory shows that this company owns an agricultural land area of 1,080 ha, a workshop for technical repairs, personnel premises, an office heated by using wood chips and firewood, a grain dryer, and cattle sheds.

After acquiring all the information regarding energy consumption and overall operation, several energy efficiency measures have been developed for each company. These measures include electricity and GHG emission reduction actions (Table 1).

Table 1. Inventory data

Company	'A'	'B'
Land area (ha)	1,120	1,080
Business directions	Livestock (milk), field crop production	Livestock breeding, field crop production
Livestock	948	740
Electricity consumption (GJ)	3,895.2	1,065.6
Produced GHG emissions (tCO_2eq)	3,282	2,525

The more data, the more precise and better improvements can be made. These data allow analysing which part of the company consumes more electricity and what measures could be introduced. Fig. 3 shows that, unfortunately, company 'A' has data only regarding energy consumption on the farm (cattle breeding) and the warehouse when company 'B' acquires information about all its compartments.

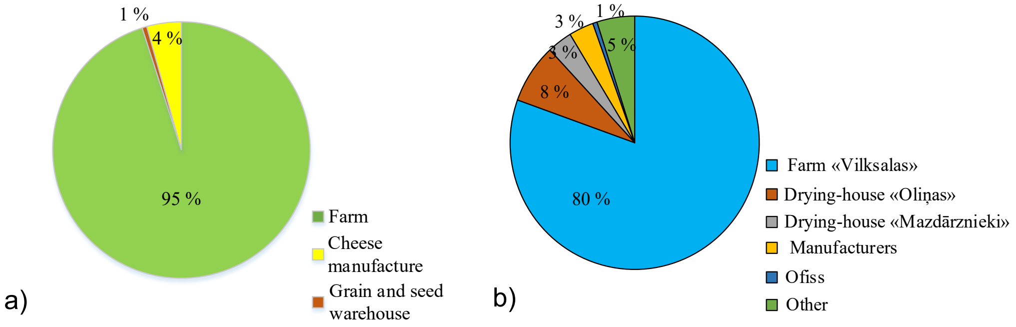

Figure 3. Share of electricity consumption by sectors in 2016: a) company 'A'; b) company 'B'.

Although the two situations are very different, depending on the information obtained, easy-to-implement proposals that do not require significant investments to increase energy efficiency and reduce GHG emissions were individually developed.

- For energy savings company ‘A’ was offered to start with such solutions as:
- Replacing inefficient lighting systems with new efficient ones;
- Use of fuel-efficient tires (if replaced by ten vehicles);
- Use of engine lubricants (if used in 10 vehicles);
- While company ‘B’ had such solutions as:
- Use of fuel-efficient tires (if replaced by ten vehicles)
- Pump replacement.

These recommendations resulted in 14% and 20% energy savings, respectively, where a suggestion for company ‘A’ is a transport use with a hybrid-type energy system, while for company ‘B’:

- Manure and agricultural residues transferred to bioenergy production facilities
- Use transport with a hybrid-type energy system
- Use of control systems for fuel economy.

If the agricultural companies implemented the GHG emission reduction measures, the emission level would decrease by about 43%. However, it is possible to conclude that there is not one specific recipe that all companies should follow because each, depending on the company’s level of development, operational specifics, and applied practices, needs to individually develop a plan for reducing emissions and increasing energy and resource efficiency to achieve maximum productivity at the lowest costs and emissions.

During the research, the indicators for farm comparison, which can be used as benchmarking, were identified and compiled in Table 3.

These indicators have been developed by analyzing the literature on this topic and summarizing other researchers’ assessments. Two indicators were retrieved from limited access to information on company consumption data and considering Table 3 - direct and indirect energy consumption per ton of crops and direct and indirect energy input per livestock. Table 5 gives a comparison of indicators in both companies.

Table 2. The Indicators for Farm Comparison

Indicator	Unit
Direct and indirect energy consumption	GJ ha^{-1}
Direct and indirect energy input per tonne of crops	GJ ha^{-1}
Direct and indirect energy input per tonne of product (livestock)	GJ ha^{-1}

Table 3. Comparison of Indicators in Companies

Company	GJ ha^{-1}	GJ/unit
‘A’	3.30	4.1
‘B’	0.98	1.4

These indicators allow us to compare different companies and analyze the benefits of energy efficiency measures and can be used in benchmarking similar size and profile farms.

Five company-level measures were identified by reviewing scientific articles and examining practices in this field of research. The most effective energy efficiency measures for the company level were determined:

Optimized fertilizer production;

Energy-saving cultivation practices;

Improved water management;

Better livestock feeding;

Use of renewable energy sources.

All found information was summarised and applied in companies, thus proving the efficiency of the developed measures. By introducing these measures, the emission level, the consumed energy and resources, also expenses can be reduced. During the research, an energy management system (Fig. 4) for the agricultural sector at the company level was developed, which can be adapted to evaluate and compare different agricultural companies.

The results have shown that using proposed indicators and benchmarking for farm comparisons is beneficial for improving the agricultural sector and reducing greenhouse gas emissions and energy consumption, leading to efficient, sustainable, and competitive farming.

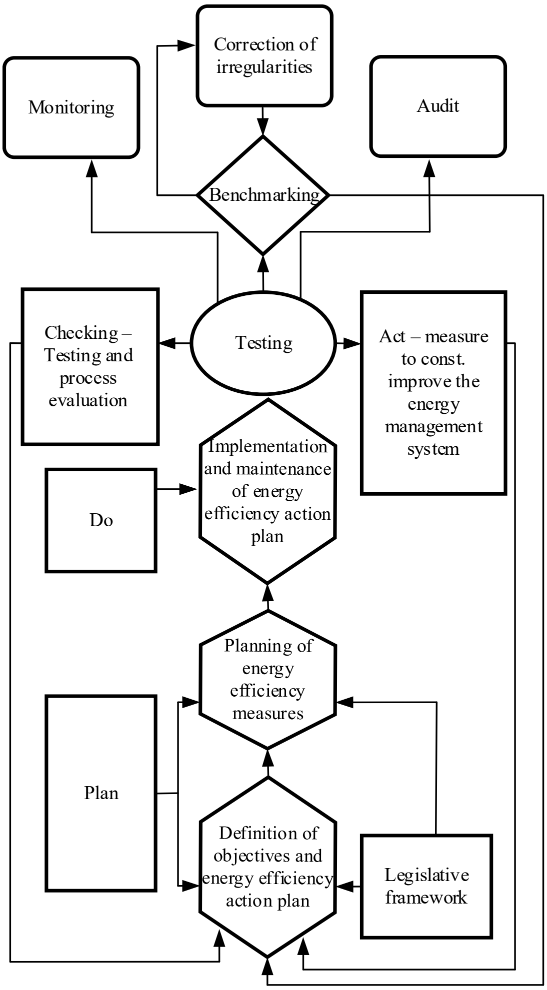

Figure 4. Energy management framework for the agricultural sector on the company level.

CONCLUSIONS

The energy management system can and should be implemented by agricultural companies. It would reduce energy consumption, optimise costs, and reduce GHG emissions. However, informative measures are required to implement these basic energy management principles in companies.

The surveyed companies should follow the initial monitoring of energy consumption data to understand where electricity and heat are consumed the most and the potential for reducing this amount. It would be advisable for agricultural companies to install an intelligent energy system. It is a sustainable energy supply system that contains information on energy consumption and options for reducing it based on monitoring the system's performance.

The energy management system can be combined with greenhouse gas reduction measures, such as organic farming and other methods and guidelines already introduced in Latvia. However, not all companies follow these guidelines. It is necessary to develop a specific policy and support program for companies to implement energy management, as implementing the basic principles of energy management or the energy system requires investment.

By implementing the energy system in an agricultural company, energy consumption in this company can be assessed, and measures can be taken to reduce energy consumption. Policy and agricultural guidelines should focus on optimizing farming and manure management.

Results show that energy efficiency improvement measures are a more effective way to reduce CO_2 emissions. If measures are applied to reduce GHG emissions from agricultural companies, the average emissions would be reduced by 43%. By implementing the basic principles of energy management, it would be possible to reduce the average energy consumption by 17%. However, it depends on the specifics of the company and what measures it can implement.

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Physiological and behavioural parameters of broiler chicks grown under different heating systems

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Abstract. This study aimed to compare the internal environment, physiological variables, and behavioural responses of chicks under two different heating systems. The experiment was performed in two commercial broiler houses located in Brazil, where 28,000 male Cobb chicks were housed during the first three weeks of life. One of the broiler houses was heated by an industrial indirect-fired biomass furnace (S1). In addition, another heating system consisting of two furnaces for indirect heating of the air using biomass (wood) as fuel, built by hand with bricks, mud, and dung on an iron structure (S2), was tested. Measurements of the dry bulb temperature (t_{db}), dew point temperature (t_{dp}), and relative air humidity (RH) were performed. Subsequently, the temperature-humidity index (THI) was calculated. In addition, the physiological variables of the respiratory rate (RR) and cloacal temperature ($t_{cloacal}$) were measured three times a day (8:00 am, 2:00 pm, and 6:00 pm) in four chicks. The behaviours were grouped by dendrograms, in which the similarity of these data was qualified. During the second and third weeks of life, the THI values were below the recommended range. The RR and $t_{cloacal}$ data were below the recommended comfort values, which may be an indicator that the chicks were subjected to cold conditions. Regarding their behaviour, the chicks exhibited calm, feeding, and sleepy behaviours most of the time. Problems in the heating system inside the broiler house could be observed, possibly affecting the chicks' thermal comfort and welfare, which consequently can result in productive and economic losses.

Key words: chicks, behaviour, cloacal temperature, respiratory rate, thermal comfort.

INTRODUCTION

The production of broiler chickens occupies a prominent position in the Brazilian economy, in addition to providing the consumer with an affordable source of protein. However, the predominant characteristics of the tropical and subtropical climate, as in Brazil, require specific care in relation to the thermal environment control in which the birds are inserted (Oliveira et al., 2018; Cândido et al., 2019; Coelho et al., 2019). The thermal environment conditions to which broilers are subjected are essential to ensure maximum comfort and animal welfare, and consequently, achieve maximum productivity (Vieira et al., 2016). For the Brazilian climatic conditions, there is a need for supplementary heating during the initial phase of the birds' life, as they are sensitive to the cold (Andrade et al., 2019).

In the early stages of chicken growth, birds have low tolerance to thermal stress, which can result in reduced productivity and reproductive performance. A chick is considered a poikilothermic animal, i.e., its body temperature varies according to the ambient temperature (Souza et al., 2015). This is because, in the initial stage of rearing, birds do not have a defined thermoregulatory system or sufficient energy reserves to adapt to adverse environmental conditions, so body temperature can vary depending on the ambient temperature (Ponciano et al., 2012; Andrade et al., 2017). When under cold stress, there is a decrease in growth performance and immune function in broiler chickens (Su et al., 2020). Thus, the first weeks of life of birds are the most critical, and errors made in this phase cannot be corrected, resulting in a decrease in the final performance (Cordeiro et al., 2010).

This highlights the importance of adapting the environment to the ideal welfare conditions for younger birds. However, the quantification of only the thermal environment to which an animal is subjected is not sufficient to meet the real welfare needs of the animal, and the creation environment directly influences its behavioural expression in physiological aspects and productive responses (Ponciano et al., 2011). The physiological responses that may be affected by heat stress include the cloacal temperature ($t_{cloacal}$) and respiratory rate (RR) (Ferreira et al., 2012; Andrade et al., 2018). In this way, great advances, investments and new forms of management have been continuously developed in the poultry sector.

Thus, the objective of the present study was to analyse two broiler houses with different heating systems based on environmental, physiological, and behavioural variables during the first three weeks of life of chicks.

MATERIALS AND METHODS

The experiment was conducted in two commercial broiler houses in the western mesoregion of Minas Gerais, Brazil, during the spring season. The two broiler houses are 13 m in width, 160 m in length, and 3 metres in ceiling height, with a 30° roof slope, 6-mm-thick asbestos cement roofing, a concrete floor, rice husk bedding, side curtains and yellow plastic canvas lining at 2.45 m height. In each broiler house, double curtains were used as side walls (one internal and one external). The internal curtains were removed on the fifth day of life of the chicks, and the external curtains were managed according to the climatic conditions throughout the experimental period.

The one broiler house had the S1 heating system, which consisted of two furnaces with indirect-fired biomass burning, built by hand with bricks, mud, and dung on an iron structure, located 80 m apart. Each furnace was 1.88 m long, 1.27 m wide, and 1.58 m high. The furnace was operated with a three-phase motor with 2,206 W of power at 1,725 RPM; this motor supplied heated air to the broiler house through a 0.10 m diameter tube. In the broiler house with the S2 heating system, the heating system consisted of an industrial metal furnace with indirect-fired biomass burning, with a length of 2.23 m, width of 1.23 m, and height of 1.85 m. The heated air was blown by a three-phase motor with 2,206 W of power at 1,725 RPM through approximately 28.6 m of galvanized steel tubing on the northeast side and 22.45 m on the southwest side installed in the central internal part of the broiler house. The tubing had a diameter of 0.23 m and holes 0.05 m in diameter separated by 1.0 m located alternately on each side to release the heated air.

A total of 28,000 male Cobb chicks aged 1 to 21 days were housed in each broiler house. The diets provided to the animals were formulated according to the nutritional requirements of the different growth stages of the birds, with the same formulations for both systems.

To characterize the thermal environment, daily measurements of the dry bulb temperature (t_{db}, °C), dew point temperature (t_{dp}, °C), and relative humidity (RH, %) were taken at a height of 10.0 cm from the litter, in intervals of five minutes, at 8:00 am, 2:00 pm, and 6:00 pm in the first three weeks of life. T_{db}, t_{dp} and RH were measured using sensors (Hobo Pro Series - Onset®), with precision of ± 3% of the reading.

The thermal environment inside the broiler houses was characterized by the temperature-humidity index (THI). The THI is considered one of the simplest indices and has stood out because it encompasses the effects of t_{dp} and RH. This index can be calculated by Equation 1, developed by Thom (1959).

$$\mathrm{THI} = t_{db} + 0.36 \times t_{dp} + 41.5 \tag{1}$$

where t_{db} = dry bulb temperature (°C); t_{dp} = dew point temperature (°C).

The RR and $t_{cloacal}$ of the chicks were evaluated three times a day (8:00 am, 2:00 pm, and 6:00 pm) in four randomly selected chicks, totalling 12 chick's broiler^{-1} day^{-1}. The $t_{cloacal}$ value was measured using a digital thermometer (precision of ± 0.2 °C). The RR was determined by direct visual observation for 15 s, and then this value was extrapolated to one minute.

The behaviour of the chicks was evaluated by observation with monitoring of the group of animals in continuous time intervals of 10 minutes for each broiler house. Evaluations were performed at 8:00 am, 2:00 pm, and 6:00 pm in the first three weeks of life. The analyses were performed daily for 21 days. The behavioural patterns were adapted from Sevegnani et al. (2005), and include the frequency of calm, scattered, prostrate, gasping, feeding, drinking water, and sleepy behaviours. These behavioural patterns were characterized by scoring as a function of time and number of chicks performing each behaviour, similar to the methodology described by Schiassi et al. (2015). For the statistical analysis of the scores assigned to the behavioural patterns, exploratory analyses using radar charts and agglomerative hierarchical clustering (AHC) were used.

Statistical design

Environmental and physiological variables

For the analysis of environmental and physiological variables, a 2×3×3 factorial design was used, composed of the following factors: broiler house (S1 and S2), week (1, 2, and 3), and period (8:00 am, 2:00 pm, and 6:00 pm), through the statistical software Sisvar 5.3 (Ferreira, 2010). The data were subjected to analysis of variance, and the means of significant interactions were compared using the Skott-Knott test, at the 5% significance level.

Behavioural variables

The behaviours of the chicks were analysed using the AHC methodology, which separates objects into groups based on the characteristics of these objects, through classification criteria, such that there is homogeneity within the group and heterogeneity between groups, allowing grouping of the treatments in which the chicks showed similar behaviours (Ferraz et al., 2014). The results of the AHC method were described using a dendrogram, which is a similarity diagram, quantified using the Ward method and Euclidean distance, as described by Lau et al. (2009). The cophenetic correlation coefficient (CCC) was used to evaluate the consistency of the clustering obtained with the AHC method, with values close to one indicating better representation.

To group the behaviour of the chicks according to their similarity, the data were subjected to multivariate cluster analysis (R Development Core Team, 2020).

RESULTS AND DISCUSSION

In the statistical analysis of the THI using the Scott Knott test, there were no differences between the two broiler houses evaluated (*P* = 0.4707) or the broiler houses x week (*P* = 0.7329). However, there were differences ($p < 0.05$) for the week x period interaction (Table 1).

Table 1. Mean THI (temperature-humidity index) values of both broiler houses for the three periods of the day studied (8:00 am, 2:00 pm, and 6:00 pm) throughout the first three weeks of life of the chicks

Week	Period	THI
1st	1 (8:00 am)	78.80 a
	2 (2:00 pm)	80.58 b
	3 (6:00 pm)	80.08 b
2nd	1 (8:00 am)	64.16 b
	2 (2:00 pm)	51.48 a
	3 (6:00 pm)	52.96 a
3rd	1 (8:00 am)	66.27 c
	2 (2:00 pm)	52.22 a
	3 (6:00 pm)	57.88 b

Means followed by different lowercase letters in the column differ by the Scott Knott test ($p < 0.05$).

Although there were no differences between the THI values of the two heating systems evaluated, there were differences between the three weeks of life of the chicks and the three periods analysed (Table 1). Week 1 had higher THI values than the other weeks studied for both broiler houses. According to Abreu & Abreu (2001), the THI values for broiler chickens should be 72.4 to 80.0, 68.4 to 76.0, and 64.8 to 72.0 in the first, second, and third weeks of life, respectively. Thus, in the first week, the THI values were within the comfort range, but in the second and third weeks, the values were below the values recommended by the literature, showing a possible failure in the heating system in these weeks. According to Schiassi et al. (2015) and Andrade et al. (2018), an inadequate thermal environment can modify the physiological response of chicks, resulting in lower productive performance.

Table 1 also shows a comparison between the THI values over the three periods of the days analysed (8:00 am, 2:00 pm, and 6:00 pm) throughout the three weeks of bird life. The data indicate variation in the environmental conditions that the chicks were subjected to throughout the days. According to Ferraz et al. (2020), it is expected that in a commercial production system, the environmental variables within the facility are homogeneous. These environmental variations within the broiler house may result in thermal conditions outside the recommended comfort range, which may cause unevenness in the lot, in addition to causing discomfort in the animals and productive and economic losses (Ferraz, et al., 2019).

Table 2 shows the values of the physiological variables, RR and $t_{cloacal}$, of the birds monitored during the experimental period. For these variables, there were no differences between the heating systems evaluated ($p > 0.05$). However, there were differences in all physiological variables between the weeks evaluated.

Table 2. Mean values of the physiological variables RR and $t_{cloacal}$ in the first, second, and third weeks of life in the both broiler houses

Week	RR (breaths min-^{1})	$t_{cloacal}$ (°C)
1	56.1 a	40.0 a
2	68.5 b	41.0 b
3	67.7 b	41.4 c

Means followed by different letters differ by the Scott Knott test ($p < 0.05$).

Table 2 shows that the mean values of the physiological variables RR and $t_{cloacal}$ were higher in the second (68.5 breaths min^{-1} and 41.0 °C, respectively) and third (67.7 breaths min^{-1} and 41.0 °C, respectively) weeks of life of the chicks. As shown in Table 1, in the second and third weeks of life of the chicks, the internal environment of the broiler houses showed THI conditions below the comfort range. According to Cordeiro et al. (2014), chicks in thermal comfort exhibit an RR of approximately 47 breaths. min^{-1}. For the three weeks evaluated in the present study, the RR was above the recommended level. These high RR values may be an indication that the birds were in thermal discomfort during the evaluated period, which generated changes in RR.

The $t_{cloacal}$ values obtained during the first three weeks of the birds' lives showed a significant difference ($p < 0.05$) (Table 2). The values obtained for $t_{cloacal}$ (40.0 °C in the first week, 41.0 °C in the second week and 41.4 °C in the third week), are below the value of 42 °C considered as comfort for the first three weeks of life for broilers, as indicated by Oliveira et al. (2006), being indicative of cold stress.

Table 3. Mean values and standard deviation from the multiple comparison test of the RR and $t_{cloacal}$ variables for the period factor, values obtained from the averages of both broiler houses

Period	RR (breaths min^{-1})	$t_{cloacal}$ (°C)
8:00 am	62.3 a	40.7 a
2:00 pm	65.4 c	40.9 b
6:00 pm	64.0 b	40.8 b

Means with different letters (for the column) indicate significant differences ($p < 0.05$), by the Scott Knott test.

According to Table 3, for the three evaluated periods (8:00 am, 2:00 pm, and 6:00 pm), the physiological variable RR of the evaluated periods differed significantly ($p < 0.05$). The lowest RR values were observed at 8:00 am, with values of 62.3 breaths min^{-1}, and the highest were observed at 2:00 pm, with a mean of 65.4 breaths min^{-1}, indicating a situation of discomfort for the animals.

Regarding $t_{cloacal}$, there were differences ($p < 0.05$) between the periods studied. In the morning, the chicks had lower $t_{cloacal}$ values than in the afternoon and evening. However, in all periods, the $t_{cloacal}$ remained below the comfort range (42 °C) (Oliveira et al., 2006).

Fig. 1 shows the exploratory analysis, in a radar chart, of the frequency of occurrence of each behaviour evaluated in each of the weeks. The behavioural evaluation serves as an indicator of the comfort or discomfort of production animals. This factor should be assessed in the early days to prevent negative effects on animal welfare and productivity. In this sense, the behaviours of the animals are monitored to find ways to improve their welfare (Garcia et al., 2015), providing a new perspective for the conventional animal production model and generating alternatives to conditions not previously considered or poorly understood in animal welfare (Pinheiro et al., 2015). This is a non-invasive procedure in which the animals themselves are the biosensors that respond to environmental variations.

As shown in Fig. 1, throughout the 3 weeks of life, the chicks showed behaviour indicative of comfort even though the THI values were below the thermal comfort range. It can be observed that the chicks spent most of the time exhibiting calm behaviour (22.6, 20.5, and 21.8% of the time for the first, second, and third week, respectively), sleepy behaviour (20.3, 18.6, and 14.3% of the time for the first, second, and third weeks, respectively), and feeding behaviour (16.0, 17.6, and 19.0% of the time for the first, second, and third weeks, respectively). Gasping behaviour was the least observed throughout the observed period (4.2, 5.5, and 4.6% of the time for the first, second, and third weeks, respectively).

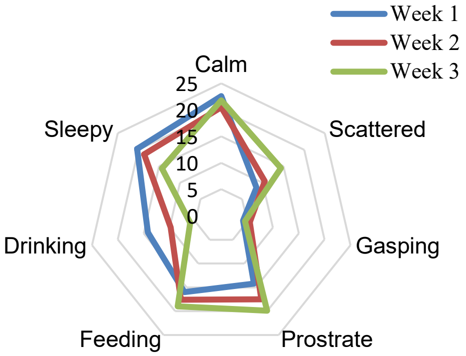
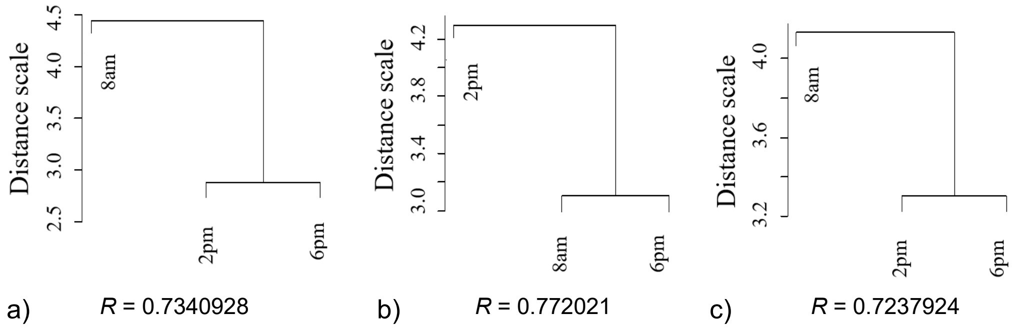


Figure 1. Analysis of the behaviour of the broiler chicks for each experimental week.

Fig. 2 shows a cluster analysis in which the behavioural patterns were grouped and the periods (8 am, 2 pm, and 6 pm) were compared and analysed according to the Euclidean distance scale. For the first week of life (Fig. 2, a), during which the THI was highest (Table 1), the behaviour of the chick’s showed characteristics of discomfort due to cold, in which the morning period differed from the other evaluated periods in the distance scale. There were no differences in the distance scale between the afternoon and evening periods.



a) *R* = 0.7340928 b) *R* = 0.772021 c) *R* = 0.7237924

Figure 2. Cluster analysis of the behavioural variables analysed for the morning, afternoon, and evening periods in a) first week of life, b) second week of life, and c) third week of life.

For the second week of life, the THI was below the thermal comfort range (Table 1), especially at 2:00 pm, when the THI was the lowest. This was evidenced in the behaviour analysis of the chicks, where the behaviour at 2:00 pm was different from that at the other times evaluated (Fig. 2, b).

In the third week of life, the behaviour was like that in the first week (Fig. 2, c), and the time of 8:00 am differed from the other two times studied. The chicks were subjected to thermal conditions below comfort (Table 1), and at 8:00 am, the THI values were higher than those at the other times. This was evidenced in the behaviour pattern of the chicks, which at 8:00 am differed from the behaviour patterns at 2:00 pm and 6:00 pm (Fig. 2, c). According to Schiassi et al. (2015), although broilers have the ability to adapt their behaviour to variations in their thermal environment, it is not recommended for there to be a large variation in the thermal conditions of the internal environment in commercial broiler houses. The large temperature variations in the environment can influence the zootechnical responses of the animals, such as feed intake, weight gain and feed conversion, which can lead to production losses not observed only by the behaviour analysis.

The CCC for all grouped behaviours was greater than 0.70. According to Ferraz et al. (2014) and Castro et al. (2017), the values found indicate agreement between the original dissimilarity values and those represented in the dendrogram.

CONCLUSIONS

Based on the results presented, the heating system evaluated had no influence on the values of the measured environmental and physiological parameters, during the trial period. However, differences in the values of these parameters were observed between the analysed periods of the day (8:00 am, 2:00 pm, and 6:00 pm).

Regarding the environmental variables, it can be concluded that only in the first week of life did the THI values show values within the comfort range considered ideal. The THI was below the comfort range in the second and third weeks, which may be indicative of failure in the heating systems of both broiler houses. The cloacal analysis and RR indicated a level outside the comfort range.

Regarding the behaviour during all weeks, the chicks showed calm, feeding, and sleepy behaviours most of the time.

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Milk yield of cows in some European countries and the implementation of automatic milking systems

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Abstract. The research study addresses the problem of implementing progress in agricultural production. This problem was developed on the basis of equipping farms with automatic milking systems (AMS). Different forms of progress can be identified on a dairy farm, including technical progress represented by AMS and biological progress expressed by milk yield of cows. The purpose of this research study was to compare whether the milk yield of cows in certain European countries meets the requirements for utilizing the milking potential of automatic milking systems. The study used information on the suggested amount of milk that an one-stall milking robot should milk per year. The second group of data was the annual milk yield of cows in the European Union countries and Great Britain. In eight countries, the annual milk yield of cows was in the range of 8,601–10,600 kg. It was found that in 2020, in these eight countries of the European Union, the milk yield of cows was at a level that meets the performance requirements of one-stall milking robot.

Key words: AMS, annual milk yield, country, cow, robotic milking, progress.

INTRODUCTION

Over the years, progress has been systematically linked to the development of various production areas, including food production (Acevedo, 2011). The growing demand for plant and animal raw materials implies the implementation of more and more modern methods of their production. Production methods in the food economy system are carriers of various forms of progress.

Global progress in agriculture is the result of the continuous search for more and more rational solutions to increase the overall level and efficiency, as well as the sustainability of agricultural production (Dillon et al., 2016). Progress in agriculture, aimed at increasing the efficiency of production, is thus directed at the continuous improvement of facilities in agricultural space.

Technical progress is particularly important for the overall development of agriculture (Michałek & Kowalski, 2000) over the centuries. The creation of simple hand tools, initially wooden and then metal, the construction of more complex agricultural tools and machines adapted to cooperation with live traction, the introduction of steam engines, tractor traction, the construction of self-propelled agricultural machines, robots and the use of satellite techniques are the most significant stages in synthetic description of technical progress identified in the history of agricultural development (Dudin et al., 2014).

Progress in construction usually leads to the creation of more and more modern models of machines and devices that allow to achieve not only higher work efficiency, but also to improve the quality of agricultural operations (Chen et al., 2020). As a result of implementing more and more perfect construction solutions into practice, gradually modifying individual plant and animal production technologies, **technological progress** is being created at the same time. One measure of this progress is the degree of substitution of the work of animals and people with the work of machines and other technical devices. The substitution of human labour in agriculture leads to the gradual automation of processes and changes in the mutual share of human labour and technical equipment (Ianchovichina et al., 2001).

Biological progress, which is an integral part of comprehensively understood progress in agriculture, is currently one of the most dynamic directions of development in scientific research. It leads primarily to the improvement of individual species of plants and animal breeds in order to develop their most beneficial functional features that determine the possible increase in production potential and at the same time meet high quality requirements (Odongo et al., 2010).

Harmonious interaction between particular categories of progress in agriculture is one of the basic conditions for development in many areas of agricultural activity. The relationships between the considered categories of progress are generally synergistic, indicating the possibility of mutually reinforcing the effectiveness of action and thus achieving increasing, primarily economic, efficiency of the process of obtaining high-quality agricultural products.

In the assessment of agricultural production activities, more and more emphasis is placed on sustainable development. The pillars of sustainable development include a set of economic, social and environmental factors (Díaz de Otálora et al., 2021). Sustainable development may also include progress, which is one of the elements stimulating the development of agriculture and its individual sectors. The various forms of progress linked to agricultural production activities give impulse to raising the issue of their sustainable implementation.

Cow milking is one typical example of an area where different forms of progress can be assessed (Gaworski, 2021). The automatic milking system reflects the technical progress that has been made over the past few decades in the field of obtaining milk from cows (Cogato et al., 2021). The production potential of cows in the milk production system identifies biological progress, expressed in the increase in the milk yield of animals. If technical and biological progress in the field of milk production are confronted, a question can be raised regarding the assessment of the effects of the simultaneous implementation of these forms of progress, which determines the sustainable development of the cow milking system on a dairy farm.

The aim of the study was to assess the conditions for the simultaneous implementation of various forms of progress on dairy farms. Equipping dairy farms with automatic milking systems (AMS) was selected as a detailed research area. The research study developed the question of whether the milk yield of cows in certain European countries meets the requirements for utilizing the milking potential of automatic milking systems.

MATERIALS AND METHODS

Milking equipment used on farms differ in the degree of complexity of the design, the level of automation of the tasks performed, efficiency, energy and water consumption per liter of milk and other characteristics (Gaworski et al., 2017). Differences in the achieved efficiency, energy input and manual work for milking determine the possibility of ranking milking systems. In this ranking, according to the value of the technological index level proposed by Nowacki (1999), five generations (G_mI-G_mV) of milking can be distinguished (Table 1). The technological index level is a ratio of machine work inputs to the sum of manual and machine work incurred for individual tasks in production technologies, including agricultural production. The value of the index ranges from 0 to 100%. The lowest values of the technological index level correspond to hand milking, and the highest to the automatic milking system. Translating the scale of the technological index level into milking generations, the lowest values of the index correspond to the first generation of milking (G_mI), and the highest values of the index correspond to the highest generation of milking (G_mV).The transition to ever higher generations of technical solutions identifies technical progress in agricultural production technologies (Nowacki, 1999). The highest level of technical progress in milking is expressed by the highest milking generation (G_mV), and this is represented by the automatic milking system (AMS).

Table 1. Generations of milking cows used on dairy farms, according to Nowacki (1999)

Generation of milking	Solution
G_mI	hand milking
G_mII	bucket milking system
G_mIII	pipeline milking system
G_mIV	milking parlour
G_mV	automatic milking system

The implementation of technical progress represented by milking robots in farms is associated with the assessment of the profitability of their use, justifying the investment in these modern devices. The profitability of using automatic milking systems is the result of many factors, among which the key is the amount of milk milked per year. The results of analyzes presented in the literature indicate that the profitability of using one-stall milking robot is achieved in the case of obtaining 515,000 kg (Meskens et al., 2001), and according to other authors (Heikkilä et al., 2010) 800,000 kg of milk per year. In practice, the one-stall milking robot is usually used in a herd of 50 to 65 dairy cows (Castro et al., 2012; Tremblay et al., 2016). Based on this data, it is possible to calculate what the annual milk yield of one cow should be in order to meet the requirements related to the profitability of using one-stall milking robot on the farm. The calculation requires the assumption that the cows in the herd are subject to rotation related to drying off and calving, therefore the number of cows in the herd operated by the milking robot increases by 15%. Dividing the annual amount of milk to be milked by the size of the herd shows that the milk yield of cows in a barn with one-stall milking robot should be in the range of approx. 8,950 to 10,700 kg of milk per cow per year.

Another approach can also be demonstrated to determine the milk yield range of cows for use in milking robot analysis. Statistical data can be included in this case. Such data, covering the milk yield of cows in geographical regions of the world in 2020, are summarized in Table 2.

The continental data in Table 2 are listed from lowest to highest milk yield of cows. It can be seen that the difference between the lowest and the highest yield is about 10,000 kg of milk per cow per year. This range has been divided into five equal ranges (categories). Value ranges are listed in Table 3.

The highest cow milk yield category ($C_{my}V$) ranges from 8,601 to 10,600 kg of milk per cow per year. This range is close to the calculated yields of cows (8,950–10,700 kg year^{-1}) resulting from the analysis of the profitability of using an one-stall milking robot.

Table 2. Annual milk yield per cow in geographical regions in 2020

Geographical region	Annual milk yield per cow (kg year^{-1})
Africa	595
Asia	1,919
South America	2,446
Oceania	4,887
Australia and New Zealand	4,922
Europe	6,667
North America	10,712
World	*2,678*

Source: www.fao.org/faostat/ [access: 01.09.2022].

Table 3. Categories (C_{my}) of annual milk yield per cow, in (kg year^{-1})

Category	$C_{my}I$	$C_{my}II$	$C_{my}III$	$C_{my}IV$	$C_{my}V$
Range	601–2,600	2,601–4,600	4,601–6,600	6,601–8,600	8,601–10,600
Average value in the range	1,600	3,600	5,600	7,600	9,600

Considering the ranges of milk yield for $C_{my}I$-$C_{my}V$ categories, the question can be raised in which countries the level of milk yield of cows in the highest $C_{my}V$ category has already been achieved. Thus, the question is in which countries the convergence of the highest level of technical progress, i.e. the highest generation of milking (G_mV) represented by milking robots, with the highest level of biological progress, represented by the milk yield of cows (identified by the $C_{my}V$ category), has been achieved. The answer to this question is the content of the research results, which have been extended by additional comparisons and discussion.

RESULTS AND DISCUSSION

The assessment of the conditions for the simultaneous implementation of two forms of progress in the area of dairy production was carried out on the example of data from European countries. Based on data from the European Union countries and Great Britain, Table 4 summarizes the milk yield of cows with assignment to the appropriate $C_{my}I$-$C_{my}V$ category of annual milk yield per cow.

The data in Table 4 show that in eight countries of the European Union, the milk yield of cows in the highest $C_{my}V$ category has already been achieved. In these countries, a convergence of technical and biological progress can be indicated in the case of milk production on farms using milking robots. The considered convergence of two forms of progress (technical and biological) in the area of milking concerns the highest, fifth level of progress: $G_mV \leftrightarrow C_{my}V$. Technical progress in the area of milking cows, identified by equipping farms with automatic milking systems, can be fully used as a result of working with herds of cows included in the highest category of milk yield, representing biological progress. Among the countries with the highest milk yield of cows, in the $C_{my}V$ category, there are four countries located in the Baltic Sea zone.

Table 4. Annual milk yield per cow in the European Union and Great Britain and their classification within $C_{my}I$-$C_{my}V$ categories, based on 2020 data

Categories of annual milk yield per cow	Country	Milk yield (kg year^{-1})	Reference ranges (kg year^{-1})
$C_{my}I$			601–2,600
$C_{my}II$	Romania	3,228	2,601–4,600
	Bulgaria	3,645	
$C_{my}III$	Croatia	5,418	4,601–6,600
	Ireland	5,880	
	Slovenia	6,357	
	Lithuania	6,389	
$C_{my}IV$	Italy	6,794	6,601–8,600
	Malta	6,949	
	Poland	6,973	
	Latvia	7,264	
	Austria	7,271	
	France	7,279	
	Cyprus	7,496	
	Slovakia	7,519	
	Greece	7,947	
	Luxembourg	8,249	
	Belgium	8,270	
	Great Britain	8,369	
	Germany	8,457	
	Portugal	8,566	
$C_{my}V$	Hungary	8,913	8,601–10,600
	Sweden	9,109	
	Czech Republic	9,153	
	Netherlands	9,256	
	Spain	9,382	
	Finland	9,414	
	Denmark	10,028	
	Estonia	10,063	

Source: www.fao.org/faostat/ [access: 01.09.2022].

The largest group of countries is in the fourth category of milk yield of cows - $C_{my}IV$ (Table 4). These are 14 countries where the milk yield of cows is in the range of 6,601–8,600 kg of milk per cow per year. This milk yield category immediately precedes the highest $C_{my}V$ category, important for the efficiency of the milking robots. If we take into account the upward trends in milk production from one cow, the question remains how many countries will soon achieve the milk yield of cows that justifies equipping farms with an automatic milking system. The situation in the following categories of milk yield of cows can be considered in different ways. Fig. 1 presents a comparison of model milk yields of cows (based on data in Table 3) and average milk yields for a set of countries in individual $C_{my}II$-$C_{my}V$ categories. Only four categories of milk yield of cows were considered, as no country was included in the first category ($C_{my}I$).

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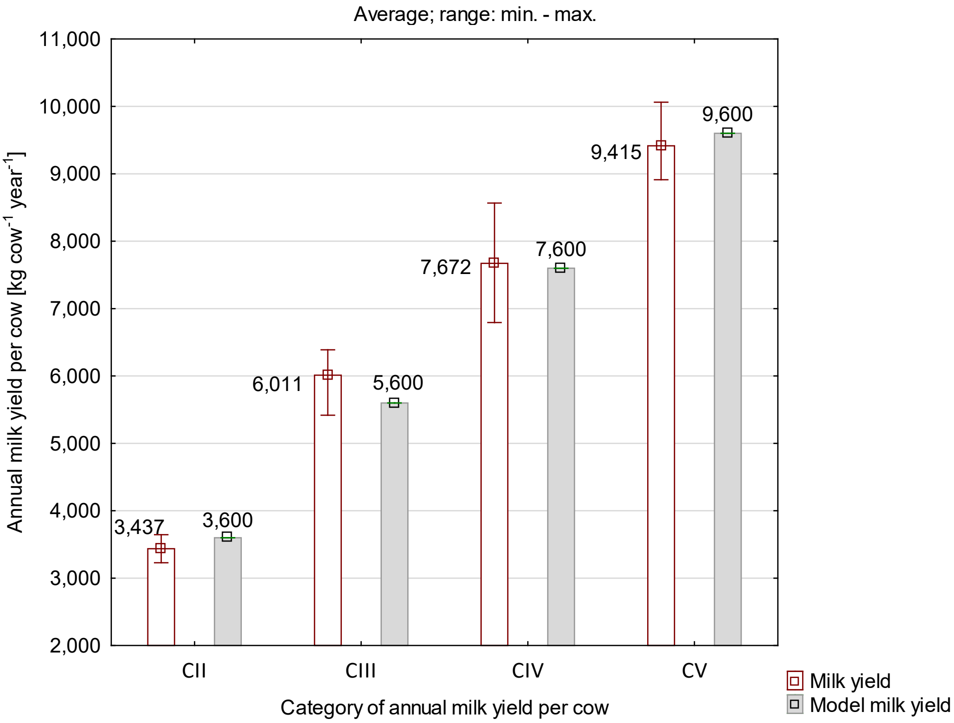

Figure 1. Model milk yields of cows (grey bars) and average milk yields (white bars) for a set of countries in individual C_{my}II-C_{my}V categories.

The greatest differences between the average model milk yield and the average for a given group of countries can be found for category C_{my}III milk yield of cows. In this category, the average milk yield of cows exceeds the model average by more than 400 liters per year (Fig. 1). The smallest difference between the averages is in the C_{my}IV milk yield category. In turn, in this category of milk yield of cows, the largest difference between the minimum and maximum value can be observed, which is more than 1,770 liters of milk per cow per year for the countries in this category. The results presented in Fig. 1 can also be considered in a different way, posing the question: How far are the average milk yields of cows in given categories from the maximum values of the range? That is, how much is missing to move to a higher milk yield category. This issue relates to three categories of cow milk yield. i.e. C_{my}II, C_{my}III and C_{my}IV. Particularly noteworthy is the fourth category of milk yield of cows (C_{my}IV), because it directly precedes the category of milk yield C_{my}V, which with its potential meets the requirements of the milking robot. The difference between the average milk yield of the C_{my}IV category and the maximum bordering on the C_{my}V category is 929 kg of milk per cow per year. Using the average milk yield of cows gives only a general picture of the comparison of the considered C_{my}IV yield category with the C_{my}V category. More valuable information is provided by comparing the milk yield of cows in individual countries of the C_{my}IV category with the maximum yield, which is already exceeded by the C_{my}V category. In this context, the question was raised: By what percentage would it be necessary to increase the milk yield of cows in individual countries to achieve a yield of 8,601 kg of milk per cow per year, which is the minimum for the $C_{my}V$ category of milk yield of cows. The answer to this question is shown in Fig. 2.

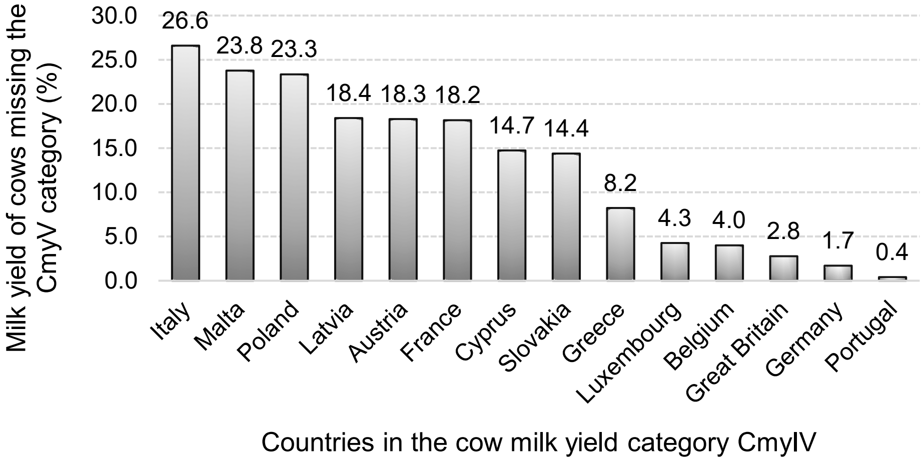

Figure 2. Missing milk yield of cows (in %) needed to achieve the minimum milk yield in the $C_{my}V$ category, by country for 2020 data.

The comparison of the data (calculation results) in Fig. 2 indicates the possibility of distinguishing two groups of countries, with the missing milk yield of cows up to 10% and above 10%. The first group includes six countries where achieving a yield of 8,601 kg of milk per cow per year would require an increase in current yield by 0.4% to 8.2%. In the second group, however, there are 8 countries with a much larger range, from 14.4% to 26.6% of the missing milk yield of cows. These are the results of the calculations for 2020, which can be verified taking into account the data in the following years. In general, the milk yield of cows shows an upward trend every year. Therefore, the number of countries in the distinguished milk yield categories may change, as well as the distance that individual countries have to cover to achieve maximum yield in a given category (C_{my}).

Regardless of the results of the comparison, this research study inspires further discussion on various aspects of improving dairy production at the farm level. Evaluation of dairy production and its improvement on a farm can be considered in the area of milk yield of cows and factors that determine this yield. The milk yield of the cows represents their biological potential, analyzed in this study in conjunction with the technical potential of the milking equipment. In practice, the technical potential does not only apply to automatic milking systems, but also to other milking systems. Gaworski et al. (2018) compared the production and other (health) indices of herds of dairy cows milked with pipeline milking systems, in milking parlours and with the use of milking robots (AMS). The milk yield of cows on farms equipped with AMS was about 24% higher compared to the milk yield of cows on farms with pipeline milking machines. Similar relationships between the productivity of a dairy cow herd and the type of milking system were indicated by Gygax et al. (2007), who compared the use of milking robots and parlours (auto-tandem type) on farms.

The links between milking equipment and the factors that determine milk production on a farm, including cow milk yield and herd size, are the subject of evaluation and optimization of milking on many farms (Gaworski et al., 2017). Such studies, but also those using stochastic models (Nitzan et al., 2006) and other mathematical simulation models (Komiya et al., 2002), have made it possible to evaluate the performance of the milking robot and other milking systems. Research on the efficiency of milking robots (Priekulis & Laurs, 2012) translates into estimation of the effectiveness of their use (Castro et al., 2012), where the production potential of a dairy cow herd is important. The effectiveness of the use of automatic milking systems on farms is also determined by other factors, including those related to the management of a robot-milked herd (Bach et al., 2009; Gaworski et al., 2016). Therefore, the production potential of a herd of dairy cows considered in this research study, which justifies the implementation of a milking robot on a farm, needs to be developed with studies of factors that favor and disrupt the full use of the technical potential of AMS. These are studies related to the assessment of the accuracy of the milking robot (Bach & Busto, 2005), milk quality (Hogenboom et al., 2019), the use of milking robots in the pasture (Lyons et al., 2013), as well as the health and welfare of dairy cows milked by a robot (Jacobs & Siegford, 2012), which directly translates into the milk yield of cows.

The milk yield of cows in robot-milked herds is of particular concern. And this is due to the possible increase in milk yield of cows, which is one of the most important benefits of milking a herd of animals with a milking robot (Tremblay et al., 2016; Filho et al., 2020). Increasing the milk yield of cows milked with a robot is the effect of increasing the frequency of their milking during the day. Milking frequency is taken into account in many studies of automatic milking systems, with for example 2.47 milkings per day (Gygax et al., 2007), 2.5 milkings per day in multiparous cows and 2.8 milkings per day in primiparous cows (Speroni et al., 2006). An important issue is also the frequency of milking cows with an automatic milking system combined with grazing (Lessire et al., 2020). In the majority of farms with conventional milking systems, cows are milked twice a day, hence the milk yields achieved there are a comparative basis for cows in robot-milked herds (Hansen et al., 2019). Considerations regarding the frequency of milking per day and the resulting milk yield of cows are an important contribution to the discussion of the results presented in Fig. 2. For some countries with milk yields in the C_{my}IV category, the missing yield from the C_{my}V category can be achieved by increasing the frequency of milking. It follows that it can be proposed to equip some farms with a herd of cows with a productivity in the C_{my}IV category with a milking robot, which will increase the milk yield of cows and their transition to the C_{my}V category. The question is how much of an increase in cow milk yield can be expected as a result of the transition from conventional to robotic milking. In response to this problem, the studies presented in the literature indicate the possibility of increasing the milk yield of cows with the increase in the frequency of milking from 2 to 3 times a day. For example, Erdman & Varner (1995) found an increase in milk yield of primiparous cows by 17.65% and multiparous cows by 18.32% when switching from 2 to 3 milkings per day.

In addition to the milk yield of cows, the size of the herd is a key factor in ensuring the appropriate production potential of a robot-milked herd. In this research study, the size of the herd of dairy cows was taken into account at the first stage of considerations regarding the determination of the range of milk yield of cows in a herd milked with an automatic milking system (AMS). The second stage of considerations was developed on the basis of data on milk yields of cows in a group of 27 countries of the European Union and Great Britain. Of course, each country is distinguished by the average size of dairy herds on farms and the structure of herds in terms of their size. This aspect of the analysis, already taken up in earlier comparative studies (Leola et al., 2021), can be a direction for further research included in the analysis of the simultaneous implementation of various forms of progress in dairy production on farms.

The presented problem of simultaneous implementation of various forms of progress on the example of milking robots is part of the direction of research devoted to the assessment and comparison of the potential of dairy production in Europe (Gaworski & Leola, 2015) and other regions of the world (Matson et al., 2021). Proposals of indicators and research tools, such as decision trees (Piwczyński et al., 2020), deep learning (Liseune et al., 2021), optimization models (Zhang et al., 2016) and management models with a decision support system (Gargiulo et al., 2022) are used to plan dairy production now and in the future, which is a premise for the sustainable development and ethical transformation (Gaworski, 2006) of the food economy system.

CONCLUSIONS

The implementation of technical progress in agriculture may generate high costs, which in the case of one-stall milking robots usually exceed 100,000 euro (at the beginning of this decade in Poland). That is why it is so important to fully use the technical potential of agricultural equipment. This may be facilitated by a balanced approach to linking technical progress with biological progress and other forms of progress that are part of the improvement of production processes in agriculture.

This research study showed significant differences in the conditions for the implementation of technical progress, i.e. milking robots, in individual regions of Europe. In 2020, in eight countries of the European Union, the average milk yield of cows was achieved that meets the criteria for their inclusion in the automatic milking system. Increasing the milk yield of cows is a continuous process and as a result, in a growing number of countries, the conditions are being created for the cost-effective implementation of technical progress in the field of milking cows.

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Comparing weight dynamics between urban and rural honey bee colonies in Latvia

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Abstract. Beekeeping is an important agricultural industry in Latvia, which has an area of 64,589 km^2 and is largely mixed forest. The natural foraging base does not provide the honey yield evenly throughout the whole season, thus the average honey yield in Latvia is about 20 kg per colony. The objective of this research was to compare the weight dynamics of colonies placed in rural and urban environments. As urban beekeeping is becoming more popular, it is important to understand whether there are enough foraging resources within the city for the bee colonies. To do this, the weight changes of ten honey bee colonies was remotely monitored and analysed during the summer period. Five colonies were located in the rural environment in Vecauce and five in the urban environment in Jelgava city. Colonies were assessed using the precision beekeeping approach and developed scale systems. It was concluded that for rural colonies in Vecauce, the main weight increase occurred in June - from 41.02 to 54.68 kg - which resulted in 94% of the total increase for the summer period. Data analysis from the urban apiary revealed that colonies increase weight during the entire monitoring period, indicating that there are foraging resources available throughout the summer period within the city.

Key words: precision beekeeping, weight monitoring, urban beekeeping, foraging activity.

INTRODUCTION

There is a growing concern about the health and number of pollinators, as insect populations are declining worldwide (Hallmann et al., 2017). Insects are responsible for pollination of up to 80% of wild plants (Ollerton et al., 2011). This fact has led to growing popularity of urban agriculture, including urban gardening and urban beekeeping.

Urban, considered also as small-scale beekeeping, is growing in many locations, like countries in North America (Ellis, 2022), major urban areas such as London, Paris, Sydney, Warsaw, Hong Kong also have a lot of city beehives (Matsuzawa & Kohsaka, 2021). This can be explained by many factors: 1) positive attitude towards the urban beekeeping in public coverage and social networks; 2) awareness about declining bee health; 3) application of pesticides in rural environments; 4) focus on biodiversity aspects. There are many recommendations on how to improve honey bee health, such as: reducing exposure to different insecticides; preventing and limiting the spread of disease and providing a greater diversity of floral resources throughout the active period of the bee colony development (Goulson et al., 2015) and placing the colonies in urban environment can be in-line with these recommendations.

There are some differences between urban and rural beekeeping. Urban beekeepers generally do not migrate their bee colonies (Ellis, 2022), but rural beekeepers in some parts of the world change apiary locations throughout the active bee colony foraging period. In Latvia the migratory beekeeping is not very common practice, only some of the beekeepers are moving their colonies around and changing the locations. Overall, 22% of the apiaries are taken out to the remote locations in Latvia (based on a beekeeper's survey in the year 2019 conducted by Latvian Beekeeping association). Among those who is placing colonies remotely, ICT solutions, like automatic scales, can help to monitor the nectar flow in the colonies to decide when to move the colonies, when the foraging activity is de-creased or the nectar flow is finished. Data about weight dynamics can provide the bee-keeper with crucial information on various important colony events (Meikle et al., 2006; Komasilovs et al., 2019).

Urban bees are potentially less exposed to pesticides as agricultural pesticides are not used in cities so much. Some authors presented results of pesticides analysis which indicated that more pesticides as well as higher concentration of them was found in rural bee samples. The analysis of pesticides shows that more polar pesticides can be found on the surface of the bodies of honey bees and more hydrophobic ones can go deep into the body. It confirms that the urban environment is more friendly for the bee colonies (Sadowska et al., 2019). But some authors states, that heavy usage of pesticides in the urban environment is a serious concern (Meftaul et al., 2020). Thus detailed investigation should be conducted per urban area samples. So, it seems, the situation can be different in different urban areas.

Urban environments can have much richer and diverse floral resources, because of the variety of plants growing within the cities (Garbuzov et al., 2015). As well, a cultivated urban environment, with its large floral biodiversity, can provide extra nutrition for bees, resulting in the production of a honey rich in nutraceutical compounds (Preti & Tarola, 2021). Honeybees living on mixed pollen showed the highest productivity, rearing more brood than bees fed monocultural pollen. The dietary quality is again reflected in longevity. Monocultural bees had a shorter life expectancy than bees nourished with mixed pollen (Szymaś & Jędruszuk, 2003; Höcherl et al., 2012).

Unfortunately, there are also some concerns about urban beekeeping, e.g., in major cities there is a huge road traffic, which is a source of dust and heavy metals, as well fuel emissions can change the odor of flowers to the extent that bees can no longer recognize them (Reitmayer et al., 2019).

There are doubts that beekeeping cannot be very productive in the cities, but we did not find any numerical results of the bee colony honey production in the city environment. Thus, the aim of this research was to identify and compare the weight dynamics of the apiary located in the urban environment (Strazdu iela, Jelgava) with the apiary located in the rural environment (Vecauce) in Latvia. Analysing the spread of urban bee-keeping in Latvia, despite the fact that beekeeping is an old and traditional branch of agriculture, there are not many examples of urban beekeeping there. It is mainly possible to see some colonies on rooftops in the capital city Riga and several other individual locations in other cities.

MATERIALS AND METHODS

Apiary location description

Experimental urban apiary was created at Latvia University of Life Sciences and Technologies (LBTU), Strazdu iela 1, Jelgava, Latvia (GPS coordinates: 56.6630, 23.7538). This apiary is located at the study centre, where some experimental green-houses, berry bushes and other garden cultures are planted. The study centre itself is located about 100 m from the main city street and various public facilities are located nearby (shopping centre, stadium, school). Five bee colonies (*Apis mellifera mellifera*) were selected for the remote observations. Colonies were placed in Latvian design type hives made from wood and in polyfoam hives.

Rural apiary was located at LBTU apiary in Vecauce, Latvia (GPS coordinates: 56.4675, 22.8878). Five bee colonies (*Apis mellifera mellifera*) were selected for the remote observations. Colonies were placed in Latvian design type hives made from wood. Within a flying radius of the bee colonies, various habitats were found around the studied apiary: agricultural land, forests, small town, roads, railways, small rivers and ditches. Most of this area was occupied by agricultural land, which was mostly used for various arable crop growing, including rapeseed and beans.

Both apiaries were monitored during the summer period from 01.06 - 31.08.2022.

Fig. 1 shows apiary locations on a Latvian map:

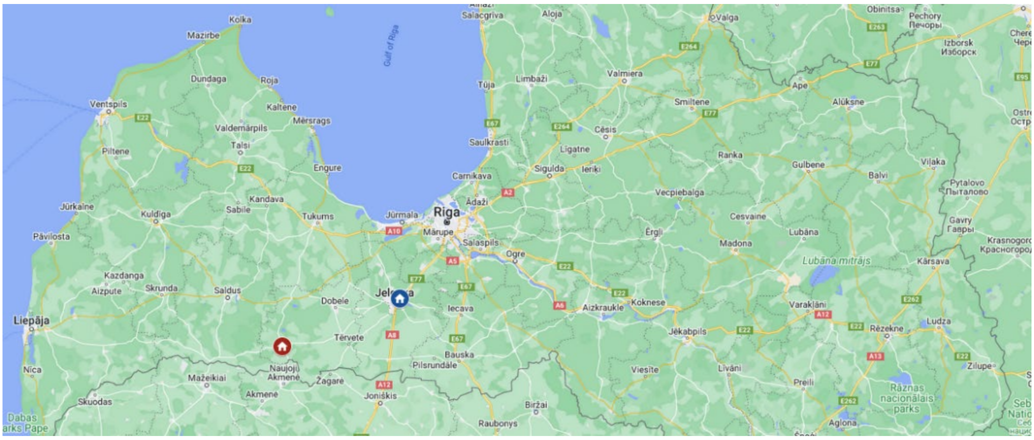

Figure 1. Location of the rural apiary (red icon) and urban apiary (blue icon) in Latvia territory.

Monitoring device

All colonies in both apiaries were equipped with a bee colony monitoring system based on the ESP8266 microchip inspired by the monitoring system developed within the SAMS project (Wakjira et al., 2021). All systems were powered by a Sony Li-ion 18650 3.7 V 3120 mAh battery. In the rural apiary a locally available WiFi router was used to transmit data to the remote server, but in the urban apiary the router was substituted with an additional communication node (powered by a solar power system) consisting of ESP32 microchip and a GSM module to transmit data via mobile network.

Each monitoring system was equipped with two Dallas DS18B20 temperature sensors and scales. For weight monitoring, a single-point load cell Bosche H30A was used. Load cell accuracy and precision were empirically evaluated by (Kviesis et al., 2020). The precision of the scale measurement system (single point load cell H30A together with the 24-bit HX711 A/D converter) was observed to be around 10 g. Maximum weight, that can be measured is 200 kg. One temperature sensor (Dallas DS18B20) per colony was installed inside the hive above the brood frames as suggested by (Stalidzans & Berzonis, 2013). Second temperature sensor was placed outside the hive to monitor the environmental temperature. Weight and temperature of the colonies were continuously measured with the time interval of 30 minutes between two measurements.

Environmental parameters

Data about environmental parameters were collected from the nearest public weather station from https://www.meteo.lv/. Figure below (see Fig. 2) summarises minimal and maximal ambient temperatures, amount of precipitation and average wind speed for the monitoring period, considering values for the time period from 5:00 till 23:00 for both lo-cations. Night period is not taken into consideration, as bees are not flying at night.

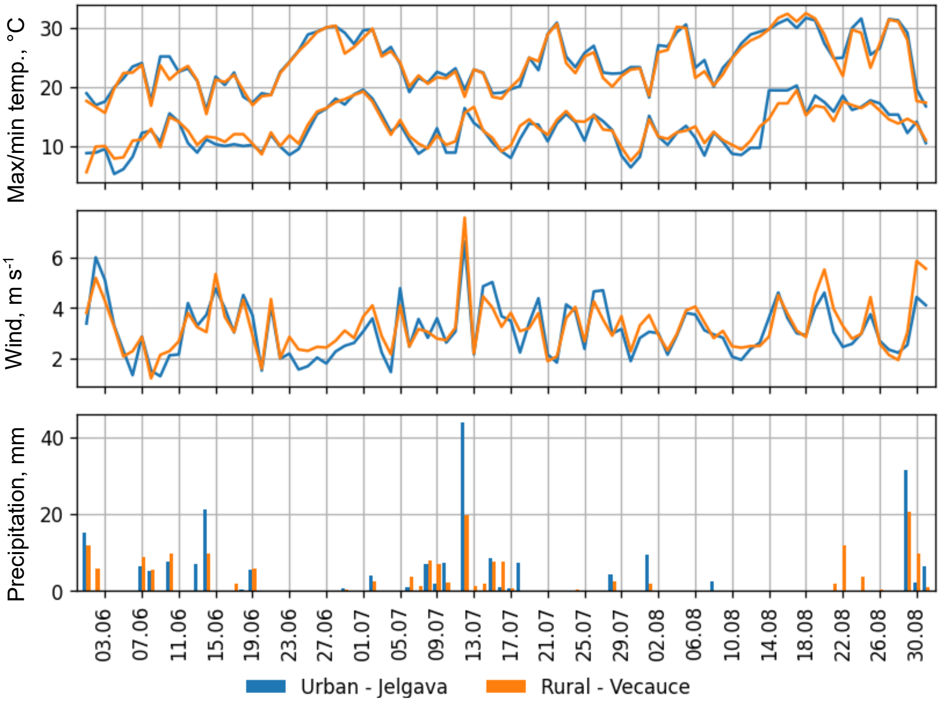

Figure 2. Weather conditions at the urban and rural apiaries during the monitoring period.

Based on (Komasilova et al., 2021) the observed conditions are considered as good for the foraging process, since the ideal conditions are defined when temperature is between 20 °C and 30 °C, wind speed less than 5 m s^{-1}, and there is no rain at the foraging site.

Weather conditions during the monitoring period were similar at both locations and almost all days of the monitoring period were suitable for bee foraging activities.

Pollen collection and analysis methodology

In addition to bee colony monitoring also pollen was collected and analysed in both apiaries. Pollen was collected using pollen traps placed outside the beehive entrance. Samples of it were collected by the beekeeper every second day from the beginning of June until the middle of August. Then the pollen samples were stored in a freezer at -18 °C until the middle of August when they were prepared for further analysis. All samples were then divided into two parts. One part of each sample was placed in a dryer at 35 degrees and dried for 24–36 hours, then sent to Quality Services International GmbH in Germany for analysis of the botanical composition. The second part of each sample was sent frozen to the Water & Life Lab analytical laboratory in Italy to identify pesticide residues in the pollen.

RESULTS AND DISCUSSION

Purpose of this research was to compare the dynamics of weight gain by colonies located in two different environment types: urban and rural, so the weight changes are de-scribed in details below. Authors analyzed the weight change of the colonies, and not the amount of the honey produced by the colony and collected by the beekeeper, as this in-formation was not provided by the beekeepers and were not available for the detailed analysis.

Overall increase of the weight during the monitoring period

The Fig. 3 below demonstrates weight dynamics of the monitored colonies during the summer period. Period from 01.06.2022 till 31.08.2022 (92 days) was taken for the analysis. Average daily weight is calculated considering 30 minutes intervals between individual measurements. Rural colonies in Vecauce are labeled by R (R_1 to R_5) and urban colonies in Jelgava by U (U_1 to U_5).

Analyzing the data day by day, it can be observed that two periods from June 5 to June 7 and from June 24 to June 29 are clearly distinguished for Vecauce. Based on the pollen analysis, the first increase in Vecauce apiary mass dynamics in hives can be explained by cruciferous plants, mainly winter rapeseed (*Brassica napus L.*) foraging (pollen composition in the sample 83%). Part of the yield mass dynamics is also formed by nearby flowering willows (*Salix sp.*) (11%), horse-chestnuts (*Aesculus sp.*) (4%) and blackberries (*Rubus sp.*) (2%). The second significant increase in colony weight can be explained by flowering of mustard (*Sinapis sp.*) fields and wild cruciferous (*Brassicaceae*) family plants (pollen composition in the sample 34%). Part of the yield is also formed by umbellifers (*Apiaceae*) (30%), clover (*Trifolium sp.*) (18%), yarrows (*Achillea sp.*) (5%), vetch (*Vicia sp.*) (4%) and linden (*Tiliaceae*) (2%).

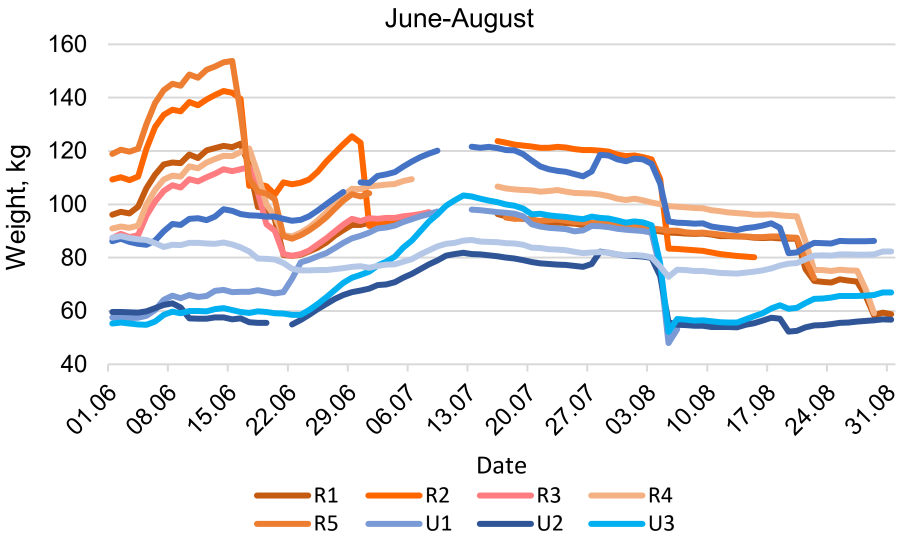

Figure 3. Dynamics of a weight change in a honeybee colonies (gaps in lines are caused by connectivity issues with Wi-Fi router in rural area, rapid weight decline indicate harvesting events).

On other days, weight gain is either very small, or generally has a negative value. Beekeepers can potentially move the rural colonies to other geographical locations, when active nectar flow is finished in the apiary, in order to increase the overall honey yield of the colonies, as well as to provide a continuous food supply for the bees. As there is a fairly even climate throughout Latvia, the use of the migratory beekeeping applies no additional stress for the bees caused by climate change, daylight changes and long traveling times. Based on the mentioned above, migratory beekeeping can be used to a large extent successfully in the territory of Latvia to provide a more diverse food base for bees, available throughout the entire period of honey collection from early spring to late summer, thereby ensuring the pollination of various crops and increasing the volumes of honey, pollen and other bee products.

For hives located in an urban environment (in Jelgava), analysing the data day by day, there are no clearly defined growth peaks, contrary to Vecauce. In general, the weight gain is quite uniform during all three months. The maximum gain per day is 3.84 kg with an average positive gain of 1.08 kg, and the number of days with a positive gain in Jelgava is 62% more than in Vecauce.

From June 5 to June 15, the weight increase in urban hives can be explained by foraging from plants of the Brassicaceae family, mainly wild weeds (e.g. field mustard (*Sinapis arvensis L.*), wild radish (*Raphanus raphanistrum L.*), wintercresses (*Barbarea sp.*)), wallflower (*Erysimum sp.*) and other cruciferous flowers, which are flowering in this time period, which made up 56% of the pollen from the obtained sample. The rest of the yield was made up of the clover genus (*Trifolium sp.*) (pollen botanical composition in the sample – 18%), the blackberry genus (*Rubus sp.*) (8%), the *Apiaceae* family (6%), the horse chestnut genus (*Aesculus sp.*) (5%) and plants of the privet genus (*Ligustrum sp.*) (3%).

From June 22 to July 3, the increase in the weight dynamics of the urban colonies can be explained by the proportion of plants of the cypress family in small gardens, urban parks, urban forest edges and natural meadows.

In the period from July 5 to July 24 weight increase is related to the active flowering of the genus Meadowsweet (*Filipendula sp.*) (botanical pollen composition in the sample 63%).

Days with a huge decrease (more than 10 kg) of the weight corresponds to honey collection events completed by the beekeeper. For the urban colonies honey collection took place on 4.08 (colonies: U_1, U_3) and 5.08 (colonies: U_1, U_2, U_3, U_4) and for rural colonies on various days: R_1 on 18.06, 21.06 and 21.08; R_2 on 17.06, 01.07, 05.08; R_3 on 19.06; R_4 on 19.06 and 22.08; R_5 on 16.06, 17.06 and 21.06.

Interruptions in data occurred due to several reasons: short-term interruptions were due to loss of a wi-fi signal and beekeepers' inspections, but long-term data interruptions were due to the low battery voltage level. These interruptions did not highly affect the whole observation period, but some periods (table cells marked in red) were excluded from the further analysis (see Table 1 below).

Table 1. Number of days with collected data for the whole observation period

Colony	R_1	R_2	R_3	R_4	R_5	U_1	U_2	U_3	U_4	U_5
June (30)	26	26	27	26	25	28	26	30	28	30
July (31)	20	18	8	21	15	27	30	31	26	31
August (31)	27	13	0	25	20	3	28	28	26	29
June-August	73	57	35	72	60	58	84	89	80	90

Overall increase of the weight during the monitoring period

Table 2 below shows the total weight gain compared to monthly weight gain. Authors define the daily weight gain of the colony as positive increase of the weight within the 24-hour period starting at midnight. To visually distinguish between high foraging months and low foraging periods colors encoding is implemented. Color scheme from red to green is used (from 0 kg increase to maximum weight gain). For the color encoding Excel Conditional Formatting was used.

Table 2. Weight gain by the honeybee colonies in kg

Colony	R_1	R_2	R_3	R_4	R_5	U_1	U_2	U_3	U_4	U_5
June-August	46.85	57.20	43.82	55.11	56.69	34.67	40.62	66.94	49.05	26.49
June	41.02	54.68	43.82	49.80	54.28	23.49	17.22	22.96	26.14	4.40
July	3.00	2.29	-	4.23	1.74	11.18	15.23	30.94	14.32	10.73
August	2.83	0.23	-	1.08	0.67	-	8.17	13.04	8.59	11.36

Honeybee colony U_5 had lower weight gain in June, because the colony swarmed, thus its development was slowed down and it was not able to fully recover after this event. This affected the overall performance of the urban apiary in June.

It should be emphasised that the weight gain for each colony differs, as colonies differ in strength, and it is also dependent on the starting weight of the colony. This starting weight included the weight of the hive box itself, weight of the bees, brood and the colony initial food storage.

Based on ANOVA single factor statistical analysis weight gains for rural and urban colonies for each month are significantly different. Calculated P-values for each month are: 0.0002 (June); 0.0146 (July); 0.0004 (August).

Table 3. below demonstrates average values for the apiaries together with the standard deviations (stdev).

Table 3. Average values for the weight gain for both apiaries

	Average values for rural colonies, kg	stdev, kg	Average values for urban colonies, kg	stdev, kg
June-August	51.93	5.51	43.56	13.83
June	48.72	5.49	22.45	3.25
July	2.81	0.93	16.48	7.44
August	1.20	0.98	10.29	2.01

Information for the weight gain by the honeybee colonies can be present in a form of a chart to provide better comparison of the honeybee colonies performance (see Fig. 4).

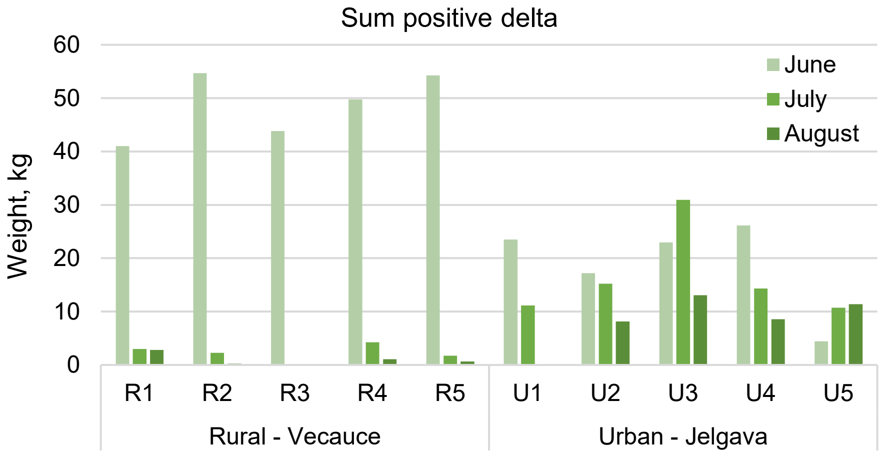

Figure 4. Weight gain of honeybee colonies in urban and rural environment.

Considering the total positive gain (delta>0) in each of the hives by months, it can be concluded that for rural colonies in Vecauce, the main weight increase occurred in June (from 41.02 to 54.68 kg), which results in 94% of the total increase for the whole summer period (June-August), while July accounts only for a 4%, and August for a 2%.

For urban colonies located in Jelgava, the following distribution of the weight gain can be observed: June accounts for a gain of 43%, July for an 38% and August for 19% of the total positive weight gain.

Detailed weight dynamics analysis per days

Analysing the number of days with a positive weight gain in the hives, it can be not-ed that for the rural apiary in Vecauce the majority of days with the positive gain (73.9% on average) are in June, 18.6% in July and 14% in August. While for the urban apiary in Jelgava the distribution of days with a positive increase for three months has the lower distribution and averages at 41.1% for June, 31.2% for July and 34.6% for August (see Fig. 5).

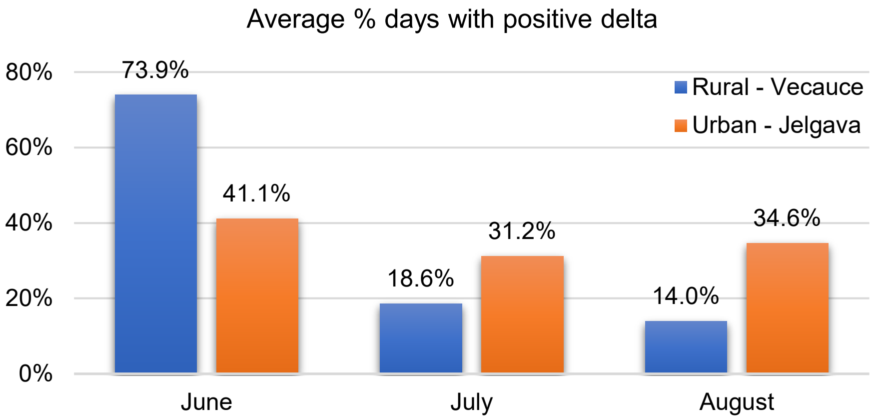

Figure 5. Average numbers of days with a positive weight gain for apiaries.

Table 4 below presents the detailed percentage of days with positive delta from all the days with positive delta.

Table 4. Percentage of days with positive weight gain from all the days with positive delta

	Rural - Vecauce					Urban - Jelgava				
	R_1	R_2	R_3	R_4	R_5	U_1	U_2	U_3	U_4	U_5
June	65.5%	75.0%	100.0%	62.5%	66.7%	64.5%	39.1%	34.7%	41.7%	25.6%
July	13.8%	20.8%	-	25.0%	14.8%	35.5%	28.3%	28.6%	27.8%	35.9%
August	20.7%	4.2%	-	12.5%	18.5%	-	32.6%	36.7%	30.5%	38.5%

The same information can be present in a form of a chart (see Fig. 6) to provide better comparison of the honeybee bee colonies in different locations.

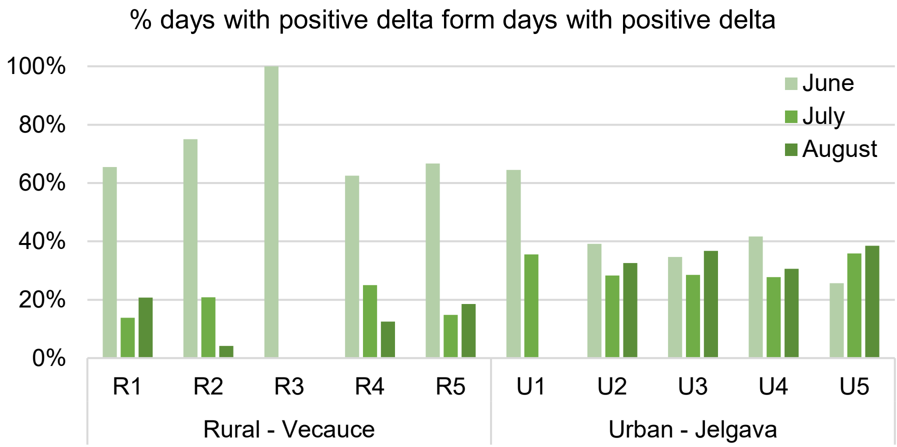

Figure 6. Percentage of days with positive weight gain from days with positive delta.

But the Table 5 below shows the percentage of days with positive delta from all days.

Table 5. Percentage of days with positive delta from all days

	Rural - Vecauce					Urban - Jelgava				
	R_1	R_2	R_3	R_4	R_5	U_1	U_2	U_3	U_4	U_5
June	63.3	60.0	63.3	66.7	60.0	66.7	60.0	56.7	50.0	33.3
July	12.9	16.1	-	25.8	12.9	35.5	41.9	45.2	32.3	45.2
August	19.4	3.2	-	12.9	16.1	-	48.4	58.1	35.5	48.4

By analysing the data, it can be observed that colonies in the urban environment have a positive increase of weight during the whole period, so there are some foraging resources throughout the summer. But the rural apiary is limited to some days of the flowering period, and it can be seen that after intensive and high foraging activity, very small amounts of other foraging sources and plants are available for bees.

Authors should stress out, that current study and bee colony monitoring is limited to one foraging season and one location for each apiary (urban and rural). If bees perform better or worse in one location, it may be because of the particularities of specific location, such as localized pesticide application or intense landscaping with bee-friendly plants, rather than due to urban or rural environments in general.

CONCLUSIONS

Based on the bee colony monitoring and evaluation of the weight gain it can be concluded that for the urban apiary foraging resources are available during the whole summer period, but rural apiary mostly has a decrease in weight in July and August.

Colonies in a rural environment can achieve high foraging performance during several intensive flowering days of the main agricultural crops, like rapeseed and beans.

Remote monitoring of the bee colonies can indicate the start and the end dates of the nectar flow and beekeepers can then decide the necessity to transfer the colonies to other locations.

The monitoring results suggested that colonies in the urban environment also can gain a sufficient amount of resources which can be converted to honey or other bee products.

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Automatic Monitoring of dairy cows' lying behaviour using a computer vision system in open barns

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Abstract. Precision Livestock Farming offers opportunities for automated, continuous monitoring of animals, their productivity, welfare and health. The video-based assessment of animal behaviour is an automated, non-invasive and promising application. The aim of this study is to identify possible parameters in dairy cows' lying behaviour that are the basis for a holistic computer vision-based system to assess animal health and welfare. Based on expert interviews and a literature review, we define parameters and their optimum in form of gold standards to evaluate lying behaviour automatically. These include quantitative parameters such as daily lying time, lying period length, lying period frequency and qualitative parameters such as extension of the front and hind legs, standing in the lying cubicles, or total lateral position. The lying behaviour is an example within the research context for the development of a computer vision-based tool for automated detection of animal behaviour and appropriate housing design.

Key words: animal welfare; computer vision; dairy cow monitoring, lying behaviour, precision livestock farming.

INTRODUCTION

Surveys of the European Union show an increasing interest and concern of citizens about animal welfare (European Commission, 2016). As a result, there is an ongoing controversial discussion about the conditions under which farm animals are kept (Vanhonacker & Verbeke, 2013). The evaluation of animal husbandry systems and the assessment of animal welfare are thus becoming increasingly important.

To date, there is no uniform indicator system for evaluating animal welfare in dairy farming. In practice, the evaluation of animal welfare is based on environmental parameters like available space or cubicle design and management-related parameters like feeding. As these parameters are related to the needs of the animals describing barn circumstances and management, they ensure good housing conditions for animals.

However, they deliver only indirect information on animal welfare (EFSA, 2012). Thus, parameters related to animal behaviour can provide further relevant insights, since animal behaviour is closely related to the physiological status (Neave et al., 2018) and animal welfare (EFSA, 2012).

One relevant behavioural parameter to assess animal welfare, health status and comfort of dairy cows is their lying behaviour (Vasseur et al., 2012; Tucker et al., 2021). In particular, changes in lying time can indicate management problems (Drissler et al., 2005), stall design problems (Gaworski, 2019; Gaworski, 2021), injuries (Ito et al., 2010; Nechanitzky et al., 2016) or a certain physiological condition (Westin et al., 2016). Since any alteration from the optimum can have a negative impact on health and reproductive capacity, several studies analysed its effect on milk production (Norring et al., 2012; Lovarelli et al., 2020), the impact on lameness (Solano et al., 2016), the association with mastitis (Cyples et al., 2012; Herskin et al., 2020) and the oestrus detection (Zebari et al., 2018). Insufficient lying time is associated with poor recovery and frustration and increases the risk for health problems (Welfare Quality Reports, 2009).

Usually, skilled operators carry out the assessment of dairy cow behaviour. However, a continuous direct observation of animal behaviour by the farmer requires a large amount of time and is therefore not feasible in practice. A variety of sensors supports the evaluation of animal behaviour. These can basically be divided into attached and non-attached sensors. Attached sensors mainly detect behaviour and range from acceleration sensors to body temperature measurement. These sensors have a high precision (Stygar et al., 2021; Fan et al., 2022), but are subject to strong mechanical influences or the penetration of dirt and moisture. In the group of non-attached sensors, a promising application for continuous monitoring of animal behaviour is computer vision technology (O' Mahony et al., 2019). The application of computer vision in dairy cow barns allows studying the behaviour of several animals as well as different behavioural patterns at the same time. In the last years, a wide range of studies investigated several use cases and methods for automatic behaviour recognition with machine vision. Determining lameness through automatic gait analysis (Van Hertem et al., 2018; Kang et al., 2020; Wu et al., 2020), location in the stable (Salau & Krieter, 2020), drinking (Tsai et al., 2020; Wu et al., 2021), eating (Porto et al., 2015; Bezen et al., 2020) and chewing behaviour (Wu et al., 2021) as well as lying (Porto et al., 2013; Adriaens et al., 2022), standing (Porto et al., 2015) and social interaction (Guo et al., 2020; Ren et al., 2021) are just some applications for machine vision. Computer vision-based detection enables non-invasive, automated and cost-effective quantification of behavioural patterns.

The cognitive subject of our research is the identification of parameters, which allow the development of an automatic computer vision-based monitoring-system of dairy cows' lying behaviour. Besides the quantitative parameters, we also focus on the qualitative assessment of the lying behaviour. On this basis, we pursue the utilitarian goal of providing information for a non-invasive and automatic approach, that describes the current status of the animals' well-being and gives recommendations for farmers. Thus, we define the following research problem:

Which parameters are suitable for a computer vision-based quantitative and qualitative assessment of the dairy cows' lying behaviour?

In order to address this research question, we conducted expert interviews and a literature review.

RESEARCH APPROACH

This study is part of the SmartMILC project, which aims to develop a holistic system for monitoring animal health and welfare of dairy cows. The project identified five use cases: 'lying behaviour', 'heat stress monitoring', 'work diary', 'barn and herd monitoring' and 'animal health tracking'. This study focusses on the analysis of the 'lying behaviour'. The theoretical framework for each use case is a combination of gold standards and expert knowledge backed up by knowledge from literature (Fig. 1).

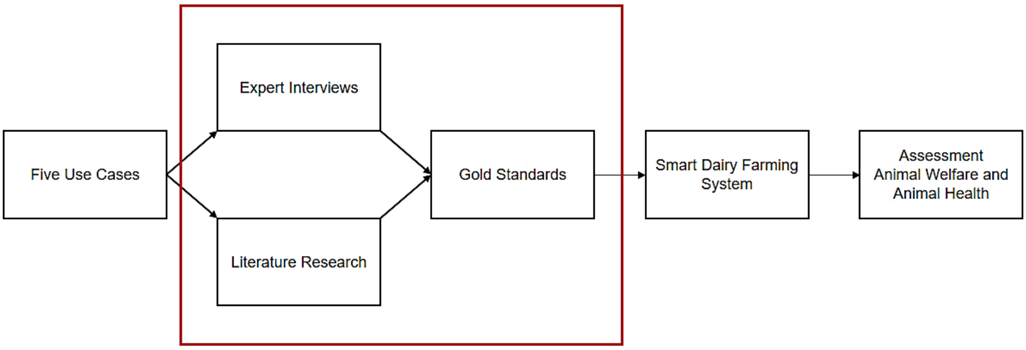

Figure 1. Research approach.

Expert interviews are widely used in qualitative empirical research. These can be used to gain an insight into the expertise of an expert and aim to capture previously undocumented knowledge. For the use case 'lying behaviour' we conducted four interviews, based on a guideline that included an introduction of the interview partners, background information on the project and use case specific information. The aim was to identify how lying behaviour is evaluated in practice, how this behavioural pattern is recorded and what kind of information the experts judge as relevant for automatic vision-based monitoring. The interviews were recorded, subsequently transcribed and key statements were identified.

Based on the literature research we identified descriptors of lying behaviour and their threshold values to derive possible methods for a computer vision-based automatic detection of the lying behaviour and a comparison with gold standards. In the context of our work, we use the term gold standard to refer to the best possible quality criterion or method for evaluation. In this paper, the gold standards are based on the results from the literature research.

RESULTS AND DISCUSSION

Expert interviews

The interviews revealed that lying behaviour is of great importance for assessing the health status of a cow. However, in practice there are clear deficits in both direct and automatic monitoring of lying behaviour. The observation is only selective and mostly carried out during barn work on the farms because direct observation is very time-consuming and subjective. Thus, acceleration sensors attached to the animals are a common alternative to direct observation, but they require a high material input.

The experts rate an automatic recording of animal health parameters, including lying behaviour, as a valuable support in their daily work. Parameters such as ‘lying time per day’, ‘length of lying period’ and ‘lying period frequency’ were named and enable a quantitative assessment of lying behaviour. According to the practitioners, these parameters should also be put into context with environmental parameters such as air temperature, humidity and wind speed and with animal-related indicators such as rumen pH, ruminant activity, milk yield, etc. Two of the four experts placed a high value on the rising behaviour, which, in contrast to horses, begins with raising of the hindquarters. In addition, the quality of lying comfort can be determined by behavioural parameters such as stretched out front or hind leg, side lying, hindquarter outside the cubicle or head resting. The experts also deduce the quality and acceptance of the cubicles from the animal behaviour: if many cows stand with two or four legs in the cubicles, it can indicate an inappropriately adjusted neck rail or an uncomfortable or dirty cubicle.

The small number of interviews is of course a limiting factor, however, the interviewees are to be seen as additional experts to the smartMILC consortium.

Derived Parameters from Literature Research

Different studies record the analysis of lying behaviour of dairy cows by computer vision. Porto et al. (2013) investigated lying behaviour using a multi-camera system and the Viola Jones algorithm. The cameras were mounted above the cubicles and provided panoramic top-view images. As the Viola Jones algorithm cannot recognize rotated images, two classifiers had to be used to recognize the cows in the opposing cubicles. Both classifiers showed a true positive rate of 0.9 but had problems with changing lighting and background conditions. Yin et al. (2020) used the Convolutional Neural Network (CNN) ‘EfficientNet’ for spatial feature extraction and the BiFPN (bidirectional feature pyramid network) to extract the features of behavioural information. Finally, the video images are aggregated into a time series by the BiLSTM (bidirectional long-term memory) module so that behavioural patterns (feeding, drinking, lying, standing and walking) could be detected quickly and accurately. The lying behaviour is detected with a precision of 98.60%. Lighting had little influence on the algorithm, the usability at night is not researched yet. Wu et al. (2021) monitored the basic behaviours drinking, ruminating, walking, standing and lying with the CNN-LSTM algorithm. The algorithm VGG16 is the skeleton of the network and is used to extract the feature vector sequences. They also used subsequently the Bi-LSTM to detect the behaviour. VGG16 is then compared with five algorithms (VGG19, ResNet18, ResNet101, MobileNet V2 and DenseNet201). The accuracy for lying behaviour was almost 0.98. Environmental factors like illumination change, rain or wind had no significant impact on the algorithm. Adriaens et al. (2022) used a cow detection and tracking algorithm (YOLOv5 for detection of changes in bounding boxes and DeepSORT for tracking) with bounding boxes to capture lying down and getting up events. The recognition of the lying down and standing up behaviour is based on changes in the bounding boxes. It has been shown that there are no uniform criteria for the best possible detection; however, the potential of this application has been demonstrated.

These studies show that lying behaviour can be successfully detected using machine vision, but a quantitative and qualitative assessment of this behavioural pattern has not been researched yet. The derivation of parameters for the evaluation of the lying behaviour requires a profound understanding: a typical lying down process begins with selecting a suitable resting location and an olfactory check of the lying place. The first front leg is bent, followed by the second one so that the cow knees on the carpal joint while the hind leg of the intended lying side is placed under the body. This is followed by lowering the body first onto the chest and then onto the hind limbs (Chaplin & Munksgaard, 2001; Pelzer et al., 2012).

Related research used the ‘daily lying time’, ‘lying period length’ and ‘lying period frequency’ as parameters in the lying behaviour context (Mattachini et al., 2019; Lovarelli et al., 2020). The daily lying time ranges from 8 to 13 hours with the most common average of 10 to 12 hours. This behavioural pattern is divided into 9 to 11 lying periods per day of 60 to 99 minutes (Tucker et al., 2021). However, the lying behaviour is subject to numerous influencing factors (Fig. 2).

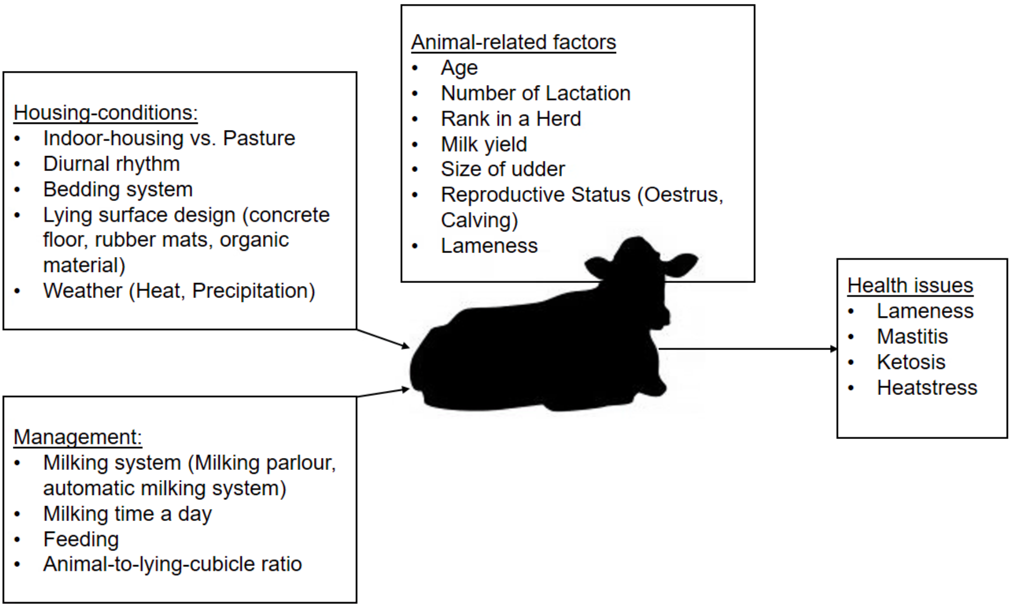

Figure 2. Lying behaviour and its influencing factors.

The lying behaviour of cows is influenced by the daily rhythm (Overton et al., 2002) and daily lying times are significantly longer for cows kept indoors than on pasture due to higher time expenditure for foraging and eating of the latter (Tucker et al., 2021). Another important influencing factor is the cubicle design. Improperly adjusted neck rails or lateral boundaries make it difficult or impossible for a cow to lie down. This is mainly manifested by cows standing with two or four legs in the cubicles (Pelzer et al., 2012). The comfort of the lying surface also has a significant influence on the daily lying time: while lying processes are shortest on pure concrete floors, they increase when the floor is equipped with organic materials or rubber mats (Solano et al., 2016). However, De Palo et al. (2006) observed that with increasing THI, cows prefer lying surfaces that allow good heat dissipation (e.g. sawdust). A lack of bedding as well as wet lying surfaces reduce lying times (Fregonesi et al., 2007; Westin et al., 2016; Schütz et al., 2019). Numerous studies investigated the influence of heat on lying behaviour using the THI. Daily lying times decrease with increasing THI (Cook et al., 2007; Allen et al., 2015). Tresoldi et al. (2019) found that daily lying time is reduced by about 20 minutes for a 1 °C increase in temperature. Besides the housing-conditions, there are management-factors, like the animal-to-lying space ratio, that also influence the lying behaviour. With increasing stock density, average lying times decrease significantly and the variability of lying times increases (Tucker et al., 2021). This effect is more pronounced in lower-ranking animals. The milking management also influences lying behaviour. Whereas in automatic milking systems the cows themselves decide when to be milked, in milking parlours the entire group is driven to get milked. This interrupts lying down processes and with increasing milking times, the animals are forced to stand for longer periods, which in turn reduces lying times (Gomez & Cook, 2010). After a long period of forced standing, cattle show a high preference for lying and neglecting of other important behaviours such as feed uptake (Munksgaard et al., 2005). Some studies show that increasing age and numbers of lactations increase daily lying times (Westin et al., 2016; Henriksen & Munksgaard, 2019) while others found no or moderate influence. A direct relationship between lying time and oestrus has been demonstrated by numerous studies (Silper et al., 2015; Zebari et al., 2018). Silper et al. (2015) found a 37% reduction in lying time on the day of oestrus. Even high milk yields have a negative influence on lying behaviour (Vasseur et al., 2012; Stone et al., 2017). Changes in lying behaviour can be both an indication of disease and a cause of it: while uncomfortable cubicles increase the risk of lameness due to reduced lying time (Thomsen et al., 2012; Bouffard et al., 2017), lameness simultaneously implies longer lying times and lying periods, but a lower lying frequency (Westin et al., 2016). In addition, a shortened lying period can be a sign of mastitis or, if it occurs shortly after birth, of ketosis (Itle et al., 2015). Due to the multiple interpretation possibilities, lying behaviour should always be seen in context with other parameters.

As the aforementioned parameters ‘lying time’, ‘lying-period length’ and ‘lying-period frequency’ just allow statements about the appearance of the lying event, the quality of lying enables animal-related statements regarding animal welfare.

CowsAndMore is a digital tool that facilitates the objective direct recording of animal-related traits. However, the tool ‘CowsAndMore’ intends an assessment twice a year and does not provide any information about the individual. To evaluate cubicle quality, the number of cows standing in cubicles (with two or four legs), lying in a cubicle and lying in the alley is recorded. The lying positions ‘breast position’, ‘stretched front leg’, ‘stretched hind leg > 45°’, ‘sleeping position’ and ‘total side position’ allow statements about both the cubicles and cow comfort. Erp-van der Kooij et al. (2019) used these parameters to find differences in lying posture between outdoor and indoor husbandry. The duration of standing in the cubicle until lying down is also evaluated by CowsAndMore. If this time is less than 30 seconds, the lying down process is assessed as quick, between 30 and 60 seconds as hesitant and over 60 seconds as refusal or aborted (Chaplin & Munksgaard, 2001; Pelzer et al., 2012).

For the evaluation of animal welfare and cow comfort, it is useful to supplement the quantitative parameters with qualitative factors. Based on the expert interviews and the literature research, the following parameters and their gold standards were defined.

The parameters summarized in Table 1 serve as the basis for the automatic analysis of dairy cows' lying behaviour. In particular, we intend to implement a computer vision system that not only recognizes lying behaviour in terms of quantity but also provides insights on lying behaviour quality. For this purpose, we make use of both a 360-degree fisheye camera for a general overview of the barn as well as depth cameras for a more precise view of individual instances of standing, lying down, lying and standing up in the lying boxes. The installed cameras provide regular image data of the recorded barn areas and additional information from the stereo depth cameras.

Table 1. Lying parameter, its description and method of measurement

Parameter	Definition	Measurement	Reference
Lying down	Starts when one carpal joint is bent and ends when the front legs are pulled out from under the torso	< 3 seconds on the carpal joints < 30 seconds: quick 30–60 seconds: hesitant	Chaplin & Munksgaard (2001); Pelzer et al. (2012); CowsAndMore
Interrupted lying down	When the process of lying down is not finished after a period of 60 seconds	> 60 seconds	Pelzer et al. (2012); CowsAndMore
Lying period duration	Duration between the end of lying down and the start of rising	60–99 minutes	Tucker et al. (2021)
Lying time per day	Sum of the lying period duration per day	10–12 hours per day	Tucker et al. (2021)
Lying period frequency	Frequency of lying down per day	9–11	Tucker et al. (2021)
Rising	Starts with head swing and rising of the hindquarter, ends with standing on four legs	Pose estimation and classifica-tion	Pelzer et al. (2012)
Anomaly while rising	Rising like a horse: at first the forehand, followed by the hindquarter	Pose estimation and classifica-tion, < 3 seconds	Chaplin & Munksgaard, (2001)
Hindquarter outside cubicle	Hindquarter overhangs the cubicle edge	Overhang in cm	Interviews
Stretched hindleg	One hindleg is stretched more than 90° from the body	Pose estimation, angle	Pelzer & Kaufmann (2018)
Stretched frontleg	One frontleg is stretched under the head and is not under the cows body	Pose estimation and classifica-tion	Pelzer & Kaufmann (2018)
Lateral position	Lateral position, legs are not under body	Pose estimation	Pelzer & Kaufmann (2018)
Head resting	Head is lying on a front leg, the ground or on the torso	Pose estimation	CowsAndMore
Standing in cubicle	Cow is standing with two or four legs in the cubicle	Pose estimation, Location	Pelzer & Kaufmann (2018), CowsAndMore
Lying outside the cubicle	Cow lies on the walking area, not in the cubicle	Pose estimation, Location	Pelzer & Kaufmann (2018), CowsAndMore

In order to enable the continuous monitoring of the cows' lying behaviour visual data needs to be analyzed automatically - i.e., by computer vision. Recent strides in this domain include an increasing quality in algorithms for object detection and tracking (Redmon et al., 2016; Chandan et al., 2018) as well as pose estimation (Munea et al., 2020; Wang et al., 2021). These are mainly used for studying human behaviour in various scenarios, but approaches for computer vision in livestock farming are gaining momentum. For example, different pose estimation models for animals are available (Lauer et al., 2022) and similar models are used for analysing dairy cows (Ter-Sarkisov et al., 2017; Li et al., 2019).

Starting from this technological progress, we implemented a Cow Detection and Cow Pose Estimation which are illustrated in Fig. 3. On this basis, we intend to develop classification models. Firstly, the classification is aimed to differentiate between standing and lying as well as between the acts of lying down and standing up. Secondly, these acts can also be classified in terms of their ergonomic quality based on training data labelled by experts. Thus, we will be able to derive quantified information regarding the listed parameters in real-time.

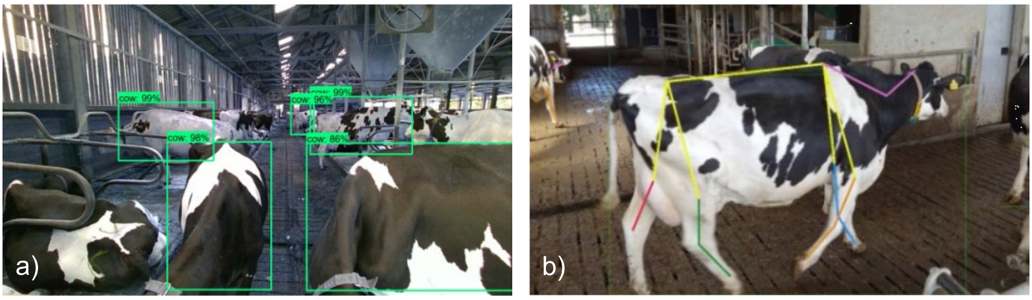

Figure 3. Example frames for (a) Cow Detection and (b) pose estimation.

However, our current challenges include reliable approaches for object detection and tracking as well as pose estimation for images in the barn environment that are characterized by visual obstructions due to overlapping cows and barn infrastructure - occlusion (Russello et al., 2022). Under these circumstances, pose estimation loses accuracy and a differentiation between lying and standing may be impossible. Here, the benefit of including additional information from the depth sensors as well as integrating information from multiple cameras becomes evident.

CONCLUSIONS

In conclusion, we identified a large number of animal-related parameters that allow a qualitative and quantitative assessment of the dairy cows' lying behaviour. This framework is the basis for the continuation of the smartMILC project. With the help of cow detection and pose estimation algorithms, first lying processes have already been successfully detected. The ongoing work includes the accuracy improvement for the detection of lying down and rising up behaviours as well as the computer vision-based detection of the derived qualitative parameters. In summary, the time-consuming direct observation of animal behaviour can be supported by computer vision and helps to improve welfare and health of animals.

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Increasing the sustainability of vegetable crops production by using intercropping

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Abstract. Some scientific reports support the idea of using plant interactions to promote the growth and yielding of vegetable crops. The plant interactions in vegetable production under intercropping conditions were investigated in ERDF funded project 'Elaboration of environment-friendly crop growing technologies identified by the Green Deal and their implementation in horticultural production in Latvia (GreenHort)' implemented in Latvia Institute of Horticulture with the aim to introduce strip cropping in the vegetable production. The investigations were carried out at the Institute of Horticulture, Latvia (57°03'44.6''N, 22°54'53.2''E), during the growing seasons of 2021 and 2022. The vegetable crops (carrots, cabbage, onions, and pumpkins) were grown in intercropping with agroecological service plants or aromatic plants as companion plants (white clover, marigolds, tagetes, lavender, sage, coriander). The investigated variants were compared with the control, where vegetable crops were grown in monoculture as usual. Each intercrop variant consists of 7 alternating rows (each 0.6 m wide) - 4 rows of service crop and 3 rows of vegetable. There was observed significant influence of the growing system on the plants productivity. The sharpest differences between variants were observed for cabbage - marigold, sage and lavender had a positive influence on the cabbage yield formation, but white clover had an extremely negative influence on the cabbage plant growth. White clover had a negative influence also on carrot and pumpkin productivity. Sage had a yield-promoting influence on the carrot crop. There was not found any significant influence of the agroecologial crops on onion productivity.

Key words: cabbage, carrot, onion, strip cropping.

INTRODUCTION

Greening measures are increasingly being introduced in agriculture to foster the implementation of environment and climate-friendly farming practices in line with EU decisions (European Green deal). These measures include a sharp reduction of pesticide use, recommendations to cultivate the legumes for the biological fixing of atmospheric nitrogen (BNF), as well as increasing the microbiological activity of the soil, in order to contribute to the proportion of biologically sequestered carbon (C) which can be achieved by introducing the green manure in the crop rotation. The abovementioned solutions can be assumed also as potentially effective in the changed geopolitical situation in the world

- a critical obstacle encumbering agricultural production is the war in Ukraine causing an increase in energy costs and reduced supply of mineral fertilisers in Europe.

The proposed greening measures are not novel per se: intercropping, catch crops, and green manure are long-known technological elements used by farmers, which, based on past knowledge and experience, can become important retroinnovations (Stuiver, 2006; Zagata et al., 2020; Kaci et al., 2022) by combining past knowledge with the needs and aims of modern society, including the promotion of sustainable farming practices. However, until now these environment-friendly green technologies have not been sufficiently widely and effectively implemented, although several studies have been carried out in this area that demonstrates the effectiveness of these technological solutions (Canali & Coopman, n.a; Talgre et al., 2012; Piotrowska-Dlugosz & Wilczewski, 2015).

It is widely known that some plants containing many flavouring substances have repellent properties that deter pests (Parker et al., 2013; Song & Han, 2020), or allelopathic influence on the soil microorganisms (e.g. nematodes) (Sharadchandra et al., 2012). In addition, aromatic plants attract beneficial insects, that help plants to pollinate, and predatory insects limiting plant pests (Parker et al., 2013; Lauren et al., 2020). By cultivating aromatic plants in intercropping with other horticultural crops, it is possible to reduce the spread of pests to horticultural cash crops. Research on this topic is more prominent in the central and southern part of Europe and other continents. Consequently, the horticultural crops whose mutual interactions are being studied are often different from the commercially important crops which are cultivated in the agro-climatic conditions of Latvia (Parker et al., 2013; Scariot et al., 2016).

However, some scientific reports are found supporting our idea of using plant interactions to promote the growth and yielding of vegetable crops commercially grown in the North Europe region. The findings report not only on the positive plant interactions but also on the depressing (allelopathic) influence. So, John (2010) reports on the inhibitory properties of Trifolium sp. plants on the growth of onions, carrots and tomatoes. Some researchers point out also the influence of soil biological and agrochemical properties on plant interactions, where allelochemicals are involved (Cheng & Cheng, 2015; Schorohodova, 2019). To get an insight into the plant interactions in vegetable production under intercropping conditions, European Regional Development Fund (ERDF) funded project ‘Elaboration of environment-friendly crop growing technologies identified by the Green Deal and their implementation in horticultural production in Latvia (GreenHort)’ is implemented in Latvia Institute of horticulture with the aim to introduce strip cropping in the vegetable production. The purpose of the research was to clarify the plants interactions in strip cropping design and evaluate the influence of intercropped plants on the soil properties.

MATERIALS AND METHODS

The investigation was carried out at the Institute of Horticulture (LatHort) located 90 km to the west of Rīga (in Pūre) (57°03’44.6’’N, 22°54’53.2’’E) during the growing seasons of 2021 and 2022. The trial was set up in a multifactorial design, where factor A – horticultural crops (carrots, cabbage, onions, and pumpkins), factor B – agroecological service plants or aromatic plants as companion plants (white clover (*Trifolium repens* L.), marigolds (*Calendula officinalis* L.), tagetes (Tagetes tenuifolia in 2021 and Tagetes patula in 2022), lavender (*Lavandula angustifolia* L.), sage (*Salvia* *officinalis* L.), coriander (*Coriandrum sativum* L.)); factor C – year (2021 and 2022). The plant combination schemes (variants) are shown in Figs 1 and 2. The investigated variants were compared with the control, where crops were grown in monoculture as usual in the institute. Each variant consists of 7 alternating rows (each 0.6 m wide) - 4 rows of service crop and 3 rows of vegetable. Variant size 4.2 m × 24 m = 100.8 m^2, where 3 replication plots are randomly dispersed for yield and quality measurements.

Year	1st variant		2nd variant		3rd variant	
2021	Carrot	White clover	Cabbage	White clover	Cabbage	White clover
2022	Cabbage		Carrot		Cucurbits	

Figure 1. Scheme of variants of vegetable strip cropping with the white clover as biological nitrogen fixation plant and living mulch plant.

Year	1st variant		2nd variant		3rd variant		4th variant		5th variant	
2021	Carrot	Sage	Cabbage	Lavender	Onion	Coriander	Carrot	Calendula	Cabbage	Tagetes
2022	Cabbage		Onion		Carrot		Cabbage		Onion	

Figure 2. Scheme of variants of vegetable strip cropping with aromatic plants.

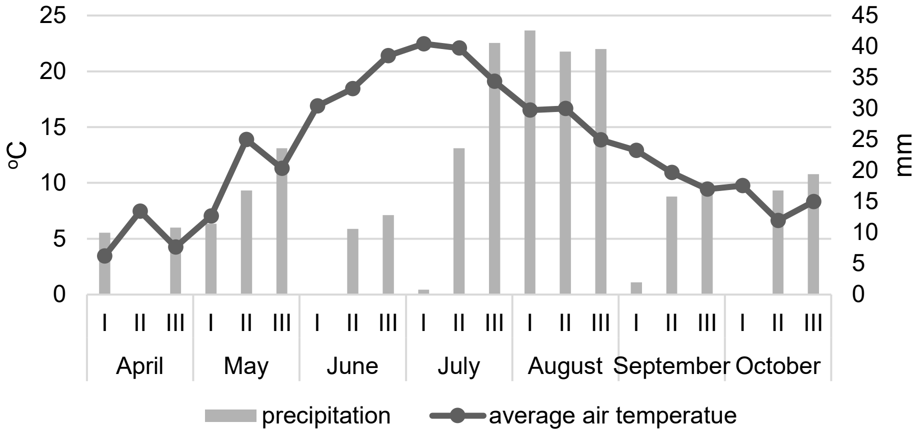
The detailed crops growing technological elements are included in Table 1 to characterise vegetable varieties, growing density, sowing and harvesting times. In monocropping onion and carrot were grown in three-row beds, with the distance between bed centres 1.5 m. Cabbage was grown in rows 0.5 m apart and 0.5 m between plants in the row. Pumpkin was grown in 1.2 × 1.4 m density.

Table 1. Summary of growing technology elements for vegetables and service crops in the intercropping trials of the 2021 and 2022 seasons

Crop	The growing scheme in the intercropping strips of 0.6 m width	Sowing/planting time		Harvest time	
		2021	2022	2021	2022
Carrot \`Solvita\`	2 rows in 0.3 m distance	June 1	May 19	October 5	September 22
Cabbage \`Holsteiner Platter\`	1 row, 0.5 m between plants	May 28	May 25	October 5	October 5
Onion \`Stutgarten riesen\`	2 rows in 0.2 m distance	May 14	May 19	August 16	August 29
Pumpkin \`Red Kuri\`	1 row, 1.4 m between plants	N.A.	June 10	N.A.	September 20
White clover	sown sparsely in all strip width	May 29	N.A.	N.A.	N.A.
Sage	1 row, 0.5 m between plants	June 1	N.A.	N.A.	N.A.
Lavender	1 row, 0.5 m between plants	June 1	N.A.	N.A.	N.A.
Coriander	sow 1 row	May 14	May 24	N.A.	N.A.
Calendula	sow 1 row	June 1	May 24	N.A.	N.A.
Tagetes	1 row, 0.2 m between plants	May 29	June 10	N.A.	N.A.

*N.A. – not applicable.

Meteorological conditions during the investigation periods (precipitation and average air temperature) were collected by an automatic meteorological station 'Davis' located at Pūre (Fig. 3). The precipitation sums of the vegetation period (May–October) for each year were 333.0 and 296.2 mm, respectively. In 2022 precipitation was spread more evenly through the vegetation period in comparison to 2021, when sharper drought periods were observed. In 2021 in May precipitation was almost regular, but in the I decade of June, there was registered a drought period. A dry beginning of the vegetation period was observed in 2022 when in the I and II decade of May the precipitation was only 0.6 and 10.0 mm. In 2022 short drought period was observed at the end of June. Both years' precipitation in July reached more than 60 mm per month. In 2021, August was very wet with 121.4 mm of precipitation, but in 2022 the first two decades were dry - only 3 mm and in the III decade a very high precipitation amount was registered - 83.4 mm (concentrated in two last days of the decade). In September for both years, the precipitation was similar. October 2022 was drier than 2021.



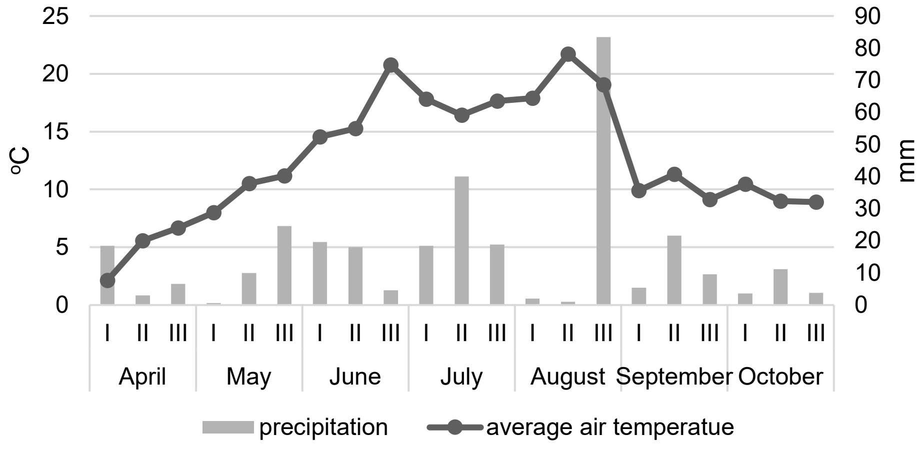


Figure 3. Meteorological conditions of growing seasons of 2021 and 2022 (per 10 days periods (decades) – I, II and III).

The air temperature overall was suitable for vegetable growth and development in both years. The average monthly air temperature during the seasons of 2021 and 2022 was 8.2–21.2 °C and 9.5–19.6 °C, respectively. 2022 was cooler from May–July (except in the I decade of May) compared to 2021. The rest of the vegetation period in 2022 was warmer than in 2021 (except I and III decades of September, when the difference was only 0.3 °C). The warmest period of the season 2021 was in the I and II decade of July but in the 2022 – II and III decades of August.

For the description of the growing conditions, particularly the balance between moisture and temperature during the vegetation period, the hydrothermal coefficient (HTC) was assessed as the ratio between precipitation to 1/10 of the sum of active temperatures (mean daily temperature of the days when it was above 10 °C). Thus, this parameter provides rational information on the correlation between the amount of precipitation in the period, when the average day temperature exceeds +10 °C, and the sum of temperature in degrees in the same period (Table 2). The HTC was calculated by applying the equation described by Selyaninov:

$$HTC = \frac{\sum x}{\sum t} \times 10$$

where $\sum x$ and $\sum t$ – the sums of precipitation and temperatures in the period, when the temperature has been above 10 °C (Selyaninov, 1928; Evarte-Bundere & Evarts-Bunders, 2012; Tchebakova, 2015).

The driest period according to the hydrothermal coefficient (HTC) in 2021 was June when HTC was 0.0–0.6. In the first decade of July, in September and October also HTC was below 1 – which indicates insufficient precipitation and too high temperature during the period. In 2022 were fewer drought periods. The driest period was the first part of August.

Table 2. Hydrothermal coefficient during the trial period in 2021 and 2022

Month	10-days period	2021	2022
May	I	6.6	0.2
	II	1.2	1.4
	III	1.9	2.4
June	I	0.0	1.3
	II	0.6	1.2
	III	0.6	0.2
July	I	0.0	1.0
	II	1.1	2.4
	III	2.1	1.0
August	I	2.6	0.1
	II	2.1	0.0
	III	2.6	4.0
September	I	0.2	1.0
	II	2.8	1.9
	III	3.2	2.3
October	I	0.0	0.5
	II	19.4	2.4
	III	5.5	0.6
Average		2.9	1.3

HTC from 1.0 to 2.0 – humidity is sufficient; HTC > 2.0 – immoderately humid; HTC < 1.0 – insufficient humidity; HTC from 1.0 to 0.7 is assumed as dry period; HTC from 0.7 to 0.4 – very dry period.

The soil type of the trial site was a sandy loam, before the experimental set-up characterized by pH_{KCl} 6.4, P – 31.68 mg kg^{-1}, K – 81.34 mg kg^{-1}, Mg 209 mg kg^{-1} and Ca mg kg^{-1} 1,125, and organic matter of 3.7%.

The yield was harvested in three replications per variant both in monocrop and intercrop variants. To compare the influence of intercropping on the yield formation, the yield outcome in the intercropped variants is calculated for a particular plot area (2 m^2) and expressed in t ha^{-1}. There is no recalculated yield outcome from ha in the intercropping, as it would be harvested from ha (not divided by two). In monocropping variants, the yield was harvested from 3 m^2 plots and expressed in t ha^{-1}.

Mathematical data processing was performed using ANOVA. The data were processed using single-factor dispersion analysis for each year separately. The least significant difference (*LSD*) between individual factor values is indicated in the graphs. A 95% confidence level was used to determine the significance of the difference between the variables.

RESULTS AND DISCUSSION

The influence of intercropping on vegetable plant development was observed for two year period in plant rotation, where the agroecological service crops were not always the same for a particular vegetable crop for both years (see Figs 1 and 2). Therefore ANOVA was performed accordingly for both years separately, and thus *LSD* is calculated for each year separately. There was observed significant influence of the growing system on the plants ability to produce yield. The sharpest differences between variants were observed for cabbage (Fig. 4).

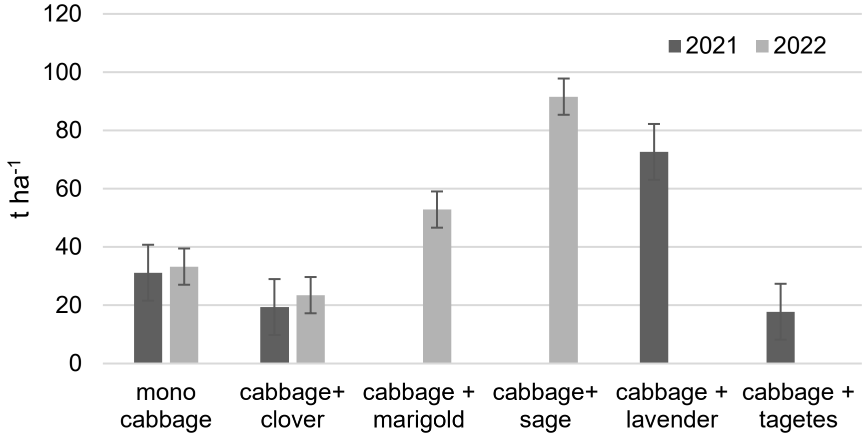


Figure 4. Cabbage yield in the trials of 2021 and 2022.

In 2021, the significantly highest cabbage yield (72.6 t ha^{-1}) was observed in the intercropping with lavender. Where the disorientation of cabbage butterfly (*Pieris brassicae*) was observed - they were strongly attracted by lavender and thus did not lay eggs so much on the cabbage as in monocrop and thus plants were less damaged. The damages in lavender intercrop were scored by 4 (out of 10), but in monocrop by 7. Probably the positive influence of lavender volatiles on cabbage growth also took place. The stimulating effect of lavender oil on the tomato was found in Turkey (Şener et al., 2018). Also sage showed a significant stimulating influence on cabbage yield formation in 2022 (91.6 t ha^{-1}). The scientific evidence on the influence of sage on the neighbouring plants is not found. Probably similar to lavender, it produces volatiles promoting vegetable growth. In 2022, also marigolds as a neighbouring plant had a positive influence on the cabbage yield formation, reaching 52.8 t ha^{-1}. The real influence of tagetes on the cabbage plant growth and development is hard to estimate, because, in 2021, when high-growing *Tagetes tenuifolia* were planted in intercropping with cabbage, they completely depressed the cabbage plants, thus creating impossible conditions for cabbage plant development. The findings of others support the positive influence of tagetes (*T. patula*) on cruciferous vegetable development (cauliflower) and other vegetables (Li et al., 2020; Mrnka et al., 2020).

The most surprising was the cabbage yield reduction in the intercropping variant with white clover as living mulch and BNF plant for both years. The harvested yield was only 19.4 and 23.5 t ha^{-1} correspondingly. It was observed that white clover is a rather aggressive plant, which spreads towards the cabbage strip every year more and more, although it was limited by a hack. We can assume that white clover has dispensed allelochemicals suppressing cabbage development. A slight phytotoxic influence of pea residues on the vegetable crops, such as carrot, eggplant, bean and Chinese cabbage is reported by John (2010). He mentions also that volatile emissions from residues of the winter cover legumes, Berseem clover (*Trifolium alexandrinum* L.), hairy vetch (*Vicia hirsute* L.), and crimson clover (*Trifolium incarnatum* L.), inhibited germination and seedling development of onion, carrot and tomato. Hydrocarbons, alcohols, aldehydes, ketones, esters, furans, and monoterpenes were identified in these residue emission mixtures (John, 2010). We can speculate that probably white clover (also belonging to *the Fabaceae* family) has an allelopathic influence on the development of cabbage. Further research is needed to confirm our assumption.

Regarding carrot yield in the trials, similarly, we can state that there are significant differences between the cropping systems (Fig. 5).

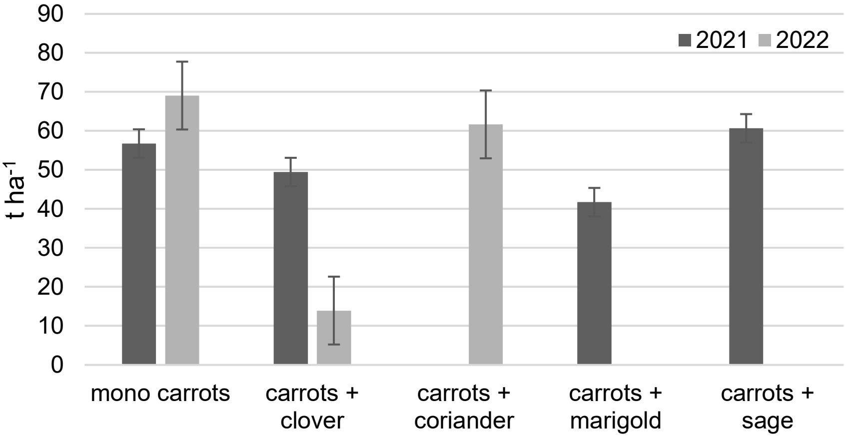


Figure 5. Carrot yield in the trials of 2021 and 2022.

In 2021, the highest yield was harvested in the intercropping variant with sage (60.7 t ha^{-1}), but the difference with the monocrop was not statistically significant. Notably lower yield in comparison to monocrop was harvested in the intercropping with marigold - 41.7 t ha^{-1}. In 2021, the variant with white clover yielded slightly lower carrot yield as it was harvested in monocrop variant (49.5 and 56.8 t ha^{-1}, correspondingly).

In 2022, there was not found a statistically significant difference between monocrop variant and intercropping with coriander - 69.1 and 61.7 t ha^{-1}, correspondingly. Contrary, in other trials where the carrot was intercropped with coriander, the positive influence of coriander on the carrot growth and yield formation was found

(Mehta et al., 2010). In our trial, similarly like in other crops, also for carrot significantly lower yield was obtained in intercropping with white clover (13.9 t ha^{-1}).

Summarizing carrot trial results, white clover had a negative influence on the yield formation as in cabbage. Contrary, the sage had a stimulating influence on the carrot yield formation, but coriander had no significant influence on the carrot yield formation.

In evaluating the onion yielding in intercropping with different agroecological service plants, some similarities were found regarding service crop influence on the cash crop (Fig. 6).

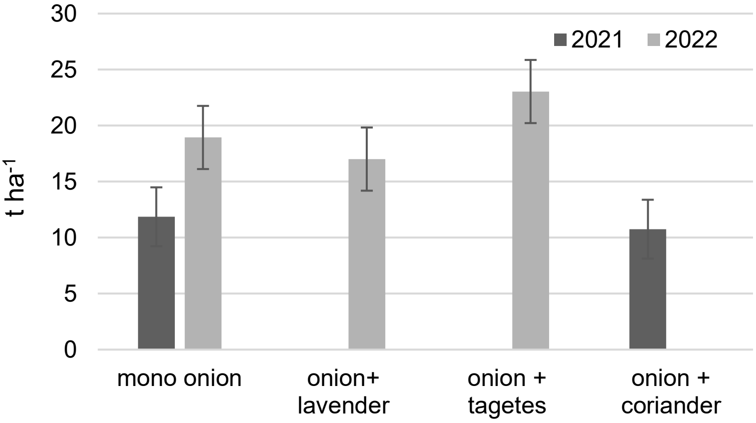


Figure 6. Onion yield in the trials of 2021 and 2022.

In 2021, there was not found a significant influence of coriander on onion crop formation. There was statistically indifferent yield harvested in both variants - 11.9 and 10.8 t ha^{-1} in monocrop and intercropping with coriander, correspondingly. Our results are supported by the findings of the trial in India, where coriander had no positive impact on the onion yield when both crops were intercropped (Talukder et al., 2015).

In 2022, the statistically significant influence of lavender and tagetes was not found in the intercropping with onions. Although higher onion yield was obtained in the intercropping with tagetes (23.0 t ha^{-1}). Also, tagetes are reported as an efficient attractant of natural enemies of onion pests, particularly trips in the trials in Brasil (Silveira et al., 2009). Also, other positive interactions of vegetable crops with tagetes are mentioned by others, mostly on the limitation of pest damages (Shiu & Wu, 2010.). In our trials thrips damages for onion crops for the years of the investigation were not found, therefore the influence of tagetes on the trips spreading was not evaluated. Obviously, also other mechanisms of plant interaction have taken place in our trial.

Summarizing the onion intercropping results, we conclude that during the two years of investigation, there was not found a statistically significant influence of tested service crops on the onion yield.

The trials on the pumpkin intercropping were performed only in the year 2022. Only one intercropping variant was tested – pumpkin with white clover. Similarly like in the trials with cabbage and carrot, also for pumpkins there was obtained sharply lower yield (4.2 t ha^{-1}) in comparison to monocropped pumpkins (24.7 t ha^{-1}).

Summarizing the results of our two-year trials, the most surprising was the negative influence of white clover on the cash crop plant development and yielding. The tendencies of the slight leek yield reduction when grown with white clover are mentioned also by den Hollander et al. (2007). They mention clover's competitive habitus character and hindered nitrogen uptake as two main reasons for the obstructive influence on the neighbouring crops. Although white clover is mentioned as a less influencing specie. In our assumptions, the hindered nitrogen uptake in cash crops and possible diffusion of allelochemicals in the soil should be investigated further as causes of the negative influence of white clover as neighbouring plant to vegetable crops.

CONCLUSIONS

Concluding the results of the two-year trials, we find out that sage and lavender have a positive influence on vegetable yield formation, similar, to calendula and low-habitus tagetes. Notable reaction on the intercropping was stated for cabbage, both positive and negative. An especially clear negative influence of white clover as a neighbouring plant was observed for cabbage and pumpkins, but also carrot yield suffered from the white clover intercropping. Further investigations on the white clover interaction mechanisms with neighbouring plants should be investigated.

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Volatile organic compounds and their generation in sourdough

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Abstract. Sourdough technology is involved in bread making process for improving the sensory, rheological, nutritional and shelf life characteristics of bakery products. More than 540 volatile organic compounds (VOCs) and other flavour precursors belonging to the chemical classes, such as aldehydes, ketones, esters, acids, alcohols, terpenes and others, have been identified in sourdoughs and sourdough breads. The synthesis of VOCs is microbial species-specific, originating mainly from fermentation process. VOCs can be used as indicators to characterize microbial processes. Other additional sources of VOCs in sourdoughs are lipid oxidation and browning reactions, the latter of which occurs during the production of dried starter cultures. The purpose of this article is to provide an overview of the composition of VOCs and their effect on the sensory properties of sourdough bread, and to describe the most common extraction methods of VOCs used in the studies of sourdough and bread aroma profile. Long-term propagated sourdough VOCs have been less studied compared to volatiles found in bread crust and crumb or sourdoughs started with defined starter culture(s) due to their complexity and diversity in metabolic pathways, including sophistication of the analytical methodology of VOCs. The relation between sourdough microbiota and its volatile profile is not fully understood and therefore, their variability and precise role as a bread flavour enhancer is not yet known in detail.

Key words: lipid oxidation, proteolysis, sourdough fermentation, volatile profile.

INTRODUCTION

The use of sourdough in food fermentation as a leavening agent for bread making is one of the oldest biotechnological processes in cereal food production (Röcken & Voysey, 1995). Sourdough is a mixture of cereal flour and water, which is fermented mainly by facultative and obligate heterofermentative lactic acid bacteria (LAB) and yeasts. Sourdough fermentation can be started spontaneously or initiated by the addition of mother sourdough or defined starter culture (s), including constantly refreshed sourdough with the mixture of flour and water. Metabolic processes of sourdough microbiota are mainly associated with the fermentation of bread dough (by LAB), the formation of aromatic compounds of bread (by LAB and yeasts) and the proofing of bread dough (by yeasts and heterofermentative LAB) (Hammes & Gänzel, 1998; De Vuyst et al., 2009, 2016).

All food products contain hundreds of volatile organic compounds (VOCs) (Maarse, 1991). VOCs in wheat and rye bread crumb and crust have been extensively studied, however much less is known about their variability and concentration in long-term propagated sourdoughs, especially in rye sourdoughs. Several studies (Schieberle & Grosch, 1994; Kirchoff & Schieberle, 2001; Pico et al., 2018) have been indicating that the flavour compounds of the bread crumb are significantly different from those of the bread crust due to the process parameters in sourdough fermentation and baking. Flavour components occurring in the bread crumb are mainly produced during sourdough fermentation while the baking process has the greater effect on the flavour of the bread crust (Heiniö, 2003; Pico et al., 2015). Although main bread aroma compounds are produced upon baking, the importance of fermentation should not be neglected since fermentation is a source of several precursor VOCs that only react at baking temperatures. Studies by Hansen (1994a, 1994b), Gobbetti et al. (1995), Ravyts & De Vuyst (2011), Settanni et al. (2013), Ventimiglia et al. (2015), Ripari et al. (2016) and Warburton et al. (2022) have demonstrated that the volatile fractions of wheat and rye sourdoughs are very complex and consist of numerous compounds with a variety of functional groups. The range of research on VOCs in sourdough has been relatively limited in terms of the diversity of microbial cultures, including regional differences in sourdough fermentation and propagation technology, and the variety of generated VOCs. Overall, the detailed knowledge of the microbial and enzymatic processes involved in sourdough fermentation, especially the evolution of aroma volatiles at the early stages of sourdough preparation, is limited and undervalued. In order to understand the impact of microbiota, raw material and technological parameters on the sensory properties of bread, it is important to know which VOCs are produced by the sourdough microbiota during its several metabolic pathways. Due to the multiplicity and complexity of metabolic pathways, including the species diversity of microbial cultures in sourdoughs, it is difficult to identify the sources of various volatile compounds more precisely. This article provides a systematic overview of the VOCs associated with sourdough and their effect on bread flavour and aroma, including a brief overview of commonly used methods for detecting these aroma compounds.

VOCs, VOLATILE ORGANIC COMPOUNDS

VOCs have a significant impact on the formation of organoleptic properties (aroma and flavour) of different fermented food products (Pinu & Villas-Boas, 2017). These are defined as a class of low-molecular-weight (< 300 g mol^{-1}), carbon-containing aliphatic and aromatic compounds characterized by their high volatility, low vapor pressure (≥ 0.01kPa at 20 °C), and low water solubility (Schulz & Dickschat, 2007; Herrmann, 2010). VOCs are comprised of hydrocarbons, acids, alcohols, aldehydes, aromatics, ketones, terpenes, thiols, and their derivatives (Pennerman et al., 2016). Their concentration varies between ng L^{-1} to g L^{-1} depending on the various metabolic processes occurring in food (Pinu & Villas-Boas, 2017). These compounds exist in the initial raw material, but can be also produced by different microorganisms (e.g. LAB, yeasts, fungi) as metabolites and by-products through catabolic pathways, including glycolysis, proteolysis, and lipolysis (Schulz & Dickschat, 2007; Pinu & Villas-Boas, 2017).

Analytical methods for detecting VOCs in sourdough

Assessment of the VOCs profile requires sample preparation, extraction of VOCs from matrix to be examined and their identification and quantification using relevant analytical instrumental method. There are several approaches for isolating and concentrating VOCs from the cereal-based products such as steam distillation/extraction, supercritical CO_2 extraction or solid phase microextraction (Chambers & Koppel, 2013; Maarse, 1991). The oldest and most common technique of VOCs characterization and measurement involves the use of analytical techniques such as gas-chromatography (GC) coupled with mass-spectrometry (MS) and GC-MS with an olfactometric port or a sniff port (Lawless & Heymann, 2010). In recent years, several online tools based on MS, such as membrane inlet MS, selected ion flow tube MS (SIFT-MS), proton-transfer-reaction MS (PTR-MS), and PTR-time-of-flight MS (PTR-ToF-MS), are being studied and applied for the assessment of VOCs. The high time resolution brought by PTR-MS is one of the direction-injection MS technologies which allows non-invasive VOC fingerprinting (Farneti et al., 2015). The coupling of PTR-MS with ToF mass analyzers has improved time and mass resolution, allowing the monitoring of VOCs during food fermentation process (Romano et al., 2015). Due to its advantages of rapid response, absolute quantification, and high sensitivity, PTR-MS has been successfully applied to detect VOCs in the field of food research (flavour studies, assessment of food quality) (Wang et al., 2012) including breadmaking process (Makhoul et al., 2014, 2015; Pico et al., 2018, 2020). While the sourdough fermentation process has been successfully examined by SIFT-MS (Van Kerrebroeck et al., 2015; Van Kerrebroeck et al., 2018) due to its simple use and direct quantification, PTR-ToF-MS has proved to be a fast high-throughput tool for the study of interactions between microbes (LAB, yeasts) and flour. Thereby manufacturers have the opportunity to choose the combination of ingredients able to yield the production of preferred volatiles serving as flavour precursors in the final baked product (Makhoul et al., 2015).

Various methods have been used to extract flavour compounds from bread and sourdoughs, but the main ones are solvent extraction methods and headspace analysis (HS) followed by identification by GC-MS (Pico et al., 2015; Petel et al., 2017). However, dynamic headspace (DHS), especially in-tube extraction (HS-ITEX), has been proposed as an alternative to optimize HS. DHS method provides better sensitivity and has fewer problems related to the selectivity of the extraction to matrix effects, but so far, only one study has investigated the optimization of DHS extractions based on sourdough (Fuchsmann et al., 2019; Dias et al., 2022).

Solvent extraction process followed by concentration under nitrogen or in a rotary evaporator was one of the first extraction methods for the separation of VOCs from foods (Zhou et al., 1999). Yet, the solvent extraction method by Soxhlet apparatus allows the extraction of a large number of volatile compounds (Sides et al., 2000) which yet may introduce potential qualitative or quantitative errors in the results (Heiniö, 2003). For instance, the solvent may limit the identification of volatile compounds (poor selectivity) and its removal requires heating that could cause thermal degradation of organic compounds (Sides et al., 2000), also flavour components may become contaminated by unwanted material such as lipids and non-aroma hydrocarbons (Zhou et al., 1999).

Nowadays the solvent-based extraction is less used due to the above-mentioned disadvantages, e.g. volatile compounds elute solvent-specifically and therefore some of the odour active compounds may disappear (Heiniö, 2003). The most preferred option for extracting volatiles from bread and sourdough is the headspace (HS) method (Ravyts & De Vuyst, 2011; Aponte et al., 2013; Settanni et al., 2013; Ripari et al., 2016). More precisely, solid phase micro-extraction (HS-SPME) coupled to GC-MS has been preferred because it is a rapid, convenient and solvent-free technique (Thompson-Witrick et al., 2015). In HS-SPME the samples are generally cleaner, containing fewer compounds than samples pre-treated with organic solvents (Grosch, 2007; Prost et al., 2012) and it may happen that important volatile substances present in sourdough in low concentrations give no electrical signal. Therefore, in order to achieve reproducibility, this method requires the precise control of many parameters (like sample volume, extraction temperature, equilibrium time, fibre coating etc.) which differ according to the food matrix (Thomsen et al., 2014; Pico et al., 2015).

In addition to above-mentioned methods, it is also conceivable to use supercritical fluid extraction (SFE) which is generally used as an alternative primary method for Soxhlet type extractions (Doane-Weideman & Liescheski, 2004). So far there is no overview of the application efficiency of the SFE method for analysing VOCs in bread or sourdough, but it has found a use in determining polycyclic aromatic hydrocarbons in toasted breads (Kayali-Sayadi et al., 2000). The main advantages of SFE for the extraction of odorant compounds from food matrices compared to conventional extraction methods are its safety (GRAS solvent in comparison to many organic solvents), higher selectivity, shorter extraction times and the possibility of its direct coupling with analytical chromatographic methods (Doane-Weideman & Liescheski, 2004; Herrero et al., 2006).

Qualitative and quantitative methods for the analysis of flavour compounds in various fermented food products have developed a lot, but the synergistic effect of several flavour substances is still difficult to evaluate due to the complexity of LAB and yeasts metabolism. Different sampling techniques offer a number of individual advantages but also suffer from specific limitations, e.g. the potential destruction of aroma components and/or production of flavour artifacts. It has been shown that the choice of analysis method for volatile compounds from cereals has a significant influence on the compounds recovered, and therefore the results obtained by using different methods should be considered carefully (Heiniö, 2003; Boyacı et al., 2015). While there are several approaches to the analysis of VOCs, there is a constant demand for a method that prevents oxidation, thermal degradation, and other chemical and biochemical changes in the sample. Currently, there is no standard method for the determination of volatile compounds in sourdough, which would enable an adequate comparison of the results of different studies, which is why there are no accurate profiles of volatile organic compounds for sourdoughs. Among the existing ones, the HS-SPME and HS-ITEX could be the most suitable methods to develop a standard extraction method for the analysis of VOCs in sourdough and sourdough bread.

Common VOCs in sourdough

Aromatic compounds in food are commonly formed in four ways such as biosynthesis, enzymatic action, oxidative decomposition and pyrolysis. The biosynthesis of VOCs mainly depends on the citric acid and amino acid metabolism pathway. The volatile profile of sourdough is rather complicated and difficult to describe thoroughly due to the variety of LAB and yeasts, fermentation substrates and flavour substances produced. (Wang et al., 2021) Sourdough VOCs are mainly produced by microbiological and enzymatic processes during fermentation (Petel et al., 2017), which depend on interactions between LAB and yeasts (Zhang et al., 2021; Fang et al., 2023). The aroma compounds produced by the sourdough microbiota can be divided into two groups: first, non-volatile compounds, including non-volatile organic acids (lactic and pyruvic acid) which acidify and contribute to the aroma of bread (Gobbetti et al., 1995) and second, VOCs such as alcohols, aldehydes, ketones, esters and sulphur, as well as volatile acids like butyric and acetic acid (Pan et al., 2014; Fang et al., 2023). The study of VOCs has mostly relied on the formation of volatile compounds in sourdough using specific microbial cultures (Kaseleht et al., 2011; Yang et al., 2020; Luca et al., 2021; Warburton et al., 2022), natural starters from local bakeries (Bianchi et al., 2008), or by creating spontaneous starters (Ripari et al., 2016; Siepmann et al., 2019; Katsi et al., 2021).

Organic acids, alcohols, esters, ether and furan derivatives, lactones, pyrazines, pyrrole derivatives, sulphur compounds and carbonyls mostly affect the sensory properties of bakery products (Hofmann & Schieberle, 2000; Czerny & Schieberle, 2002; Kirchhoff & Schieberle, 2002; Pacyński et al., 2015; Giannone et al., 2018; Gancarz et al., 2021). The metabolism of VOCs during sourdough fermentation is influenced by the interaction between LAB or/and yeasts, and the type of fermentation pattern where the activity is mainly attributed by endogenous or non-controllable parameters (e.g. nutrient composition) and, exogenous or technological parameters (e.g. fermentation time and back-slopping, temperature), affecting the amount of formed metabolites (Vrancken et al., 2011). Petel et al. (2017), Liu et al. (2020) and Wu et al. (2022) have reported that many VOCs begin to accumulate after 12 h of fermentation. According to Hansen & Hansen (1996), Decock & Cappelle (2005) and Catzeddu (2019), sourdough bread have more VOCs compared to yeast leavened bread.

Hydrocarbons (e.g. benzene, propane) have been the most abundant chemical class detected in sourdough fermentation followed by esters, alcohols, ketones, aldehydes, and sulphur compounds. Origins of hydrocarbons are not yet clearly identified in the literature but their occurrence is more common in studies that employed HS extraction (Seitz et al., 1998; Bianchi et al., 2008) compared to solvent extraction (Kirchhoff & Schieberle, 2001; Czerny & Schieberle, 2002; Pizarro & Franco, 2017). The most frequent VOCs detected in bread crust and sourdough are 2- methyl-1-butanol, 3-methyl-1-butanol, 2-methyl-1-propanol, phenylethyl alcohol, hexanal, benzaldehyde, 3-methylbutanal, ethyl acetate, ethyl octanoate, acetic acid and butanoic acid. These compounds originate from the fermentation process, lipid oxidation, and Maillard reactions (Birch et al., 2014; Pico et al., 2015; Pizarro & Franco, 2017).

Isoalcohols, e.g. 2-methyl-1-propanol, 2,3-methyl-1-butanol, with their respective aldehydes and ethyl acetate, are synthesized inside the yeast cell due to the degradation of the flour amino acids (Damiani et al., 1996; Birch et al., 2014). According to Hansen & Schieberle (2005), the different amount of alcohol production may be related to the distinct degradation reaction of amino acids occurring during sourdough fermentation via the Ehrlich pathway, leading to formation of aldehydes or the corresponding alcohols. Alcohol formation involve the initial transamination of an amino acid producing an α-keto acid, irreversible decarboxylation of the acid to the fusel aldehyde, and reduction by alcohol dehydrogenase to fusel alcohol (Birch et al., 2014).

Generation of VOCs in bread and sourdough is also influenced by both raw material and technological parameters (Hansen et al., 1989; Saa et al., 2019; Kirchoff & Schieberle, 2002). The formation of principal VOCs are mainly induced by the sourdough fermentation process, lipid oxidation and Maillard reaction, while volatile compounds from raw materials have only a minor effect on the amount of VOCs in the sourdoughs (Pozo-Bayon et al., 2006; Prost et al., 2012). Due to the lack of studies, precise data on the effect of the interaction of microbial communities, technological parameters and raw materials on the concentration of VOCs is not available. From few studies (Lund et al., 1989; Kirchhoff & Schieberle, 2002; Kaseleht et al., 2011) it appears that the most abundant amounts of ethanol, iso-alcohol, hexanol, acetic acid, hexanoic acid, dimethyl sulfide, ethyl acetate, ethyl lactate, ethyl hexanoate, pentanone and 2,3-butanedione are present in rye sourdoughs. Extruded rye sourdough has been reported to contain high levels of furfural, ethyl acetate, 3-methylbutanol, 3-methylbutanal and 2-methylbutanol (Heiniö et al., 2003). The characteristic strong flavour of rye bread is induced by the alcohols, 2-phenylethanol (phenolic, plastic), 2-methylbutanol (roasted, fruity) and benzyl alcohol (vinegar) and acetone (essential, fruity, acidic) (Hansen et al., 1989). Esters in sourdough and bread are associated with a pleasant fruity (apple, banana) and sweet aroma (Reale et al., 2016; Pizarro & Fransco, 2017) although their concentration declines due to their hydrolysis by the heat during dough baking (Hanis-Syazwani et al., 2018). Ethyl esters originate from the reaction of alcohols and acetyl coenzyme A derivatives of fatty acids, but fatty acid esters may be a product of free fatty acids from further oxidation of aldehydes, reacting with some alcohols (Pico et al., 2015). Hexadecanoic acid and 1-(hydroxymethyl)-1,2-ethanediyl ester have been identified as the most common esters in rice bran sourdough. Other esters found in the rice sourdough are 9-octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, methoxyacetic acid, 3-tridecyl ester, and pentanoic acid, 4-methylethyl ester (Bolarinwa et al., 2019; Özgül-Yücel & Türkay, 2002). A study by Özgül-Yücel & Türkay (2002) and Bolarinwa et al. (2019) indicate that the types and amounts of esters are influenced by the temperature and time of fermentation. Reale's et al. (2016) data showed that the production of ethyl acetate, isoamyl acetate and ethyl hexanoate was improved when the yeast was co-cultured with *L. casei* N87 grown under respiratory conditions. In the evaluation of volatile organic compounds, it was observed that the respiratory metabolism of *L. casei* N87 significantly affected the flavour of the sourdough, demonstrating that the type of cultivation of microbial starter can have an important factor in the formation of the profile of volatile compounds in sourdough. Low concentrations of hexyl acetate and isoamyl lactate were found only in dough inoculated with *L. casei* N87. The increase in their content can be facilitated by the use of heterofermentative LAB in wheat/rye firm sourdoughs. Moreover, Lanciotti et al. (2003) proved that hexyl acetate, hexanal and (*E*)-2-hexanal have significant inhibitory effects against pathogenic microorganisms.

FORMATION AND ORIGIN OF VOCs IN SOURDOUGH

Raw material as a source of VOCs

In addition to chemical and microbiological generation of volatile compounds, some of the flavour-active compounds are also detected naturally in the cereal flours, which, based on their flavour activity values (ratio of concentration to flavour threshold), have an impact on both the overall aroma of sourdough and the bread itself. The flour in the sourdough is a substrate for microorganisms with its heterogeneous nutritional composition. Most of the sourdoughs are made of wheat and/or rye flour (Meroth et al*.,* 2004; Valcheva et al*.,* 2005; De Vuyst et al*.,* 2009), but corn, cassava, barley, emmer, oat, rice and teff flours and flours based on legumes (fava bean, pea, chickpea) and pseudo-cereals, such as buckwheat, amaranth and quinoa, have also found application in the sourdough fermentation. Some investigations refer to using teff flour in sourdough fermentation which has a great influence on the aroma profile of the baked products due to its high contents of unsaturated fatty acids and phenolic compounds (Kirchhoff & Schieberle, 2001; Gänzle, 2015; Campo et al., 2016). In general, scarce information is available on the VOC profile of fava bean (*Vicia faba* L.), although it has been subjected to several studies in last decade for increasing cereal food nutritional quality (Rizzello et al., 2014; Coda et al., 2017; Verni et al., 2019). Fava beans are characterized by off-flavours resulting from non-VOCs that cause bitterness and unpleasant odours, which are mainly promoted by auto- and enzymatic oxidation of free fatty acids and degradation of free amino acids during seed processing. Its off-flavours are defined for instance as dried or fresh pea, mouldy, rancid and yeast (Karolkowski et al., 2021, 2022; Tuccillo et al., 2022). Although beans have off-flavours and their addition can compromise the gluten network, they are still considered sustainable and highly nutritious gluten-free alternatives to cereal flours. Huang et al. (2023) have shown that using the exopolysaccharide-producing strain *Weisella confusa* QS813 in red bean (*Vigna angularis*) sourdough fermentation can improve the rheo-fermentation and viscoelastic properties of red bean dough, as well as the aroma profile of bread made from it. Wheat-red bean sourdough bread was enriched with new aroma compounds, such as acetic acid and a higher content of 3-methyl-1-butanol and 2,3-butanediol. Their findings were consistent with other studies (Cho & Peterson, 2010; Pico et al., 2015), which observed that the use of sourdough in breadmaking reduces the total content of aroma compounds, but increases other types of aroma compounds in the bread crumbs. Perri et al. (2021) also identified the positive effect of using dextran producing LAB (e.g *W. confusa* SLA4) and sprouted lentil sourdough on the sensory (synthesis of bread key-aroma compounds) and nutritional quality (high fiber content) of white sourdough bread.

Insect flour (house cricket *Acheta domesticus* L.) is also a new promising and sustainable raw material for the production of sourdough due to its nutrient richness and suitability to support the growth of the *Lactiplantibacillus plantarum* strain. Beldean et al. (2022) study highlighted that during controlled insect flour sourdough fermentation with *L. plantarum* ATCC 8014 strain, higher level of benzaldehyde, 2-methyl-5-propan-2-ylcyclohex-2-en-1-one, p-cymene and β-myrcene were produced; meanwhile, the spontaneous fermentation enhanced the formation of benzoic acid and disulphide dimethyl, with a faint and unpleasant odour perception. Among these, the odour of 2-methyl-5-proparn-2-ylcyclohex-2-en-1-one is associated with spicy, minty, caraway, bread, and rye bread. The formation of benzaldehyde and 3-methyl-1-butanol could be the result of degradation of phenylalanine and leucine, respectively (Pico et al., 2015; Fang et al., 2023). In a recent study, Bartkiene et al. (2023) found that non-fermented cricket flour contained higher concentrations of acetic acid, hexanal and decane, but their content decreased after fermentation by *L. plantarum* no. 122. After 48 hours of fermentation, hexanal was not detected in the fermented samples. Furthermore, the content of several other compounds, including 1-octen-3-ol, 2-pentylfuran, 4-methyldecane, 3,6-dimethyldecane, 3-methylundecane, ethyl octanoate, and dodecane, decreased during fermentation. Meanwhile, the concentration of various VOCs such as acetoin, 2,3-butanediol, butanoic acid, 3-methylbutanoic acid, 2-methylbutanoic acid, 1-hexanol, 2,6-dimethylpyrazine, 2,2-dimethyl-3-heptanone, 2,6-dimethyl-4-heptanol, 2-hydroxy-

3-methylpentanoic acid methyl ester, benzaldehyde, phenol, benzeneacetaldehyde, 3-ethyl-2,5-dimethylpyrazine, nonanal, and phenylethyl alcohol, increased due to the metabolic activity of LAB. Nissen et al. (2020) concluded in their study that insect flour gives bread a unique bouquet of volatile compounds consisting of nonanoic acid, 2,4-nonadienal (*E,E*), 1-hexanol, 1-heptanol and 3-octene-2-one. The most interesting results were those obtained from sourdough fermentation, where the amount of acetoin in the cricket sourdough samples (24.42 ± 0.23 mg kg^{-1}) was twice that of the control sourdough samples (13.74 ± 0.35 mg kg^{-1}). Similarly, higher concentrations of acetoin, ethyl acetate and acetic acid have been noticed in breads made with teff sourdough compared to breads made with LAB-initiated wheat sourdough. It can be associated with the high content of unsaturated fatty acids and phenolic compounds in teff flour, which could influence the formation of VOCs (Campo et al., 2016). The high content of acetic acid has a positive effect on the aroma of the bread and an antimicrobial effect against molds and rope-forming bacilli (Rosenquist & Hansen, 1998).

The first studies on the composition of VOCs in rye flour were carried out by Markova et al. (1970), Hougen et al. (1971), Prince & Mackey (1972). Several studies indicate that the concentrations of aroma compounds vary widely in straight-grade, low-grade, and wholemeal flours, and therefore the choice of flour type affects the aroma quality of sourdough bread (Hansen & Hansen, 1994a; Czerny & Schieberle, 2002; Kirchhoff & Schieberle, 2002; Galoburda et al., 2020).

When bakery products are made from flour rich in phenolic acids, such as wholemeal flour, the ability of LAB and yeasts to reduce or decarboxylate phenolic acids is significantly altered (De las Rivas et al., 2009). Some LAB have strain-specific properties to decarboxylate and/or reduce phenolic acid. The use of free phenolic acids increases the bioavailability of phenols as antioxidants. In addition, the reduction of phenolic acids is important for the elimination of bitter taste compounds in bread (Gänzle, 2014). A study by Kirchhoff & Schieberle (2002) suggested that many compounds already present in flour belong to the important odorants in sourdough, such as methional or (*E,E*)-2,4-decadienal. Some aldehydes that are already present in the flour and undoubtedly resulting from the peroxidation of flour lipids, for example, hexanal or (*E*)-2-nonenal, are clearly degraded during the fermentation process. Czerny & Schieberle (2002) identified fatty-smelling odorants (*E,Z*)- and (*E,E*)-2,4-decadienal, (*E*)-2-nonenal, (*E, Z*)-2,6-nonadienal, and (*E,Z*)-2,6-nonadienol, 2- and 3-methylbutanal, 2- and 3-methylbutanoic acid, phenylacetaldehyde, phenylacetic acid, 3-(methylthio) propanal, acetic acid, pentanoic acid, and vanillin to be present in whole wheat flour in higher concentrations compared to wheat flour type 550. It can be seen in Table 1 that the concentrations of acetic acid, 3-methylbutanol or 2,3-butanedione in sourdough increased compared to these concentrations in flour. Although these three compounds have also been found in flour, they are mainly produced by LAB metabolism during fermentation. Since Czerny & Schieberle (2002) used commercial starter in their laboratory sourdough fermentation, resident microbiota remained unidentified and no association between volatile profile and starter species could be established.

The main chemical classes of volatile compounds identified in flour and flour blends are alcohols, carboxylic acids, terpenes, aliphatic aldehydes (e.g. pentanal and hexanal, formed as fatty acid oxidation products), alkanes and esters (Hansen, 1995; Galoburda et al., 2020), including derivatives of pyrazines and pyridines, which are also often found as a result of heat treatment (Heiniö, 2003). The following volatile compounds have been detected mainly in rye flour: alcohols (ethanol, n-propanol, 2-methyl-1-propanol, 2-butanol, acetone), a homologous series of n-alkanals (from propanal to heptanal) as well as (E)-2-hexanal, benzaldehyde, 2-heptanone, 2-pentylfuran, and 2-furaldehyde, esters ethyl acetate and methyl formate (Markova et al., 1970; Lund et al., 1989, Kirchhoff & Schieberle, 2002). The listed VOCs above are produced through technological processes from flavour precursors present in cereal grains, such as amino acids, fatty acids and phenolic compounds (Galoburda et al., 2020). Major phenolic compounds in wheat and rye grains are phenolic acids (e.g. ferulic acid, caffeic acid, dihydrobenzoic acid and sinapic acid) and alkylresorcinols (Shewry et al., 2010). Microorganisms may decompose the phenolic acids into chemical compounds with a strong flavour; for example, ferulic acid is the source of 2-methyl-4-vinylphenol, which is described as having a burnt or tar-like flavour (Hansen, 1995). Saccharomyces cerevisiae have two enzymes (phenylacrylic acid decarboxylase and ferulic acid decarboxylase) responsible for the decarboxylation of hydroxycinnamic acids (Dzialo et al., 2017). Several additional methods can be used to improve the taste characteristics of cereals, e.g. germination, extrusion and grinding. Germinated cereal grains are a good source of amino acids, peptides and sugars that act as flavour precursors for odour-active compounds. Germinated, extruded rye is characterized by grainy and fresh taste and hard texture related to the concentration of dimethyl sulphide and 2-methylbutanal (Heiniö et al., 2003).

The chemical characteristics of flour, such as ash content and falling number, also have a significant impact on the fermentation of sourdough. The falling number of flours is an indicator of the enzymatic activity of the flour - the lower it is, the higher the amylase activity of the flour and the more free sugars are available for microorganisms. The ash content of flours varies depending on the extent of flour extraction. The higher the flour extraction, the higher the ash content in the flour. The bran fraction contains more minerals and micronutrients that are essential for the growth of LAB. (Spicher & Stephan, 1999) Thus, the use of a higher extraction flour prolongs the fermentation time of the sourdough and more lactic acid and VOCs are produced. The higher nutrient content in higher-grade flours stimulates the microbial growth and their biochemical activity, resulting in the production of more flavour and aroma compounds (Hansen & Hansen, 1994a).

The chemical composition and quality of flour determine the dynamics of the microbial communities in the fermentation process and the kinetics of their metabolites (De Vuyst et al., 2014). The amount of fermented carbohydrates in the flour varies depending on the cereal, but above all on the activity of endogenous enzymes. These enzymes may originate from the cereal flour, from the microbial strains (e.g. yeast or LAB) or additives used. The activity of flour enzymes (amylases, xylanases and peptidases) is important in the release low molecular weight carbohydrates and amino acids (Hellemann et al., 1988; Hansen, 2012). LAB do not normally degrade starch, and the content of fermented mono- and disaccharides is less than 3% in wheat sourdoughs and 1% in rye sourdoughs. Rye has a higher arabinoxylan content (9–12%) compared to wheat. During sourdough fermentation, flour enzymes can degrade arabinoxylans into xyloses and arabinoses which affect the consistency of the sourdough (Hansen, 2004). It has been observed that the fermentation of sourdough with *Leuconostoc mesenteroides* increases the maltose content from 1.5%–2.4% and the fructose content from 0.05–0.45%. The glucose content remains unchanged at 0.17% due to the balance between enzymatic and microbiological processes (Lefebvre et al., 2002).

Table 1. Comparison of the amount of odour-active compounds in flours and sourdoughs made from wholemeal wheat flour (WWF), white wheat flour (WF) and rye flour (RF) (Kirchhoff & Schieberle, 2002; Czerny & Schieberle, 2002)

VOCs	Odour description	Amount in flour (µg per kg of dry weight)			Amount in sourdough (µg per kg of wet weight)*		Amount in sourdough (µg per kg of dry weight)
Aldehydes		WF	WWF	RF	WF-S	WWF-S	RF-S
3-methylbutanal	malty	97	153	265	105	452	899
2-methylbutanal	malty	30	74	205	54	96	180
hexanal	fresh, green, fatty	11,000	112,00	3,080	5,600	5,900	503
phenylacetaldehyde	honey-like	183	508	121	144	325	170
(*E,Z*)-2,4-decadienal	fatty	389	1,810	n.d	65	148	n.d
(*E,E*)-2,4-decadienal	deep fat fried	355	1,690	80	82	136	78
vanillin	vanilla-like	583	2,910	1,270	265	823	2,790
3-(methylthio)propanal	cooked-potato-like	25	127	n.d	12	41	n.d
Ketones							
2,3-butanedione	buttery	n.d	n.d	55	n.d	n.d	744
Alcohols							
3-methylbutanol	malty	n.d	n.d	76	n.d	n.d	15,200
2-phenylethanol	flowery	n.d	n.d	416	n.d	n.d	9,100
Organic acids							
2- and 3-methylbutanoic acid	sweaty	342	629	1412	524	1,030	3,519
acetic acid	vinegar	134,000	218,000	50,000	601,000	1,220,000	3,100,000
butanoic acid	sweaty	5,900	6,980	5,100	13,900	13,,400	21,800
pentanoic acid	sweaty	6,900	11,600	n.d	12,100	13700	n.d
2-phenylacetic acid	honey-like	142	418	2,260	327	852	4,990

*sourdoughs contained 40% water; n.d – no data; WF – wheat flour (type 550), WWF – wholemeal wheat flour, RF – rye flour, -S – sourdough.

A comparison of the key odorants identified in wheat and rye flour has shown significant differences in the qualitative and quantitative composition. For instance, compounds such as (*E,E*)-2,4-nonadienal (deep fat fried), (*E,Z*)-2,6-nonadienol (cucumber-like), hexanoic acid (sweaty), γ-nonalactone (coconut-like), and 4-methylphenol (faecal), are not associated with the aroma of rye flour, whereas 2,3-butanedione (buttery), octanal (fruity), (E)-2-octenal (fatty), 4-vinyl-2-methoxyphenol (apple, spicy) and 3-hydroxy-5-ethyl-4-methyl-2(5H)-furanone (sweet, fruity) have been characterized in rye flour but not in wheat flour (Czerny & Schieberle, 2002). The origin of the mild, cereal-like aroma of native rye flour is still unclear and complex.

Another aspect beside the type of cereal flour is the grain milling procedure used. Although nowadays the stone milling procedure has been almost completely replaced by the roller milling method, the main advantages of slow and low-temperature stone milling are the minimal oxidation of numerous compounds and the reduction of components (e.g amino acids as a precursors of aromatic substances) which are essential nutrients for microbes. Cardinali et al. (2022) study is currently the only one where stone-ground soft wheat flour was analyzed for its aromatic profile. The most characteristic compounds belonged to classes of aldehydes and alcohols. Within aldehydes, pentanal (strong acid, pungent) and hexanal (green, grassy, tallow) were found in appreciable amounts. Ethanol (alcoholic), 1-hexanol (green grass, flowery, woody) and isoamyl alcohol (pungent, cognac, fruity) were the most represented alcohols in stone-ground soft wheat flour. The results of the study confirmed that flour already contains many important substances contributing to the aroma of baked bread, and the choice of flour type is also one major factor affecting the quality of the aroma of bread.

In addition to the impact of flour, the effects of a natural pre-ferment aroma mixture, purified exogenous enzyme preparations and cell-free enzyme extract on the formation of VOCs have also been investigated (Niçin et al., 2022). In the biotechnology of bakery products, exogenous enzymes are used more as technological aids to replace additives, such as oxidants and emulsifiers, because in addition to the aroma formation, they increase the dough volume, improve crust and crumb colour, and have antiaging effects (Gänzle, 2008). Cavallo et al. (2017) found higher levels of alcohols in bread samples prepared with cell-free enzyme extract (CFE) of *Fructilactobacillus sanfranciscensis*, *Hafnia alvei* and *Debaryomyces hansenii* compared to bread samples without enzyme extracts. Some alcohols (e.g. acetaldehyde, propanal, 3-methylbutanal) were found at the highest levels in the bread produced with *F. sanfranciscensis* and especially *H. alvei* CFEs. The level of ketones, mainly 2,3-butanedione (or diacetyl), was not affected by the addition of CFEs in the sourdoughs. 2,3-butanedione is characterized by buttery odour and according to Pico et al. (2015) it may be produced by both lactic acid bacteria and yeasts from the glycolysis intermediate pyruvic acid or during baking upon oxidative decarboxylation of acetohydroxyacids (Pico et al., 2015). The addition of CFE of *D. hansenii* affected negatively the levels of five ester compounds in sourdough breads. While CFE of *F. sanfranciscensis* improved the synthesis of ethyl acetate; and CFEs from *H. alvei* and *D. hansenii* showed the ability to increase the level of heterocyclic compounds (e.g., pyrrole and pyrazine containing molecules) in sourdough breads. This study showed that microbial CFEs combined with sourdough fermentation could be an effective biotechnological tool to improve the sensory properties of sourdough bread (Cavallo et al., 2017).

The production of volatile compounds can be further influenced by the addition of nonconventional ingredients such as milk, fruits, by-products of beer and wine (e.g brewers' spent grain, cava lees), additional carbohydrates (including fibers) and other chemical compounds (e.g. citrate, glutamate) that stimulate the growth of certain microbial communities. The use of an additional source of fructose as an electron acceptor in sourdough increases the activity of mannitol dehydrogenase and acetate kinase and decreases the activity of lactate dehydrogenase, resulting in an increase in acetate production and a decrease in lactic acid production (Gobbetti et al., 1995). Similarly, naturally occurring citrate in milk acts as a substrate for microbial activity, resulting in production of 4-carbon flavour compounds such as acetoin and diacetyl (nutty and buttery aromatic notes) via citrate metabolism in LAB (Laëtitia et al., 2014). Since citrate metabolism affects the aroma of sourdough and sourdough bread, Comasio et al. (2019) investigated the effect of citrate addition on acetoin and diacetyl production by the citrate-positive *Companilactobacillus crustorum* strain LMG 23699 during wheat sourdough fermentation. Butter flavour compounds were detected both sourdough and bread, but compared to other studies (Kang et al., 2013; Gänzle, 2015) that have found an increase in growth rate or cell yield of LAB (e.g. *Limosilactobacillus panis*, strains of *Lactococcus lactis* and *Leuconostoc* spp) when citrate was added to sourdough, no growth stimulation of the *C. crustorum* strain was observed.

Previously, studies conducted by Hernández-Macias et al. (2021) and Martín-Garcia et al. (2022a) reported that Cava lees have a growth-promoting effect on some LAB species (mainly *L. sakei*, *L. curvatus*, *L. fermentum*, *L. casei*) both *in vitro* and in sourdough. Cava lees are a good source of soluble and insoluble fibers (β-glucans and mannan-oligosaccharides) derived from the yeast cell wall (Alonso et al., 2002). Their prebiotic effect and ability to support the growth and survival of LAB has been well recognized by several studies (Shi et al., 2018; Liu et al., 2021). Martín-Garcia et al. (2022b) study referred that Cava lees increased the concentration of 1-hexanol, acetic acid, hexanal, and ethyl decanoate in wheat sourdough. Compounds characteristic of sparkling wine, such as 1-butanol, octanoic acid, benzaldehyde, and ethyl hexanoate, were also detected in sourdoughs. This indicates that Cava lees not only support the production of volatile compounds in sourdough but also provide VOCs found in sparkling wines. Additionally, many studies have focused on the use of flaxseed (*Linum usitatissimum* L.) and its by-products, mainly seed cake, as a functional ingredient in bread making (Ozkoc & Seyhun, 2015; De Lamo & Gómez, 2018; Sanmartin et al., 2020). Sourdough bread fortified with flaxseed cake flour has been characterized by a more complex VOC composition compared to bread not containing flaxseed cake flour. Specifically, increasing the content of flaxseed cake flour increased the levels of esters (e.g. ethyl acetate), 2-butanone and isobutyl alcohol. The bread had a higher acetic acid content even when flaxseed cake flour was not added to the dough (Sanmartin et al., 2020).

Formation of VOCs during sourdough fermentation

The predominant LAB in sourdough are a group of gram-positive bacteria which are catalase-negative, non-motile, non-spore-forming rods or cocci that produce lactic acid mainly through lactic acid fermentation (Hammes & Vogel, 1995; Gänzle et al., 1998). The main genera of LAB identified in sourdoughs are *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* (Hansen & Schieberle, 2005). The primary function of the LAB in sourdoughs is to ferment cereal carbohydrate substrates (e.g. maltose, sucrose, fructose and glucose) into organic acids (mainly lactic acid) and many other metabolites that affect the nutritional, sensory, and technological properties of fermented foods (Chiş et al., 2020).

According to whether the enzyme aldolase is used in the production of lactic acid, LAB are divided into homolactic and heterolactic fermentation types. Homofermentative LAB generally produce diacetyl, acetaldehyde and hexanal, while heterofermentative LAB are responsible for the production of ethyl acetate, alcohols, and aldehydes. (Wang et al., 2021) Studies conducted between 1987–2017 in Europe (mainly Italy, France, Germany and Belgium), U.S. and Canada show that 95% of the sourdoughs contain heterofermentative LAB alone or in combination with homofermentative lactobacilli. The most frequently detected species were (taking into account the revised taxonomy of the genus *Lactobacillus*) *Fructilactobacillus sanfranciscensis*, *L. plantarum* and *Levilactobacillus brevis*, species in the *Companilactobacillus alimentarius* group (*C. paralimentarius*, *C. crustorum*, *C. mindensis* and *C. nantensis*), *Leuconostoc* spp. and *Weissella* spp. (Gänzle & Zheng, 2019) All homofermentative lactobacilli produce 2,3-butanedione (particularly *Lacticaseibacillus casei*), which is not seen in heterofermentative species. Probably, the heterofermentative bacteria lack the diacetyl synthesis pathway or/and the redox potential is too high to allow acetoin oxidation (Kaseleht et al., 2011). Homofermentative LAB (*Lactobacillus (Lb.) delbrueckii*, *Lb. acidophilus*, *C. farciminis*, *Lb. amylovorus*, *C. mindensis*) convert almost all hexose into lactic acid via the Embden-Meyerhof pathway (EMP). This metabolism is characterized by the breakdown of 1,6-diphosphate fructose into two triose phosphates, which are converted into lactate. Other hexoses, such as mannose, fructose, and galactose, enter the EMP after different stages of isomerization and phosphorylation of glucose-6-phosphate or fructose-6-phosphate. Heterofermentative LAB (*Fr. sanfranciscensis*, *Furfurilactobacillus rossiae*, *Le. brevis*, *Limosilactobacillus pontis*, *Li. fermentum*) decompose degrade hexose into lactic acid, acetic acid, ethanol and carbon dioxide via the phosphoketolase pathway (Axelsson, 1998; Hansen & Schieberle, 2005).

Specifically, monosaccharides are used in glycolysis to produce pyruvic acid, which is the basis for fermentation by LAB and yeasts. Several compounds are produced from pyruvic acid, such as e.g. lactic acid, propionic acid, propanol, ethyl lactate, ethanol, acetic acid, 2,3-butanedione, 2-butanone or butanol. Therefore, the production of VOCs is highly dependent on the sourdough microbiota, including fermentation time. The fermentation time required to produce a sufficient amount of VOCs is at least 12 h (Petel et al., 2017; Wu et al., 2022). When *Saccharomyces cerevisiae* is used, the fermentation of the sourdough takes place within a few hours and the resulting bread is therefore less aromatic (Hansen & Schieberle, 2005), despite the higher concentration of volatiles compared to single LAB starters (Hansen & Hansen, 1994b; Meignen et al., 2001; Annan et al., 2003).

Moreover, LAB release precursors of aromatic compounds, such as free amino acids, which are degraded to aldehydes or alcohols as a result of metabolic processes (Hansen & Schieberle, 2005). The main amino acid conversion pathways of LAB cells include decarboxylation, transamination (such as the conversion of glutamine to glutamate), and deamidation (various α-carboxylic acids) (Fernandez et al., 2006; Gänzel et al., 2007; Wang et al., 2021). Sourdough fermentation with LAB has been shown to increase amino acid content, while dough fermentation with yeasts decreases free amino acid concentration. The enhancement of proteolysis during sourdough fermentation can be attributed to the proteolytic activity of sourdough LAB or to enhanced proteolysis by cereal enzymes (Thiele et al., 2002). Amino acid metabolism has also a crucial role in the adaptation of LAB to the acidic environmental conditions prevailing in sourdough (Wang et al., 2021).

Ketones, aldehydes, carboxylic acids and alcohols are synthesized in catabolic (e.g. deamination, decarboxylation, transamination) pathways, which have a significant impact on the sensory properties of bakery products (Kieronczyk et al., 2001). For example, expression of the arginine deiminase pathway promotes ornithine production (LAB metabolism), which in turn enhances the formation of 2-actetyl-1-pyrroline, that is responsible for the roasty note of bread crumbs (Gänzle et al., 2007). Peptides and free amino acids are substrates for microbial conversion or are converted into volatile compounds (e.g. methylbutanol, acetaldehyde, methionic acid) during baking. The catabolism of free amino acids in sourdough by LAB not only has a sensory effect, but also improves the acid tolerance of LAB and promotes microbial development in poor nutrient conditions (Su et al., 2011; Gänzle & Gobbetti, 2013).

During spontaneous sourdough fermentation, butyric acid and acetic acid, butyl formate, butanol and 4-methyl propanol are the main volatile compounds produced (Kam et al., 2011). Although lactate is the end-product of lactic acid fermentation, under aerobic conditions it can be catabolised by lactate oxidase or NAD+ (nicotinamide adenine dinucleotide) -independent LDH (lactate dehydrogenase) of some LAB (e.g. *Latilactobacillus curvatus*, *Lat. sakei*, *L. casei*, *La. plantarum*) to produce pyruvate, which is further catabolised by pyruvate oxidase into acetate and carbon dioxide. Under anaerobic conditions, some LAB (e.g. *Le. brevis*, *Lentilactobacillus buchneri*, *La. plantarum*) can via NAD+-independent LDH catabolise lactate into acetate and formate. (Liu, 2003) A study by Damiani et al. (1996) concluded that in laboratory-produced sourdoughs containing single-strains of *Le. brevis* subsp. *linderi* and *La. plantarum*, had most complex VOCs profiles. In addition, they also determined that sourdoughs initiated by microbial starter cultures produce an even greater amount of volatiles.

Studies in the past decade (Minervini et al., 2012; Zhang & He, 2013; Lhomme et al., 2015; Li et al., 2016; Ripari et al., 2016; Comasio et al., 2019) have reported the occasional occurrence of acetic acid bacteria (AAB) in spontaneous wheat, rye and maize sourdoughs. AAB are not considered typical members of sourdough microbiota probably because of their growth is highly dependent on the availability of molecular oxygen. However, some studies indicate that a number of AAB can still grow despite anaerobic conditions during sourdough fermentation. Their oxygen requirement cannot be fulfilled through aeration, as this would also enhance mold growth and oxidation of flour compounds. Further, AAB oxidation of ethanol and lactic acid would increase the flavour-impacting concentrations of acetic acid and acetoin to a great extent and hence result in variations in VOC compositions. Finally, they may have a retarded impact on the dough rise (De Vuyst et al., 2021).

The ratio of lactic acid to acetic acid, defined as the percentage of fermentation, is also an important parameter that may affect the flavour profile of bread, as well as play an important role in the structure of the final product (Lorenz, 1983). To give the bread a balanced flavour and aroma, the ratio of lactic to acetic acid should be about 4:1. If the concentration of acetic acid is low, the bread has too weak taste and no characteristic odour. However, if the relative amount of acetic acid is too high, the taste and aroma may be excessively sharp (Spicher & Stephan, 1999). AAB are obligate aerobic bacteria with a unique oxidative fermentation pattern. Their membrane-bound aldehyde dehydrogenases oxidize a variety of sugars and sugar alcohols, ethanol, organic acids, and then release the oxidized products (aldehydes, ketones and organic acids). The presence of AAB in the sourdough may induce oxidative stress in LAB and yeasts, which is why the latter are able to produce various volatile compounds in sourdough (Saichana et al., 2015; Li et al., 2021).

Yeasts, mainly *S. cerevisiae* strains, occur naturally or in added form in sourdough, contributing to the formation VOCs. The extensive variation in the number of yeast species and strains depends on several factors, e.g. degree of sourdough hydration, type of cereal, fermentation temperature and storage temperature of the sourdough (Corsetti et al., 2007). VOCs synthesis is also influenced by microbial interactions. Yeasts are often associated with LAB in the sourdough, and the ratio of yeasts to LAB is usually 1:100 (Guerzoni et al., 2013). Yeasts identified in sourdough belong to more than 20 species, including *Kazachstania exigua*, *Candida humilis* and *Issatchenkia orientalis* (Corsetti et al., 2007). Sourdough fermentation which has been initiated by *Fr. sanfranciscensis* and other homo- and heterofermentative LAB and/or *K. exigua* communities are characterized by a balanced aroma profile. Sourdough based on the combination of *Fr. sanfranciscensis* and *S. cerevisiae*, contain a higher concentration of metabolites produced by yeast fermentation (1-propanol, 2-methyl-1-propanol and 3-methyl-1-butanol) and a lower concentration of bacterial compounds (Meignen et al., 2001). However, the presence of *S. cerevisiae* does not significantly affect the formation of VOCs by *Fr. sanfranciscensis* during sourdough fermentation. In a study by Liu et al. (2022), the content of 1-butanol and 1-penten-3-ol decreased as the fermentation progressed, which are known to be novel findings in sourdough fermentation by *Fr. sanfranciscensis*. This may mean that *Fr. sanfranciscensis* S1 strain can utilize these two alcohols. It is evident that LAB, including *Fr. sanfranciscensis* and *L. plantarum* species, contribute to a higher and broader spectrum of VOCs than baker's yeast (Arora et al., 2021).

Yeast fermentation mainly produces carbon dioxide and ethanol, the concentration of which depends on the microbial strain (Hansen & Hansen, 1994a, 1994b). Moreover, the formation of metabolites depends on the concentration of fermentable carbohydrates (mainly maltose) in the sourdough, which in turn is due to the activity of microbial amylase, which breaks down starch into simple sugars (De Vuyst et al., 2016). The carbon dioxide produced by yeasts causes the proofing of bread dough. Although ethanol has an impact on the properties of bread, most of it evaporates during baking. Furthermore, yeasts produce a small amounts of organic acids (acetic and succinic acid), which lower the pH of the bread dough and thus contribute to the taste of the bread (Jayaram et al., 2013, 2014).

In addition to ethanol and carbon dioxide, yeasts produce a variety of metabolites, such as higher alcohols with branched chain amino acids via the Ehrlich metabolic pathway (Fig. 1) and derived esters, that have an effect on the aroma of bread (Hazelwood et al., 2008; Pico et al., 2015). For instance, higher alcohol 3-methyl-1-butanol (acidic, fruity odour) originates from the conversion of the L-leucine amino acid via α-ketoisocaproic acid and already at very low concentrations has a significant effect on the development of bread aroma (Rehman et al., 2006; Birch et al., 2013; Pico et al., 2015). Xu et al. (2020) and Fang et al. (2023) have also obtained similar results regarding the alcohol content of yeast-containing sourdoughs. The study of Fang et al. (2023) indicates that phenylethyl alcohol content increases in mixed fermentation of yeasts and LAB due to the promotion of the Ehrlich pathways of yeasts.

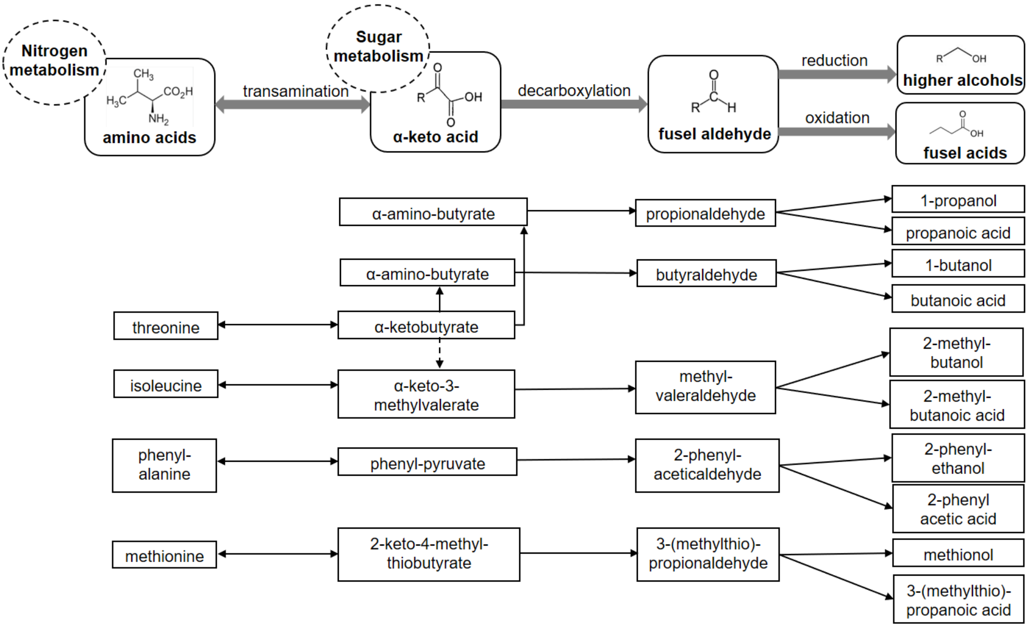

Figure 1. The three step Ehrlich pathway: catabolism of amino acids into fuse acids and fusel alcohols (Hazelwood et al., 2008; Dzialo et al., 2017).

Although *S. cerevisiae* prefers maltose as an energy source, specific yeasts such as *C. humilis* and *K. exigua* are maltose-negative (i.e. do not ferment maltose) and form a trophic relationship (mutualism) with the heterofermentative lactic acid bacterium *Fr. sanfranciscensis*, hydrolysing sucrose and glucofructan, and consuming glucose during the process (Gobbetti et al., 1994; Gobbetti, 1998). 2,3-butandione (diacetyl) produced by both LAB and yeasts (via pyruvate catabolism or Ehrlich metabolic pathway) supports the formation of a characteristic aroma (buttery flavour) of bread, while this compound is also formed by Strecker degradation (α-amino acid conversion to aldehydes) during bread baking, yielding pyrazines, for example (Hansen & Schieberle, 2005; Cho & Peterson, 2010; Birch et al., 2013; Pico et al., 2015). It has been observed that when yeast strains are added to the sourdough, the yeasts use the available oxygen for growth, thereby increasing the metabolite content. At low concentration of yeasts, oxygen is available for flour lipoxygenase, which oxidizes flour lipids to aldehydes (Pico et al., 2015).

Of the compounds produced by LAB and yeast fermentation, 36 are characteristic of both wheat and rye sourdough. These are for example hexanal (fresh, fatty, sweaty), acetylaldehyde (sharp, pungent), hexanoic acid (sour, fatty), 1-hexanol (ethereal, alcohol), 1-pentanol (oily, sweet), 2- and 3-methyl butanol (oily, alcohol, sweet, roasted), 2-pentylfuran (fruity, metallic) and dimethylsulfide (sulphur, fresh), associated with

LAB fermentation (Gobbetti et al., 1995; Kaseleht et al., 2011). 2,3-butanedione (strong, buttery, sour, caramel) is mainly produced by homofermentative LAB (Damiani et al., 1996; Liu et al., 2020), while the production of acetic acid and esters is specific to heterofermentative LAB and their compounds (ethyl acetate, ethyl lactate (sharp, caramel) (Hansen et al., 1989; Petel et al., 2017). Homo- and heterofermentative LAB can produce the same volatile compounds, but their content varies. The study by Kaseleht et al. (2011) shows that heterofermentative LAB has greater activity in reducing aldehyde (2- and 3-methylbut-anal, 2-hexenal, (*E*)-2-heptenal, hexanal, heptanal and 2-octenal) content than homofermentative LAB.

Thermally induced generation of VOCs

Volatile compounds formed during baking of dough or drying the sourdough (production of dry sourdough, Type III sourdoughs) are directly dependent on the precursors - aldehydes (usually reduced sugars) and amines (amino acids or proteins) (Parker et al., 2000; Nursten, 2002). The colour of the bread crust and its roasted aroma are caused by non-enzymatic reactions, e.g. compounds obtained by the Maillard reaction. The Maillard reaction is mainly induced by high temperatures (roasting, baking, extruding), while the free amino groups of lysine, peptides or proteins react with the carbonyl groups of reducing sugars (Silván et al., 2006). Nowadays, the Maillard reaction is one of the most important reactions in terms of food quality. Its importance lies in the formation of compounds that affect the organoleptic quality and preservation of food (Somoza & Fogliano, 2013). Since the chemical structures of the aldehydes generated during the Ehrlich pathway and the Strecker reaction are identical, it is a challenge to quantitatively distinguish the amounts of compounds formed from the respective pathways (Chavan & Chavan, 2011).

According to Hodge (1953) and Martins et al. (2001), the Maillard reaction consists of three main stages: initial, intermediate and final. At each stage, the special chemical structures are formed. The initial stage is related to sugar-amine condensation and Amador rearrangement. In the following intermediate stage, sugar dehydration and fragmentation, and amino acid degradation takes place, which continues in the final stage with aldol condensation, aldehyde-amine condensation and the formation of heterocyclic nitrogen compounds. The final step of Maillard reaction itself is under-explored, but it is known that the reaction consists of polymerization and condensation reactions of carbonyl compounds with the participation of amino compounds. As a result of the reactions, a mixture of cyclic compounds containing nitrogen is formed: pyrazines, pyrroles, furans and sulphur-containing compounds that are insoluble in water and have a brown colour, and lipid degradation products, e.g. alkanal, 2-alkene, 2,4-alkadenane (Parker et al., 2000). Pyrazines are nitrogenous cyclic compounds with very strong odour properties (Ji & Berhard, 1992). Zhou et al. (2022) detected pyrazine compounds (e.g 2,5-dimethyl pyrazine, 2,6-diethylpyrazine, 2-methylpyrazine, 2,3-dimethyl-5-ethylpyrazine) as the dominant volatile compounds in Tatary buckwheat sourdoughs. The study also found a significant negative correlation between most pyrazines and microbial diversity.

Individual Maillard reaction and Strecker degradation compounds, for instance 2-methylbutane (fruity), 3-methylbutanal (sweet, roasted) and 2,3-butanedione (buttery) and furfural (almond) have been identified in wheat and rye bread (Heinio et al., 2003). These compounds may be derived from fermentation associated with LAB and *K. exigua* yeasts (Kratochivil & Holas, 1983; Gobbetti et al., 1995) and most of them contribute to the desired roasted (or toasted), caramelized and sweet flavour. In addition to the Maillard reaction, caramelization of sugars occurs during dough baking (above 140 °C), while VOCs produced during fermentation (Hadiyanto et al., 2008; Bianchi et al., 2008) and low molecular weight components, e.g. ethanol, smaller esters (ethyl acetate (ethereal, floral) and ethyl 3-methylbutanoate (sweet)) and acids, evaporate (Birch et al., 2013). In sourdough, the Maillard reaction affects gluten proteolysis, resulting in free amino acids (Gobbetti et al., 1995). The addition of enzymes (e.g., amylase and glycosidase) to sourdough during fermentation increases the concentration of sugars, thereby contributing to the Maillard reaction. Some compounds, e.g. methylpropanol (isobutanol), 2- and 3-methylbutanol (iso-pentanols), ethyl acetate and ethyl lactate identified in bread have been shown to be related to their respective concentrations in sourdough (Chavan & Chavan, 2011). The formation of these compounds can be attributed to Maillard reactions, influenced by the amount of free amino acids. An increase in free amino acid content has also been observed in sourdough fermentation (Hansen et al., 1989).

Free amino acids can also be a source of nitrogen for both yeast growth and metabolism, resulting in higher alcohols such as 3-methylbutanol (Heiniö, 2003), or are converted to flavour compounds during steaming (production of steamed bread). Cereal protein hydrolysates, including amino acids and peptides, are important precursors for VOCs (Gänzle, 2008), but degradation and depolymerisation of cereal proteins are complicated to achieve due to the low and limited activity of cereal and microbial enzymes in sourdoughs. According to Gänzle (2008) proteolysis in both wheat and rye sourdough is less than 5% of cereal proteins and malt or fungal enzymes are required to increase protein degradation. Liu et al. (2015) have demonstrated that the addition of 1% Hyd-SPI (hydrolysed soy protein isolate) promotes the growth and fermentation of the selected starter due to its content of peptides and amino acids (especially phenylalanine, tyrosine, arginine, leucine and glutamic acid). Higher concentrations of volatile compounds such as 3-methyl-1-butanol (apple, banana), 2-pentylfuran (fruity), phenylethyl alcohol (floral) and variety of esters responsible for the flavour of steamed bread were detected due to the presence of Hyd-SPI. Moreover, the activity of cereal proteinases depends on the pH of the nutritional environment. A drop in pH during sourdough fermentation is known to activate aspartic proteases, which are the major proteinases in resting grains of wheat and rye (Bleukx et al., 1998).

VOCs originating from enzymatic processes

Lipids are a minor component of flours, but they have a great impact on the bread quality (Castello et al., 1998). Lipid oxidation is a complex of reactions involving several molecular mechanisms such as the generation of reactive oxygen precursors, free radicals and peroxides (Ahmed et al., 2016). Oxidation of lipids in sourdough is caused by the presence of active enzymes (e.g. lipoxygenase), the addition and type of fatty substances (e.g. shortenings and margarines), and mixing (aeration) of the sourdough (Maire et al., 2013). Lipid oxidation is not sourdough or process specific, but it creates precursors that become a source for volatile compounds (Petel et al., 2017). Lipoxygenase oxidizes polyunsaturated fatty acids to free radicals, peroxides, and hydroperoxides, which are common VOCs during bread baking. Hydroxyperoxy acids are decomposition products of linoleic acid oxidation. In the presence of cysteine, hydroxyl-fatty acids are also produced, which may have a bitter taste (Shahzadi, 2011). Lipoxygenase of wheat origin is capable of forming 9 hydroperoxy linoleic acids in contrast to lipoxygenase of rye, whose lipolysis produces 13 hydroperoxy isomers (Belitz et al., 2004). Oxidation of lipids in sourdough and breadcrumbs mainly produces aldehydes (hexanal, heptanal, octanal (fresh, fatty, aldehydic)), ketones (2-octanone (grass, woody)), alcohols and esters, depending on the primary material of the fatty acids (Martínez-Anaya, 1996; Birch et al., 2013; Maire et al., 2013). Studies by Waraho et al. (2011) and Frankel (2005) have shown that free fatty acids are more prone to oxidation than esterified fatty acids. The effect of vegetable oils on sourdough fermentation is still little studied. Wu et al. (2022) determined that addition of corn oil to sourdough had a positive effect on the concentrations of aldehydes, ketones and furans. Important odour-active compounds including (*E,E*)-2,4-decadienal, 2-pentylfuran, 1-octen-3-ol, 3-methylthio-1-propanol, and (*E*)-2-nonenal were produced by *Lb. lactis*, corn oil, and lipase individually or via their interactions.

Vermeulen et al. (2007) have reported that the metabolic reactions of LAB in sourdough can favor lipid oxidation during fermentation. Lipid oxidation is enhanced by homofermentative LAB, meanwhile obligate heterofermentative LAB (*Li. reuteri*, *Fr. sanfranciscensis*) decrease the oxidation-reduction potential of sourdoughs and accumulate glutathione or thiol compounds and convert decadienal and nonenal rapidly to the corresponding unsaturated alcohols. The activity of lipoxygenase can be affected by yeasts activity. While yeast content is reduced, there appears to be an increase in the amount of oxygen available for lipid oxidation (Czerny & Schieberle, 2002; Gänzle et al., 2007; Poinot et al., 2007). Some LAB can convert lipid oxidation compounds into alcohols (Vermeulen, 2006). For example, 3-methylbutanoic acid (cheesy, sweaty) is produced by the oxidation of 3-methylbutanal with aldehyde dehydrogenase (Guerzoni et al., 2007). The following volatile compounds from lipid oxidation have been identified from wheat and rye sourdoughs: hexanal (fresh, fruity, fatty), acetaldehyde (ethereal, aldehydic), benzaldehyde (strong, sharp, bitter, almond), nonanal (citrus), (E)-2-octenal (fresh, nutty, roasted), (E)-2-heptenal (sour, fatty), (E)-2-nonenal (fatty, citrus), 1-pentanol (oily, sweet), 1-hexanol (essential), 1-heptanol (musty, sweet) and 2-pentylfuran (green, fruity) (Martínez-Anaya, 1996; Birch et al., 2013; Maire et al., 2013; Liu et al., 2020). According to Fernández Murga et al. (1999), the ability of lactic acid bacteria to convert fatty acids is probably related to their membrane homeostasis.

Research on the lipolytic activity of sourdough lactic acid bacteria has been limited to studies of the esterase and lipase activities of several strains of *Lb. sanfranciscensis*, *Lb. plantarum*, *Pd. pentosaceus*, *T. delbrueckii* and *S. cerevisiae* (De Angelis et al., 1999; Paramithiotis et al., 2010). In a study conducted by Wang et al. (2020), it was found that *Lactobacillus* spp, *Kazachstania* spp and *Candida* spp had close linkages with lipid metabolism, suggesting these microbes genera exhibited higher metabolic capacity of lipid metabolism; and thus during lipid metabolism they can produce a variety of organic acids with different carbon chain lengths.

Proteolysis during sourdough fermentation has been poorly investigated, despite the fact that protein content and its degradation can noticeably affect flavour and odour development. It is unclear whether the proteolysis during sourdough fermentation is attributed to the proteolytic activity of starter cultures (LAB, yeasts) or cereal enzymes, e.g. aminopeptidase, carboxypeptidase, and endopeptidase (Thiele et al., 2002; Di Cagno et al., 2014). Thiele et al. (2002) results indicate that the amino acid levels in wheat dough mainly depends on the pH level of the dough, the fermentation time, and the consumption of amino acids by the microbiota. Proteolysis has been studied indirectly by determining the content of free amino acids and peptides after the fermentation process (Gobbetti et al., 1994; Thiele et al., 2002). Free amino acids and peptides produced during microbial and enzymatic reactions are important growth substrates for microbes or are converted into flavour precursors or flavour compounds during baking. LAB proteolysis involves three main processes. First, degradation of extracellular proteins into oligopeptides by proteinases associated with the cell wall. Next, the transport of peptides into the cell by peptide transporters and finally the degradation of peptides into shorter peptides and amino acids by several intracellular peptidases (Rizzello et al., 2016; Chiș et al., 2020). The proteolytic activity of sourdough LAB has been studied most thoroughly, although extracellular proteinase activity has not been detected in most sourdough lactobacilli (Gerez et al., 2006; Zotta et al., 2007; Paramithiotis et al., 2010), but the peptidase activity of sourdough LAB significantly contributes to the hydrolysis of peptides (Gänzle, 2008).

The effect of microbiota on proteolysis and peptide hydrolysis during fermentation is well known, but the contribution of the metabolic activity of microbes involved in the formation of flavour-active peptide derivatives remains unknown. Flavour-active amino acids and their derivatives and peptides are thought to be catalyzed by lactoyl-transferase, succinyl transferase, pyroglutamyl cyclase or γ-glutamyl-transferase (Zhao et al., 2016). Zhao & Gänzle study (2016) demonstrated that *Limosilactobacillus reuteri* 100-23 and *L. reuteri* 100-23Δ*gadB* produce γ-glutamyl dipeptides during growth in sourdough and suggested that these peptides influence the flavour of bread. Zhao & Gänzle (2016) and Zhao et al. (2016) studies refer to the formation of flavour-active peptides, such as glutathione and some γ-glutamyl dipeptides and tripeptides, through proteolysis, which give rise to kokumi taste (described as a sensation of enhancement of sweet, salty and umami tastes). The addition of vital wheat gluten, proteolysis, and fermentation time were the most relevant indicators contributing to γ-glutamyl peptide synthesis, but the supplement of microbial transglutaminase did not support production of γ-glutamyl peptides. It is worth highlighting and further investigation that flavour-active peptides may be a new innovative tool for improving several flavour characteristics. Although kokumi-active compounds are not classified as flavour-active compounds, these compounds have the ability to increase the flavour intensity of other compounds. For instance, Zhao & Gänzle (2016) observed that the taste of bread with higher kokum peptides was rated as more balanced than the taste of bread with lower content of kokum peptides, which was rated as saltier.

The extent of proteolysis in sourdoughs fermented with LAB (exhibit proteinase and peptidase activities, which are mainly bound to the cell wall) has been found to be higher, while sourdough fermentation with yeasts produces less free amino acids (Gobbetti et al., 1994; Thiele et al., 2002). Sourdough fermentation has been shown to significantly increase levels of the branched-chain amino acids leucine and isoleucine, branched-chain amino acid metabolites, as well as several small peptides containing branched-chain amino acids. This effect is more prominent in rye than in wheat sourdough most likely due to intensive proteolysis in acidic rye sourdough (Koistinen et al., 2018). Amino acids, e.g. glutamate, improve the taste of sourdough bread, while other amino acids (incl. ornithine, leucine and phenylalanine) are flavour precursors, which are converted to 2-acetyl-pyrroline, 3-methyl-butanol and 2-phenylethanol (Hansen & Schieberle, 2005; Gänzle, 2014).

The content of amino acids, e.g. ornithine, methionine, phenylalanine, leucine, isoleucine, and valine in the dough is essential for the development of bread flavour. Ornithine, a precursor compound of 2-acetyl-1-pyrroline (roasty note of the crust odour), is derived from the yeast biomass or arginine metabolism of LAB. *Lb. amylolyticus*, *Le. brevis*, *Li. fermentum*, *Li. frumenti*, *Li. pontis*, *Li. reuteri*, *Lat. sakei*, and few strains of *Fr. sanfranciscensis* convert arginine to ornithine via the arginine-deiminase (ADI) pathway. (Vermeulen, 2006) Ornithine is not a proteinogenic amino acid and therefore its presence in dough is the consequence of microbial metabolism (Thiele et al., 2002). In addition to LAB, the addition of baker's yeast has been observed to increase the level of ornithine in wheat doughs and corresponding levels of 2-acetylpyrroline in wheat bread crust (Schieberle, 1990). Moreover, ornithine can react with the carbonyl compound 2-oxopropanal during Maillard reactions in the bread baking process, to form 2-acetyl-1-pyrroline, which is responsible for the aroma of bread crust (Gänzle, 2014; Pico et al., 2015).

CONCLUSIONS

An abundant literature shows the various functional properties of sourdough fermentation that contribute to the production of VOCs. The formation of VOCs in sourdough and sourdough bread is therefore mainly related to the sourdough microbiota and their metabolic activity, raw materials and technological parameters of fermentation and proofing. The aroma profile of sourdough bread is mainly influenced by the breakdown of carbohydrates and amino acids during sourdough fermentation, as well as by thermal reactions occurring during baking of the dough, such as caramelization of sugars and nonenzymatic Maillard reactions. Improvements in VOC analysis methods, diversity of starter cultures and novel ingredients as microbial growth substrate have expanded and created new directions in VOC research, enabling a better understanding of the complex and versatile metabolism of sourdough. VOCs not only play a role in developing flavour or preventing the growth of spoilage microorganisms, but are also gaining popularity for their potential health-promoting role in human health. Principally, the scientific literature on VOCs in sourdough bread is more focused on sensory descriptive analysis, and less research has been done using an analytical approach to determine the VOC content in sourdough and sourdough bread. In-depth knowledge of microbial and enzymatic metabolism increases the understanding of the effect of VOCs on the sensory and nutritional properties of sourdough bread and thus enables the bakery industry to more effectively control and optimize technological processes. In the future, more detailed and systematic studies are needed to evaluate the influence of sourdough fermentation on the formation of non-volatile flavour-active compounds, which has so far been little studied.

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A cost-effective imaging system for monitoring poultry behaviour in small-scale kenyan poultry sheds

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Abstract. The objective of this paper was to develop a low-cost prototype poultry behaviour imaging and analysis system for monitoring intensively-reared flocks suitable for small-scale Kenyan poultry sheds. An image processing and analysis programme was developed using Python programming language and the OpenCV image processing package. This was tested on overhead images of Ross 308 birds collected over a number of days using a Raspberry Pi V2 camera. A second experiment using toy-chicks was conducted with an angled camera (Wansview W3). Linear transformation (LT) and background subtraction (BS) methods were applied and compared for effectiveness at detecting yellow and brown toy-chicks on woodchip bedding. Perspective transformation (PT) was applied and evaluated for its ability to transform the angled images into two-dimensional views. In the first experiment, where white birds were detected against a dark background, LT object detection successfully detected 99.8% of birds in the sampled images. However, in the second experiment, the LT method was just 56.5% effective at detecting the yellow toy-chicks against the light-coloured background. In contrast, the BS method was more effective, detecting 91.5% of the yellow toy-chicks. The results showed that BS detection success was worse for yellow toy-chicks in the far section, detecting 83% as opposed to 100% of those in the near-section. Edge processing of the image processing algorithm was tested on a Raspberry Pi 3 series B+ computer. This prototype provides a solid foundation for further development and testing of low-cost, automated poultry monitoring systems capable of reporting on thermal comfort inferred from cluster index.

Key words: background subtraction, cluster index, image processing, linear transformation, poultry.

INTRODUCTION

Poultry farming to improve the lives of those in poor, rural regions of Sub-Saharan-Africa has become increasingly established. Whilst poultry farming is well-established in Kenya, with studies finding over 88% of households own chickens (Thumbi et al., 2015; Otiang et al., 2020), productivity has historically been low due to the prevalence of traditional, free-range rearing methods (Njue et al., 2006; Magothe et al., 2012). The introduction of intensive rearing systems, whereby birds are housed permanently and supplied feed, water and health treatments, has been shown to significantly improve productivity (Menge et al., 2005; Ochieng et al., 2011). As such, in the past few decades there has been a shift towards intensive systems across low-income countries worldwide (Hedman et al., 2021).

In Kenya, raising chickens has traditionally been the responsibility of women, who constitute around 75.8% of those engaged in small-scale rearing enterprises (Ochieng et al., 2011). However, Dumas et al. (2018) highlight that many women face difficult decisions when balancing livestock rearing and other household tasks. This leaves the time-burden of intensive systems as a key limitation, generating a need for low-cost solutions that can automate labour intensive tasks. By providing enabling solutions for women in poultry rearing, they can become empowered through greater financial autonomy (Dumas et al., 2018), thus contributing toward the United Nations 'Sustainable Development Goal Five' for gender equality (UN, 2021).

The use of intensive systems in tropical climates such as Kenya also poses thermal management challenges, as poultry shed temperatures must be carefully controlled. Whilst the most common type of chicken used in small-scale enterprises are indigenous chicken (IC) breeds, which are adapted for improved thermal tolerance (Piestun et al., 2008; Magothe et al., 2012), thermal stress remains a cause of productivity loss in shed conditions (Nyoni et al., 2018). This generates a need for systems which can monitor the thermal comfort of poultry flocks and alert farmers remotely when thermal regulation actions are required.

For measurement of bird core temperature, cloacal insertion of thermometers can be used (Maman et al., 2019; Aluwong et al., 2017). However, this method involves handling, which has been shown to induce stress-related core temperature increases in birds, impacting accuracy. For use in continuous flock monitoring, the method is invasive, requires training and is highly time consuming (Cabanac & Aizawa, 2000; Cabanac & Guillemette, 2001). Alternatively, infra-red thermography (IRT) can be used to measure skin surface temperatures - Giloh et al. (2012) have shown a strong correlation between the core and skin temperatures in chickens aged between eight and 36 days. IRT is a non-invasive approach enabling continuous sampling and requiring significantly lower time costs to implement (Jerem et al., 2015). However, there is not clear evidence that this approach will work with chicks under eight days of age.

The cluster index algorithm for distinguishing agglomeration of birds under different temperature conditions, and computer vision were suggested to be suitable tools for assessing poultry thermal comfort automatically by Pereira et al. (2020). Current commercially available systems for automated poultry flock monitoring (Greengage, 2021; Scout Monitoring, 2021; Speller, 2022) are prohibitively costly for typical rural Kenyan farmers. However, automated monitoring, which can increase productivity and reduce labour requirements, offers a tool for accelerated sustainable development in these regions. Thus, the research problem, small-scale farmers need an affordable poultry monitoring system which alerts on welfare issues.

The objective of this paper is to develop a low-cost prototype poultry behaviour imaging and analysis system for monitoring intensively-reared flocks to meet the needs of small-scale farmers.

MATERIALS AND METHODS

Overhead camera system experiment

The low-cost image capture hardware setup consisted of a Raspberry Pi Zero 2 and a Raspberry Pi Camera V2 (8 MP full colour) (incl. suitable power supply and flash drive) mounted with the camera facing down above a 1.2 m × 1.5 m pen of 25 Ross 308 birds, equating to a stocking density of 13.8 birds m^{-2}. A height of 2.7 m was used as this was the highest of the minimum roof heights identified on a site visit to Kenya by the authors, providing the greatest challenge to camera resolution from an overhead view. Due to ethical constraints, exposure of the flock to thermal stress was not possible, therefore sampling over a number of days enabled a range of degrees of clustering to be captured as the birds naturally moved around the pen. The birds were males, aged 42 days on the last day of data collection, the average weight on the last day of data collection was 3.1 kg, there were no incidents of mortality in the pen used for data collection, bedding was not changed throughout the trial and not topped up. The birds were provided with optimum conditions and comfort to meet the health and welfare requirements for growing birds i.e. fresh food and water, ventilation, friable dry bedding and optimum temperature and lighting. The study procedures were approved by Harper Adams University Research Ethics Committee and reported here in accordance with the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020).

Images were collected at a sample rate of 15 s, with 69 h of data collected over eight days (Table 1). Additional lamps were not used. A 15 s sample rate was deemed sufficient to balance the need to manage the volume of data and to maximise data capture, being between 1 s (Pereira et al., 2020) and 30 s (Del Valle et al., 2021).

Table 1. Data recording and darkness hours

Date	Recording			Darkness			Visible imaging (h)
	Start time	End time	Total (h)	Start time	End time	Total (h)	
04-11-21	15:56			20:47			
05-11-21		16:04	24.13		03:05	6.30	17.83
05-11-21	16:46			20:46			
06-11-21		00:19	8.55		00:19	3.55	5.00
08-11-21	17:47			20:47			
09-11-21		16:05	22.30		03:05	6.30	16.00
09-11-21	16:27			20:47			
10-11-21		11:24	18.95		03:05	6.30	12.65
11-11-21	10:54			20:28			
12-11-21		11:53	24.15		03:05	6.62	17.53
TOTAL			98.08			29.07	69.02

The image processing code was developed using Python language via the Anaconda development platform, with the OpenCV (OpenCV, 2022) image processing package installed. A sequence of linear transformations (LT) were conducted for object detection. Linear transformation (LT) methods have been successfully used for object detection in recent studies (Fernández et al., 2018; Pereira et al., 2020; Del Valle et al., 2021), where white birds were detected against a dark background.

The first step was to crop and rotate the gathered images, to focus on the individual pen of birds. This was then blurred, using the OpenCV function 'cv2.blur()' (Nixon & Aguado, 2020) to reduce pixel noise before binarization (Fig. 1).

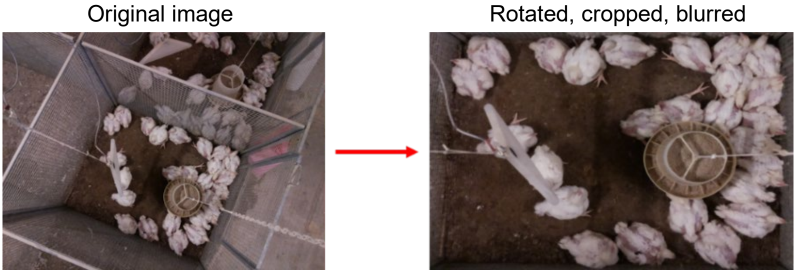

Figure 1. An example of rotation, crop and blurring.

The image was then converted into grayscale for binarization using OpenCV which converts each pixel value from 0–255 into either 0 (black) or 1 (white), with a threshold of 110 being used as the cut off point for conversion either way (Fig. 2). The threshold was determined iteratively by comparing visually input and output images until a threshold value was found which outputs most complete information about the objects of interest and the least anomalous information.

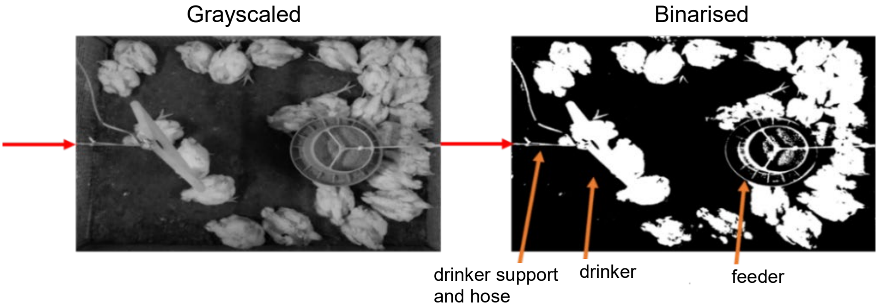

Figure 2. An example of grayscaling and binarisation.

Erosion and dilation were then performed to eliminate noise and reduce the occurrence of unwanted objects such as feeder and drinker (Fig. 3).

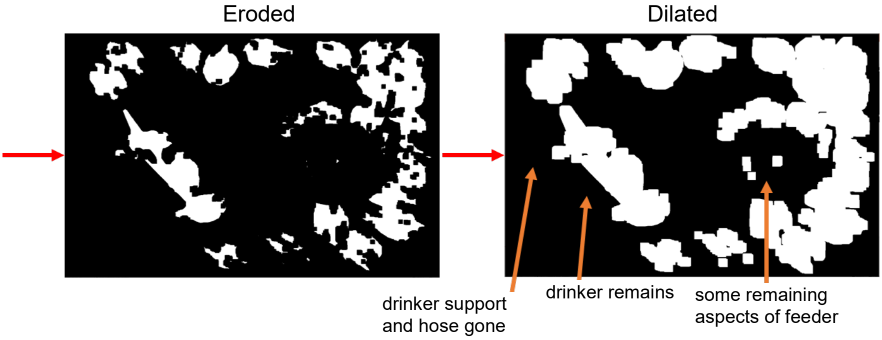

Figure 3. An example of erosion and dilation.

Contour detection was conducted using the OpenCV function ‘cv2.findcountours()’. These contours were then filtered by perimeter length to eliminate final non-bird containing objects (Fig. 4).

Figure 4. An example of contour-detection and contour-filtering.

Once the contours had been detected and filtered, the following variables required for the cluster index equation (Pereira et al., 2020) could be identified: $\underline{A}$ – average area of detected objects, $\underline{P}$ – average perimeter of detected objects and N_A – number of objects, whilst x – image’s horizontal pixel length and y – image’s vertical pixel length are known from the camera’s resolution. To detect the average distance between objects, $\underline{D}$, the object centre points were required. A minimum bounding rectangle was drawn around each object, using the OpenCV function ‘cv2.boundingRect()’, and the centre coordinates were computed:

$$Centre\ Coordinates\ (x,y) = x1 + \frac{width}{2}, y1 + \frac{height}{2} \tag{1}$$

where $x1$ and $y1$ are the coordinates for the vertex closest to the origin (Fig. 5).

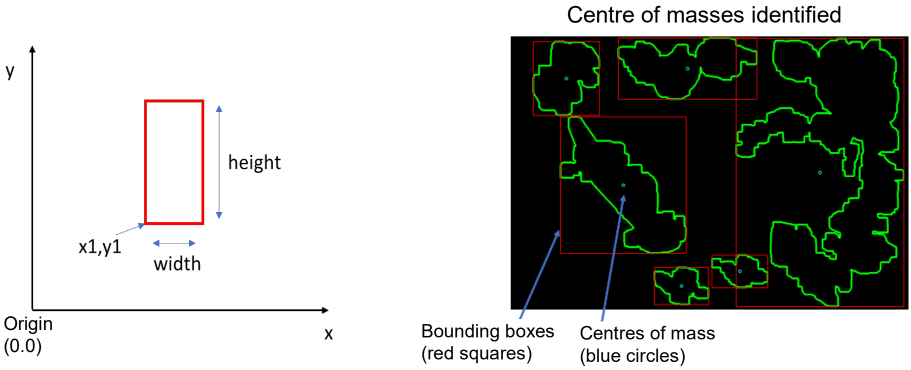

Figure 5. Calculation of bounding box centre coordinates and an example of the bounding box and object centres overlaid onto object contours.

An open-source package ‘scipy.spacial.distance’ was used to find the average Euclidian distance between each object centre and the others (SciPi, 2022). This was repeated for all object centres, which were then averaged to find $\underline{D}$. A random set of ten images from each recorded day were selected and the object detection output was compared to its rotated and cropped original (Fig. 6). For each image, any birds not captured within a green contour line were recorded, along with any detected anomalous objects.

Figure 6. An example of the comparison method for checking contour detection accuracy.

Cluster Index variables were generated using the Python image processing script. Using a Raspberry Pi 3 series B+, ten sample images from each day of testing were processed using the developed code.

Angled camera system experiment

A second trial was conducted to test the applicability of an angled camera set-up. This would increase the area covered by a camera, reducing hardware requirements per shed and lowering installation costs.

A 16 m × 5 m area was marked out onto the floor of an empty poultry house, to demarcate the boundaries of the size of a representable Kenyan shed. An image capturing and storage system (a Wansview W3 camera (1 MP full colour, outdoor rated, WiFi enabled), Raspberry Pi 3B+, and a laptop) was set-up with the camera at a height of 2.7 m and located at the edge of one end of the demarcated boundaries. The camera's floor area coverage was measured by observing the live feed from the camera and marking the floor at the vertices of its field of vision. These points were measured in comparison to the demarcated shed.

An 8 m × 1.5 m strip of woodchip bedding was laid out within the camera's field of vision and outlined with white tape. In order to test the applicability of the vision system with chicks under 10 days of age, 20 yellow-coloured toy-chicks with dimensions roughly 80 mm × 80 mm × 60 mm were used to simulate young chicks.

Two 1m × 1.5m sections of the woodchip strip were selected for locating toy-chicks at the front and rear sections (Fig. 7). This would provide data at the extremes of the imaging systems capability. By using 1.5 m^2 sections, 20 birds could be used to ensure that the stocking density of 12.5 birds m^{-2} was achieved.

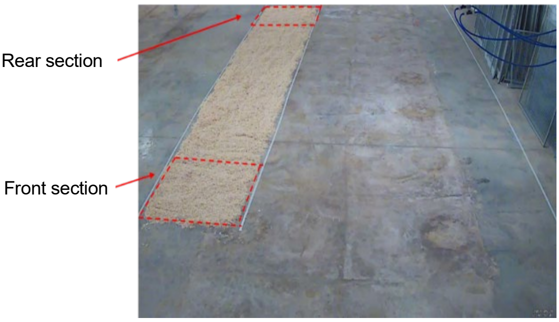

Figure 7. A visualisation of the front and rear sections of woodchip strip used in the angled camera experiment.

Images were taken using the 20 yellow toy-chicks. Later, ten toy-chicks were painted brown, to replicate the colour characteristics of Kenyan indigenous varieties. Five images of the background and then 50 images in five sets of yellow, yellow and brown, and just brown birds, were then collected.

A perspective transform (PT) Python script was developed using the image set of yellow birds in the front and rear section. The coordinates of the four corners of the woodchip strip were identified using Microsoft Paint, and fed into the algorithm. This algorithm highlighted the location of the given coordinates, then cropped and warped the images, using the OpenCV function 'cv2.warpPerspective()' to provide a two-dimensional, birds-eye view of the bounded area (Fig. 8). This method was repeated for all data sets to provide a basis for object detection testing.


Original image with coordinates highlighted

Perspective transform output

Figure 8. An example of Perspective Transform conducted in the angled camera experiment.

To test the impact of object stretching resulting from PT, as highlighted by Dawson-Howe (2014), the contour detection algorithm used in the overhead camera system was applied images after background subtraction (BS). LT is highly dependent upon lighting and object-background contrast (Okinda et al., 2020), thus BS method is more successful as found by Van Hertem et al. (2013) in an experiment with cows of varied colours.

Contours were filtered to leave only individual birds, with no multi-bird groups (Fig. 9). The area of each object in the front and rear sections was calculated using the OpenCV function 'cv2.contourArea()'and then averaged for each group. This was repeated on 5 random images from each image set.

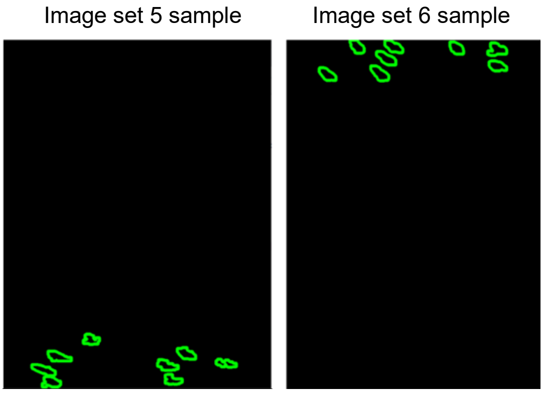
Image set 5 sample

Image set 6 sample

Figure 9. An example of the contours used to test the impact of PT on object size.

The linear transfer (LT) and background subtraction (BS) methods were applied for direct comparison with the angled camera application. Using the PT images generated, the linear transformations used in overhead camera experiment were applied. As the birds in this instance were darker than the bedding, the binarisation stage outputs dark objects of interest against a white background. Contour detection requires white objects against a black background, so the image was inverted by applying inversion to each pixel (Fig. 10)

$$Inverted\ Pixel = 255 - Binarised\ Pixel \tag{2}$$

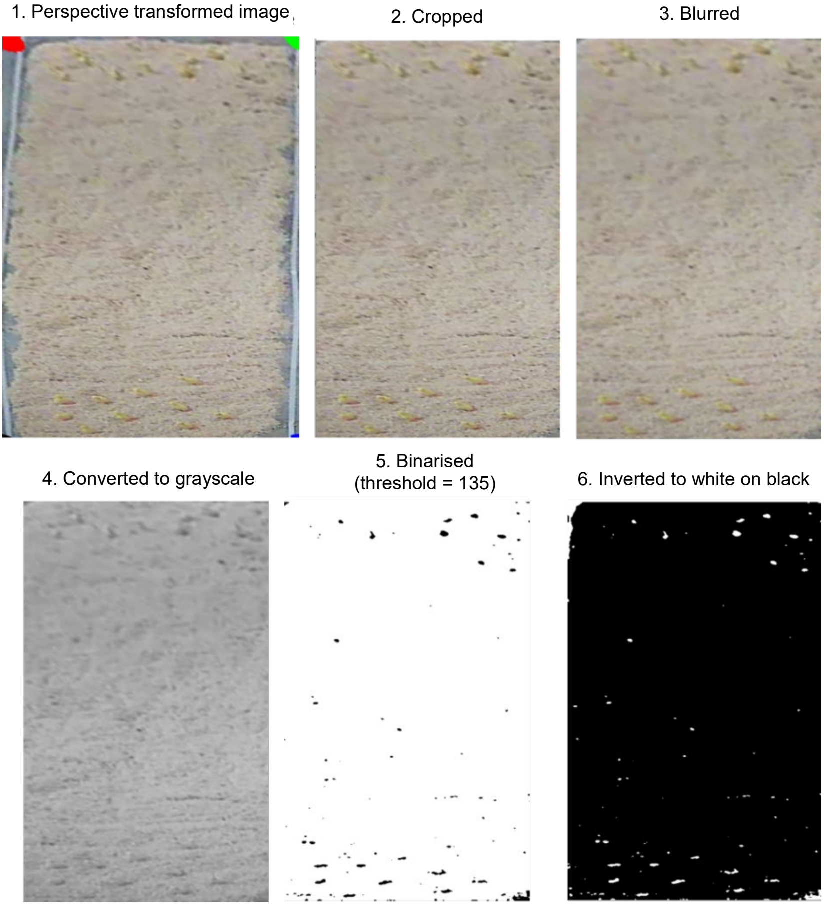

Figure 10. The stages of linear transformation object detection in angled camera experiment for image set of yellow birds in the front and rear section (threshold of 135 was found iteratively).

Background subtraction was again initially applied by taking a background image and subtracting it from the 'current frame' containing yellow birds in the front and rear section. A threshold of 35 was selected to filter out noise based on iterative adjustments as described above (Fig. 11). The variation in the threshold values selected between the tests was due to the change in contrast between the birds and the background, resulting from their changed colours.

To compare LT and BS methods, a foreground mask was generated using each method for all images of birds. Contour detection method as described above was then applied. Post contour-detection, the number of birds missed and anomalous contours generated were manually recorded for each of the images.

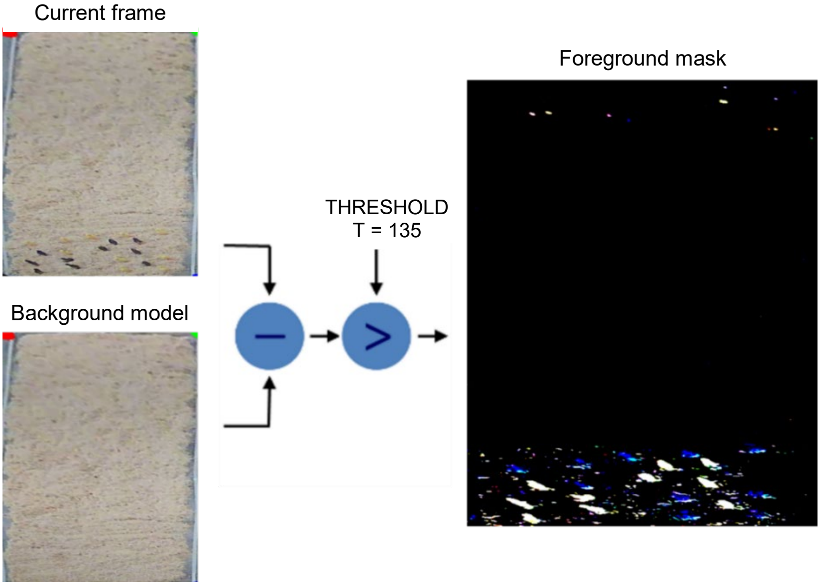

Figure 11. A visualisation of the background subtraction method applied in angled camera experiment for image set of yellow birds in the front and rear section.

RESULTS AND DISCUSSION

In the overhead camera system experiment, where white birds were detected against a dark background, a randomly selected ten-image sample from each of the eight days of collected footage (i.e. 25 birds in each image × 80 images totals 2000 birds) was processed for contour detection. The image processing code ran on the Raspberry Pi was able to generate the variables required to calculate the cluster indices. LT object detection successfully detected 99.8% of birds in the sampled images (just four birds were identified as being missed). This correlated with the success of Pereira et al. (2020) and Del Valle et al. (2021) in similar scenarios.

However, in the angled camera system experiment, the LT method was just 55–56% effective at detecting the yellow toy-chicks against the light-coloured background (Fig. 12). This finding agrees with Okinda et al. (2020) that object-background contrast is critical for LT applications. In contrast, the BS method was more effective, detecting 83–100% of the yellow toy-chicks (Figure 12). This correlates with the results of Van Hertem et al. (2013) who found BS methods superior to LT methods in their scenario detecting cows of inconsistent colours. With Chesoo et al. (2021) finding that Kenyan indigenous chickens phenotypes tend to be varied combinations of brown, black and white, the BS method for object detection is recommended for this prototype system. Nonetheless, the angled camera system experiment did not involve live birds as Kenyan indigenous chickens were unavailable, and so the background stability issues highlighted by Okinda et al. (2020) could not be analysed. Whilst the background update method used by Guo et al. (2020) may have combatted this, testing this was also not possible with the resources available. The results showed that BS detection success was worse for yellow toy-chicks in the far section, detecting 83% as opposed to 100% of those in the near-section. The impact of camera-resolution upon object detection using the BS method offers an avenue for future research in this area with a higher resolution camera.

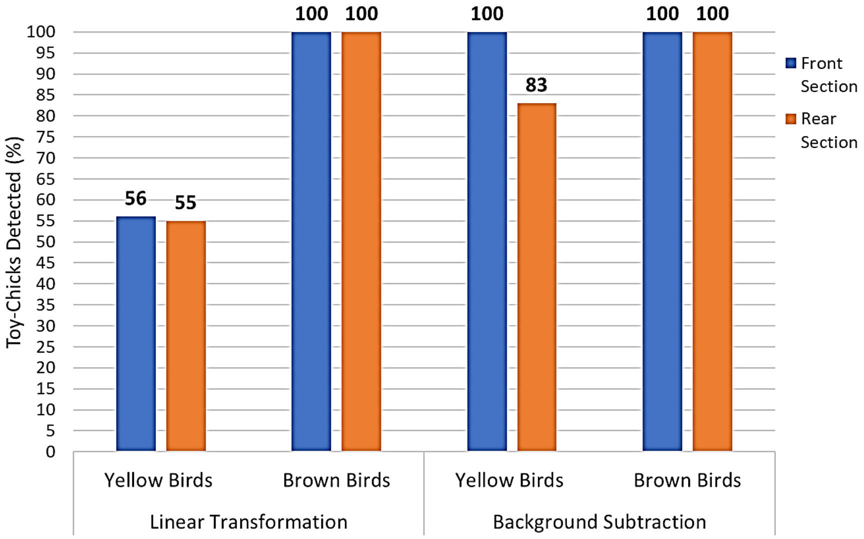

Figure 12. A comparison of LT and BS methods for detecting toy-chicks, by colour and location.

The floor area covered by a single overhead camera was 2.45 m × 3.26 m. A typical 16 m × 5 m Kenyan poultry shed would require 12 Raspberry Pi V2 cameras for full floor area coverage. This would entail a camera cost of 288 GBP, which is prohibitive even before the subsequent increased processing requirements were factored in.

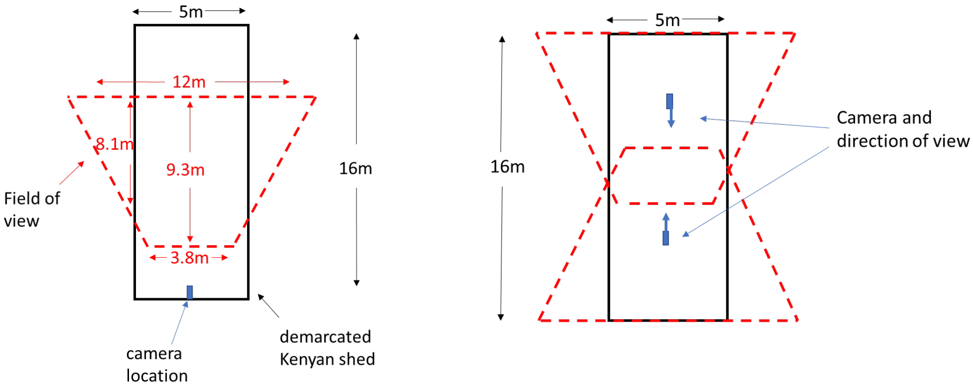

Figure 13. Left: The measured camera floor area coverage; Right: projected total floor area coverage with two angled cameras.

Due to this, an angled camera approach was tested, and the use of PT techniques to enable cluster indexing using this set-up was demonstrated to successfully transform the three-dimensional view into two-dimensions. The area covered by a single angled camera was 56% of the demarcated Kenyan shed (Fig. 13 left) - it would be possible for the entire floorspace to be covered by just two cameras as shown in Fig. 13 right. This brings camera costs down by 240 GBP, whilst also reducing the processing requirements. However, the results support the assertion of Dawson-Howe (2014) that objects further from the camera become stretched and enlarged during PT. The impact of this upon the cluster index was not measured. The impact will vary depending upon the size and density of the birds in the sheds and thus is a necessary avenue of investigation before this system can be developed further.

Edge processing of the developed image processing algorithm was tested using a Raspberry Pi 3 series B+ computer, which was found capable of generating the required cluster index variables for individual images. Further testing would be required to better understand the processing capacity for multiple images of larger flock sizes at 15 second sampling intervals. SMS communication to alert the farmer is suggested to be optimal for rural Kenya and is applicable with a communications module with the Raspberry Pi 3 series B+. An early-stage prototype has been recommended (Table 2 and Fig. 14).

Table 2. Proposed prototype hardware

Hardware	Quantity	Total cost GBP
Wansview W5 IP66 security camera	2	119.98
Raspberry Pi 3 Series B+	1	33.90
MicroSD card	1	6.00
5V power supply	1	8.00
SIM800X Communications HAT	1	18.50
SIM card	1	–
TOTAL		186.38

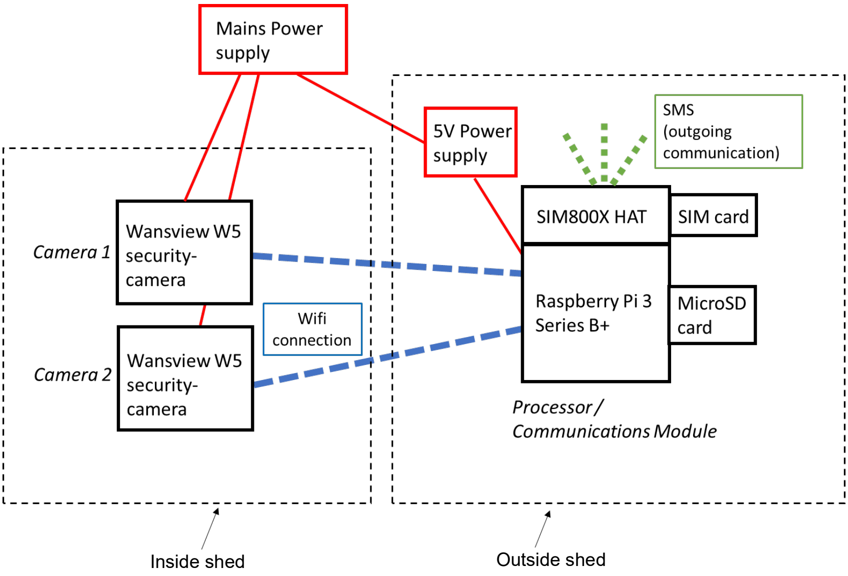

Figure 14. The proposed prototype system.

The inbuilt WiFi capability of the computer and cameras enables wireless communication without a dedicated router. This set-up enables the computer assembly to be located outside of the shed, in a protected environment, limited by the range of the WiFi connection and access to a power supply. By using the edge processing capability of the Raspberry Pi 3 Series B+, the need for further computing hardware is negated. The use of Python, and the open-source OpenCV package requires no additional software cost to run the inference algorithm on the Raspberry Pi.

Nonetheless, LT has shown potential to reduce processing and installation capital requirements, lowering the barriers to implementation of poultry monitoring systems in rural Kenya. The new knowledge can be used to monitor remotely and in real time conditions and welfare of birds in small-scale farmers' poultry sheds, reducing labour costs and bird mortality and increasing performance and incomes.

CONCLUSIONS

An overhead camera system using Raspberry Pi V2 camera was used to collect images of Ross 308 birds over eight days. An image processing algorithm was developed using Python and OpenCV. Linear transformation (LT) object detection successfully detected 99.8% of birds in the sampled images white birds against a dark background. A single overhead camera covers 2.45 m × 3.26 m floor area, thus requiring 12 cameras to cover the total floor area in a typical poultry shed opposed to just two with an angled camera approach. An angled camera system using Wansview W3 camera was tested on a 16 m × 5 m area on yellow and brown toy-chicks. The LT method was just 56.5% effective at detecting the yellow toy-chicks against the light-coloured background. In contrast, the background subtraction (BS) method was more effective, detecting 91.5% of the yellow toy-chicks. The results showed that BS detection success was worse for yellow toy-chicks in the far section, detecting 83% as opposed to 100% of those in the near-section. The suggested prototype provides a solid foundation for further development and testing of low-cost, automated poultry monitoring systems.

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The selection of maize parent lines within marker assisted selection (MAS) by crtRB1-3'TE marker for Steppe zone of Ukraine

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Abstract. Maize has a large genotypic diversity and a broad scale of economically important traits. Therefore, it is extremely important for breeding to obtain hybrids which can ensure high yield even under severe growing conditions, such as in Steppe zone of Ukraine. This study aimed to determine the optimal allele ratio by crtRB1-3'TE marker in parental components of maize modified hybrids. There were investigated four hundred sixteen maize hybrids which are modified hybrids of heterotic model (Iodent × Iodent) × Lancaster germplasm. SCA (specific combining ability) effects for grain yield and grain moisture content were calculated in maize hybrids with different allele combinations of crtRB1 gene. As results, the stable positive SCA effects were calculated for hybrids with allele combination (296 bp + 875 bp) × 543 bp × 296+875 bp during both 2019 and 2020 (1.23 and 0.99 t ha^{-1}, respectively). The lowest SCA effects for grain moisture content were obtained for modified hybrids with allele combination (543 bp × 543) × 296 + 875 bp both in 2019 and 2020 (-0.54 and -0.36%, respectively). The greatest influence SCA effects for grain yield had the interaction of allele combinations and year weather conditions (39%), the impact the allele combinations was 36%. The year weather conditions had the greatest impact on SCA effects for grain moisture content (44%), the allele combination (36%). Thus, it was determined that SCA effects for studied indicators of heterotic model (Iodent × Iodent) × Lancaster under contrasting weather condition are resulted from both genotypes of hybrid parents and favourable allele presence.

Key words: *Zea mays* L., carotenoids, crtRB1 gene, favourable allele, SCA effects.

INTRODUCTION

Maize (*Zea mays* L.) is currently one of the most important crops in the world. Maximizing its productivity and yield while maintaining the quality is one of the primary goals of corn producers (Bojtor et al., 2021). Maize is a crop with enormous diversity of economically important traits. Maize, being a C4 plant, can be considered a potential source of bioenergy as it possesses all the essential traits like wide adaptation, superior carbon sequestration, and efficient nitrogen utilization (Choudhary et al., 2020; Bojtor et al., 2021). Meanwhile, maize can be used directly for human food, processed into various types of food products, such as flour, cornmeal, grits, starch, snacks, tortillas, and breakfast cereals, or used for animal feed (Gayosso-Barragán et al., 2020; Sun et al., 2022).

Maize has the potential to meet the vitamin A requirements by providing precursors for vitamin A biosynthesis. Carotenoids are an important source of vitamin A. Maize kernel has considerable variation for the levels of carotenoids content and breeding for these economically important compounds is feasible. An understanding of the plant carotenoid biosynthesis pathway has opened an avenue for the deployment of functional markers to improve carotenoid accumulation in maize grain (Gebremeskel et al., 2018). The main genes related to the accumulation of carotenoids in maize grain include the lycopene ε-cyclase (lcyε) and β-carotene hydroxylase (crtRB1). The crtRB1 gene polymorphism is connected with increased β-carotene rate in maize grain and revealed by crtRB1-3'TE marker. It is possible to identify three allele variants: 543 bp (favorable allele), 296 bp and 296 + 875 bp (Muthusamy et al., 2015). The efficiency of using this marker in MAS for maize line with high carotenoids level selection and strong correlation between the presence of favourable allele and carotenoids content in kernel was demonstrated by our previous study and many other authors (Senete et al., 2011; Messias et al., 2014; Muthusamy et al., 2015; Zunjare et al., 2018; Prysiazhniuk et al., 2022a). However, introgression of favorable alleles of crtRB1 gene can have dramatically different effects depending on the genetic background (Diepenbrock et al., 2021).

According to the State Statistics Service of Ukraine, the sown area of maize over the past three years increased to 5.5 million hectares (Prysiazhniuk et al., 2022a). The Steppe zone of Ukraine has the longest growing season, but receives the lowest precipitation and often suffers from drought. Therefore, it is extremely important for plant breeding to obtain hybrids which can ensure high yield under such growing conditions. The one of important agronomical traits is also harvest grain moisture content. High grain moisture contents at harvest necessitate grain drying prior to the transport and storage of ears and requires the extra expenses for the grain basic moisture content (Petkevičius et al., 2008; Li et al., 2021).

Combining ability may be considered as the potential of an individual inbred line to contribute better fitness-related traits to hybrid progeny. In maize breeding programs, knowledge of specific combining ability (SCA) of hybrid combinations as well as the identification and exploitation of heterotic groups, are crucial for successful hybrid production (Gami et al., 2018; Iseghohi et al., 2020). Thus, we aimed to determine the optimal allele ratio by crtRB1-3'TE marker in parental components of maize modified hybrids by assessing SCA effects for grain yield and grain moisture content.

MATERIALS AND METHODS

Plant material and laboratory measurements

Four hundred sixteen maize modified hybrids of heterosis model (Iodent × Iodent) × Lancaster germplasm (three-way cross hybrids) were used in this study. The effects of specific combining ability (SCA) were assessed by the top-crossing method. Test crosses were made with using sister sterile hybrids (Lancaster germplasm) as testers. All breeding materials were provided by Research Institute of Agrarian Business (Dnipro, Ukraine).

The laboratory studies were carried out in Laboratory of molecular genetic analysis, Ukrainian Institute of Plant Variety Examination (Kyiv, Ukraine) in 2019. The DNA marker crtRB1-3'TE was used to identify the allele state of crtRB1 gene (Muthusamy et al., 2015). DNA extraction procedure, PCR parameters and amplicons visualisation were described in our previous study (Prysiazhniuk et al., 2022a). To assess the impact of alleles combinations on grain yield and grain moisture content, genotypes of modified hybrids with all combinations of detected alleles of crtRB1 gene were used.

Field measurements and environments

The field experiment was carried on during 2019-2020 on pilot plots of Research Institute of Agrarian Business (Vesele village, Dnipro region, Ukraine). The field experiment was designed according to classical crosspollinated plant breeding methods (Dospekhov, 1985). The grain moisture content was estimated during harvesting using the plot combine (Wintersteiger, Germany). The weather conditions rates were provided by Sinelnykove weather station (Table 1).

Table 1. The amounts of precipitation and air temperature during 2019–2020 maize growing season

Month	Amounts of precipitation, mm			Air temperature, °C		
	Normal daily average	2019	2020	Normal daily average	2019	2020
May	50.0	21.3	2.9	15.8	18.1	13.7
June	59.0	1.0	0.0	19.1	23.9	22.1
July	61.0	33.7	1.3	20.9	20.9	23.2
August	35.0	73.4	14.0	20.1	20.9	21.5
September	36.0	6.4	0.0	15.0	16.1	19.5

Statistical analysis

The coefficient of agrometeorological indicators from normal daily average amounts during 2019–2020 was computed using the equation below:

$$Dc = \frac{X_i - \bar{X}}{\sigma}$$

where Dc – deviation coefficient; X_i – indicator of current weather; X – normal daily average amounts; σ – mean-square deviation. The rate of deviation coefficients was determined according to scale: $Dc = 0–1$ – close to normal conditions; $Dc = 1–2$ – strong different conditions; $Dc > 2$ – close to unique conditions (Yeremenko et al., 2017).

The significant differences of studied indicators and the rate of factors impact on SCA for grain moisture content were determined by ANOVA using STATISTICA 12.0 software (trial version).

The correlation between the allele state of crtRB1 gene and SCA effects for grain yield and grain moisture content was evaluated with Mantel test (Pearson corelation) using XLSTAT software (trial version) (Prysiazhniuk et al., 2022b). The distances matrices were obtained based on allele combination and SCA effects values. The unweighted pair-group average amalgamation rule was used for genetic distances calculation based on allele state, single linkage rule - for distances based on SCA effects.

RESULTS AND DISCUSSION

For the crtRB1-3'TE marker, all three possible allele variants were identified among the lines which were parental components of studied modified hybrids accept three lines variants. There were not identified any expecting alleles. For each type of allele combinations of modified hybrids, SCA effects for grain yield was calculated (Table 2).

Table 2. SCA of modified hybrids with different allele combinations for grain yield and grain moisture content 2019–2020

Allele combinations of crtRB1 gene			Average SCA for grain yield, t ha^{-1}		Average SCA for grain moisture content, %	
♀	♂	pollinator	2019	2020	2019	2020
296	296	543	-2.08	-0.52	-0.26	-0.13
296	296	296	0.63	-0.35	0.21	-0.15
296	296	296 + 875	3.16	0.03	0.61	0.36
296	543	543	0.38	0.07	0.16	0.03
296	543	296	0.29	0.06	-0.15	-0.03
296	543	296 + 875	0.36	0.09	-0.36	-0.07
296	296 + 875	543	-0.35	0.60	-0.05	-0.12
296	296 + 875	296	-0.06	0.43	0.02	-0.05
296	296 + 875	296 + 875	0.99	-0.55	0.07	0.20
543	296	543	0.18	-0.77	0.20	-0.03
543	296	296	0.38	0.05	-0.17	-0.04
543	296	296 + 875	0.53	1.26	-0.25	0.15
543	543	543	-1.36	-0.96	0.11	0.24
543	543	296	0.70	-0.03	-0.15	0.14
543	543	296 + 875	0.84	2.58	-0.54	-0.36
543	296 + 875	543	0.24	0.22	-0.03	0.04
543	296 + 875	296	0.14	-0.18	-0.02	0.06
543	296 + 875	296 + 875	-0.68	-0.32	0.08	-0.07
296 + 875	543	543	2.17	-1.17	0.33	0.14
296 + 875	543	296	-0.01	0.34	-0.04	0.00
296 + 875	543	296 + 875	1.23	0.99	-0.79	0.17
296 + 875	296 + 875	543	1.04	-0.89	-0.18	0.07
296 + 875	296 + 875	296	-1.26	0.58	0.13	-0.07
296 + 875	296 + 875	296 + 875	-1.93	1.21	0.03	-0.05
296 + 875	-	543	0.94	-0.56	-0.28	-0.07
296 + 875	-	296	0.35	-0.05	0.21	-0.09
296 + 875	-	296 + 875	0.35	-0.66	0.20	0.27

According to obtained results, SCA effects for grain yield of modified hybrids were characterised by diversity depending on studied year. It was shown that in general, hybrids which had high positive SCA effect in 2019 or 2020, demonstrated low SCA in another year. The highest SCA effect for grain yield was observed in hybrids with allele combination (296 bp ×2 96 bp) × 296 + 875 bp in 2019 p. (3.16 t ha^{-1}). Meanwhile, the SCA effect in hybrids with this combination in 2020 was low (0.03 t ha^{-1}). The same situation was observed for hybrids with allele combination (543 bp × 543 bp) × 296 + 875 bp. These hybrids demonstrated low SCA in 2019 (0.84 t ha^{-1}), but the high SCA effects were observed in 2020 (2.58 t ha^{-1}). Hence, the stable positive SCA effects were calculated for hybrids with allele combination (296 bp + 875 bp) × 543 bp × 296+875 bp during both 2019 and 2020 (1.23 and 0.99 t ha^{-1}, respectively). Hybrids with allele combination (296 bp × 543 bp) × 543 bp had also low positive SCA effects during 2019–2020 (0.38 and 0.07 t ha^{-1}, respectively).

The lowest SCA effects both in 2019 and 2020 were obtained for hybrids with allele combination (296 bp × 296 bp) × 543 bp. They were -2.08 and -0.52 t ha^{-1}, respectively. The hybrids with allele combination (543 bp × 543 bp) × 543 bp demonstrated low SCA effects during 2019–2020 (-1.36 and -0.96 t ha^{-1}). However, hybrids with allele combination (296 + 875 bp × 296 + 875 bp) × 296 + 875 bp shown low SCA effects in 2019 (-1.93 t ha^{-1}), meanwhile in 2020, the SCA was positive (1.21 t ha^{-1}).

It was determined that the lowest SCA effects on grain moisture content were obtained for modified hybrids with allele combination (543 bp × 543) × 296 + 875 bp both in 2019 and 2020. They were -0.54 and -0.36%, respectively. In 2019, the lowest SCA effects were calculated for hybrids with allele combination (296 + 875 bp × 543 bp) × 296+875 bp (-0.79%). In 2020, the hybrids with this allele combination showed low positive SCA effects (0.17%). The positive SCA effects for grain moisture content during the experiment were noticed for hybrids with allele combination (296 bp × 296) × 296 + 875 bp (0.61 and 0.36% in 2019 and 2020, respectively). The hybrids with allele combination (296 bp × 296 bp) × 543 bp demonstrated low SCA effects for grain moisture content during 2019-2020 (-0.26 and -0.13%, respectively). However, these hybrids have shown negative SCA effects for grain yield as it was described.

Considering the weather conditions during the maize pollination stage in 2019 the weather conditions were close to normal, *Dc* for air temperature and the amount of precipitation were 0 and -1, respectively. In 2020 during this period the weather conditions characterized by the high air temperature and the lack of precipitation, *Dc* for weather condition indicators were 2 and -2 respectively. On the other hand, the weather conditions during physiological maturity period in 2019 were close to normal in contrast to 2020. The air temperature during August-September 2019 was close to normal temperature (*Dc* was from 0 to 1). In comparison to 2019, in 2020 *Dc* was 2 which indicates strong different conditions. The deviation coefficients for amounts of precipitation during August-September 2019 were from 1 to -2. In 2020, during this period *Dc* has negatiive values (from -1 to -2).

To assess the rate of factors impact on SCA effects for grain yield and grain moisture content of maize modified hybrids, the ANOVA was used. Partitioning mean squares into its components revealed significant influence of allele conbination, year wheather condition and their interaction on SCA effects (Table 3).

Table 3. Mean squares from the analysis of variance of grain yield and grain moisture content of maize modified hybrids evaluated during 2019–2020[1]

Effect	DF[2]	Grain yield	Grain moisture content
Intercept	1	4.37	0.02
Alleles combination	26	2.69	0.20
Year	1	1.87	0.25
Alleles combination*Year	26	2.97	0.11
Errors	108	0.007	0.008

[1] Significant at 0.05 probability levels; [2] DF – Degree of freedom.

According to the obtained results, it was determined that the greatest influence of SCA effects for grain yield had the interaction of allele combinations and year weather conditions (39%). Partitioning mean squares into its components revealed that the impact of the allele combinations on SCA effect for grain yield was 36%. Furthermore, the year weather conditions had the lowest impact on SCA effects (25%) (Fig. 1).

Menkir et al. (2014) reported that environments, hybrids and hybrid × environment interactions had significant effects on carotenoid content in their study which was examined the effect of crossing parental lines from two AFLP-based groups on carotenoid accumulation and agronomic performance in hybrids. It was determined that environments, hybrids and hybrid × environment interactions had significant effects on grain yield. In this study, similar results were observed with the greatest influence of allele combination and the interaction between allele combination and year weather conditions.

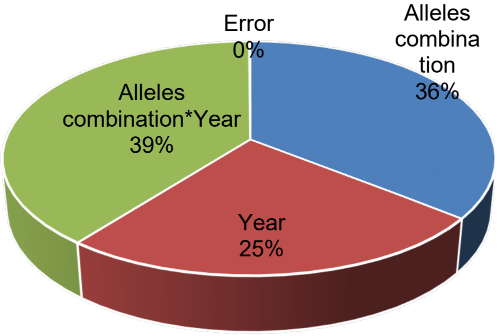

Figure 1. The rate of factors impact (mean squares) on SCA for grain yield of maize modified hybrids during 2019–2020.

It was reported that the one of the critical stages for the formation of main yield components is the pollination. Maize plants are sensitive to high air temperature and moisture deficit. Stress can cause kernel abortion at the cob tip, and wilted leaves from moisture stress in the morning can lead to a yield loss of up to 7% per day (Baum et al., 2019). Moreover, the critical stage of grain yield is physiological maturity. Stress at this point can reduce the number, size and weight of the harvestable kernels.

Muthusamy et al. (2016) assessed the combining ability and nature of gene action for kernel carotenoids and identified superior hybrid combinations for specific carotenoids over commercially available hybrids. As obtained results, investigated maize genotypes were stable across environments.

According to the obtained data in this study, 2020, during critical stages of maize growing season, characterized of high air temperature and precipitation deficit. These factors could cause modified hybrids grain yield decrease. Meanwhile, in 2019, there was the softer weather condition which was the reason of higher rate of grain yield. It should be noted that the high impact rate of interaction of year weather conditions with allele combinations on SCA effects for grain yield could be expressed as the adaptation of modified hybrids to different weather conditions. On the other hand, genotypes which were shown the positive SCA effects both in 2019 and 2020 could be considered as stable hybrids with ability to enable stable grain yield rate under limited environmental conditions (Bonea & Dunăreanu, 2022). There was no corelation between the presence of the favourable alleles by crtRB1-3'TE marker and SCA effects for grain yield for studied modified hybrids. Muthusamy's et al. (2016) investigation demonstrated the similar conclusion that the grain yield did not show association with carotenoids.

As a result of analysis, the year weather conditions had the greatest impact on SCA effects for grain moisture content of modified hybrids (44%). The allele combination revealed a weaker influence (36%). It was shown that the interaction of allele combination and year weather conditions impacted on SCA effects for grain moisture content significantly. The rate of impact was 19% (Fig. 2).

Researchers have previously reported that the physiological maturity is also the critical stage to determine grain moisture content at harvest. Water loss from kernels occurs in two phases. Before physiological maturity, the decrease in grain moisture content is due to successive accumulation of dry matter via grain filling and the water loss rate is constant and highly dependent on genetic factors. After physiological maturity, the accumulation of dry matter ceases, and the reduction in grain moisture content is primarily due to water evaporation from kernels and thus can be greatly affected by environmental factors (Liu et al., 2020).

On the other hand, the grain dehydration rate before and after physiological maturity is also closely related to grain moisture content at harvest. Maize genotypes with a fast dry-down rate generally have low ear moisture at harvest. The grain dehydration rate of maize is affected by many factors, such as variety, endosperm type, planting density, temperature, and humidity.

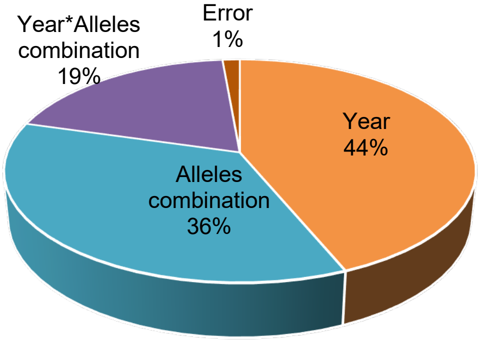

Figure 2. The rate of factors impact (mean squares) on SCA for grain moisture content of maize modified hybrids during 2019–2020.

Taking into account that during maturity stage of studied maize modified hybrids the dry period occured, the lowest SCA effects for grain moisture content were observed in 2020. However, it is more attractive to breeding to consider hybrids with allele combinations which demonstrated low SCA effects under mild weather condition as 2019 was characterized. It is because this weather conditions allows to assess the genetic potential of studied genotypes excluding the weather impact. Kang & Zuber (1989) reported that white hybrids had slightly higher grain moisture content than yellow hybrids because white maize lacks phytoene synthase, an enzyme involved in both carotenoid and abscisic acid biosynthesis. This study shows that majority of modified hybrids, which demonstrated low SCA effects for grain moisture content, had at least one favorable allele connected with high carotenoids content.

In order to assess relationship between the presence of favorable alleles by crtRB1-3'TE marker and SCA effects for grain moisture content in modified hybrids and testers, the Pearson’s correlation coefficient was estimated. It was determined that there was no correlation between the presence of favorable alleles and SCA effects of modified hybrids. However, the analysis revealed the weak correlation between the presence of favorable alleles and SCA effects of testers. The coefficient of correlation $r = 0.31$ at 0.05 probability levels. It could be explained by the fact that three-way maize hybrids possess broader adaptation than single-cross hybrids (Makinde et al., 2022). Meanwhile, the SCA of sister sterile hybrids used in this study were estimated through characteristics obtained for modified single cross hybrids during top-crossing. Thus, testers demonstrated higher genotype impact on SCA effects for grain moisture content than modified hybrids. Hence, as a result of this study, it was shown that factors as genotypes with different allele combination by crtRB1-3'TE marker and year weather conditions impacted significantly on grain yield and grain moisture content of maize heterotic model (Iodent × Iodent) × Lancaster.

CONCLUSIONS

In the study, SCA effects for grain yield and grain moisture content of maize modified hybrids were found to be significant. By assessment of the rate of factors impact on SCA effects for grain yield it was found that the interaction of allele combinations and year weather conditions had the greatest influence on modified hybrids (39%). This can indicate of the modified hybrids' ability to adapt to different weather conditions. The high rate of influence SCA effects for grain moisture content revealed by the year weather conditions and allele combination (44 and 36%, respectively). It is concluded that taking into account the different weather condition in Steppe zone of Ukraine during studied year, there were selected genotypes with allele combinations of crtRB1 gene (296 bp × 543 bp) × 296 + 875 bp, (543 bp × 296 bp) × 296 bp, (543 bp × 296 bp) ×296 + 875 bp, (543 bp × 543 bp) × 296 + 875 bp, (296 + 875 bp × 543 bp)× 296 + 875 bp with provided positive SCA effects for grain yield and low negative SCA effects for grain moisture content. These newly identified cross combinations hold promise in breeding programme to enhance carotenoids in maize with combination important agronomical traits such as high grain yield and low grain moisture content.

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Measuring and alleviating drought stress in pea and lentil

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Abstract. Water deficit in the soil can cause drought stress in plants and drastically affect plant growth and crop yield. Therefore, early detection of drought stress in plants followed by the timely application of agronomic measures to alleviate plant conditions is crucial. This research aimed to study the agronomic practices that could reduce the sensitivity of pea and lentil to drought stress. The practices included (i) soil amendment with moisture retainer (hydrogel), (ii) seed treatment with a growth regulator to promote root formation, (iii) application of a biological formulation to boost soil mycorrhizal biota, and (iv) foliar application of micro fertilisers. The research was carried out in Ukraine in 2015−2020. Drought stress in plants was detected by measuring chlorophyll fluorescence with a portable fluorometer Floratest and calculating the ratio of variable to maximum fluorescence Fv/Fm of the photosystem. The content of proline, high values of which in vegetative organs point out to stress in plants, was determined by colorimetric analysis using ninhydrin.

In pea, the incorporation of hydrogel (Aquasorb) and growth regulator (Mycofriend) combined with seed treatment (Kelpak SC) and foliar application of micro fertiliser (Biovit or Freya-Aqua Legumes) at BBCH 14 led to obtaining Fv/Fm values from 0.81 to 0.82. Similarly in lentil, the maximum value of Fv/Fm (0.67) was obtained with the application of all studied agronomic practices, with the correlation coefficient between yield and Fv/Fm at the flowering stage (BBCH 61) $r = 0.97$. In pea, the correlation between yield and Fv/Fm at the budding stage (BBCH 51) was $r = 0.99$. The content of proline in photosynthetic plant organs was species-specific; however, in the control treatment, where plants were exposed to drought, its maximum value was 1.10 μmol g^{-1} in pea and 1.40 μmol g^{-1} in lentil, while with the application of the proposed agronomic practices proline content was only 0.56 μmol g^{-1} in pea and 0.36 μmol g^{-1} in lentil. Obtained strong correlation between proline content in plant vegetative organs and the ratio of variable to maximum fluorescence Fv/Fm of the plant photosystem indicates that measurement of Fv/Fm with portable fluorometer might be an effective method of early identification of drought stress in pea and lentil.

Key words: chlorophyll fluorescence, portable fluorometer, hydrogel, growth regulator, micro fertiliser.

INTRODUCTION

Drought stress is the main cause of considerable yield losses in many crops, including leguminous lentil (*Lens culinaris*) and pea (*Pisum sativum*) traditionally grown in Ukraine (Prysiazhniuk et al., 2020). Water deficit causes considerable inhibition of plant growth and development and negatively affects root and leaf formation resulting in yield shortage. Drought stress is a major cause of the low yield of lentil in many regions of the world (Zeroual et al., 2023). For example, in the Mediterranean region, lentil yield can be highly affected by fluctuations in seasonal precipitation, as the intensive rainfalls occur in winter, while in the period from March to May, plants are exposed to drought and high temperatures (Choukri et al., 2020).

Water deficit in the critical stages of growth and development of pea and lentil may result in falling buds, flowers and fruits, low seed weight, and, consequently, low yield (Coyne et al., 2020). Drought stress negatively affects the majority of C3 crops that do not have mechanisms for alleviating the negative impact of drought (Guidi et al., 2019; Marchin et al., 2020; Zhuang et al., 2020; Mihaljevic et al., 2021). While we can provide plants with water at the beginning of vegetation by adjusting the timing of seedbed preparation and sowing (Sen et al., 2016), in the rest of the vegetation season, the plants are defenceless against drought.

Crop resistance to drought can be increased through a breeding approach, i.e., the development of drought-tolerant varieties (Ghanem et al., 2015; Larouk et al., 2021; Snowdon et al., 2021). Another approach to alleviating drought stress in plants may be agronomic, i.e., application of certain agronomic practices, for example, conservation tillage, seed treatment, proper fertilisation, application of growth regulators, plant-promoting rhizobacteria and arbuscular mycorrhizal fungi that have proven to be useful in diminishing the adverse effects of drought stress (Rosa et al., 2023). A well-developed root system is crucial for efficient water uptake from the soil, while a weak root system may be a reason for slow growth and development of plants. A weak root system makes plants vulnerable to the drying up of the soil layer. Our previous research demonstrated that seed treatment with growth regulators can help prevent the issue of a weak root system (Prysiazhniuk et al., 2020). However, a limited number of research on such practices in lentil and pea has been reported.

Agronomic practices will be successful if they are applied at the right time; this is especially true in the case of growth regulators. To determine the right time, portable fluorometers may be used. Such devices detect changes occurring in the plant photosynthetic system by determining the fluorescence state of the plant photosystem and transforming it into an electrical signal with subsequent processing of the signal (Tsai et al., 2019; Suárez et al., 2022; Tsytsiura, 2022). Drought stress in C3 plants can be identified by the Templer protocol - calculating the ratio of variable to maximum fluorescence Fv/Fm of plant photosystem (Templer et al., 2017). Another (and the only alternative) method that can provide reliable results in the case of moderate drought stress is the determination of Fs/Fo (Flexas et al., 2000; Flexas et al., 2002). However, this method is effective only under moderate drought stress and is not suitable for most crops. Measuring Fv/Fm has been proven to identify even severe drought stress in plants (Arrobas et al., 2016). Interestingly, chlorophyll fluorescence can be used not only to assess plant resistance to abiotic stress factors (Simeneh, 2020; Legendre et al., 2021; Larouk et al., 2021; Moore et al., 2021; Oláh et al., 2021; Lin et al., 2022; Wang et al., 2022), specifically water deficit (Li et al., 2020; Kimm et al., 2021) and high or low temperatures (Baldocchi et al., 2020; Kim et al., 2021) but also to detect plant diseases and pests (Hupp et al., 2019; Amri et al., 2021; Sloat et al., 2021), even pollution by heavy metals (Van Zelm et al., 2020; Javed et al., 2022).

One of the conventional methods of detecting drought stress in plants is determining proline content in plant vegetative organs (Ain-Lhout et al., 2001; Al-Khayri, 2002). Its accumulation is species-specific and is considered a stress reaction but not an indicator of tolerance to drought stress (Liu & Zhu, 1997; Hoai & Shim, 2003). Proline also influences cell proliferation and initiates plant recovery after stress; therefore, it can be found in plants even at the stage of their recovery from stress (Yamada et al., 2005; Valliyodan & Nguyen, 2006; Szabados & Savoure, 2009). Contrary to the proline method, express analysis with the use of a portable fluorometer can detect stress in plants more selectively and precisely (Larouk et al., 2021).

The purpose of the research was to develop a method of early detection of drought stress in lentil and pea with the use of a portable fluorometer and study the efficiency of proposed agronomic practices to alleviate plant conditions.

MATERIALS AND METHODS

Place and crop rotation

Field experiments on lentil and pea were carried out in the Uladivske-Liulyntsi Experimental Breeding Station (49°34'30.7"N 28°22'39.5"E) of the Institute of Bioenergy Crops and Sugar Beet National Academy of Agrarian Sciences of Ukraine in 2015−2020 (lentil) and 2015−2019 (pea). Pea and lentil were grown in conventional grain and beet crop rotation with the following crop alternation: leguminous crops - winter wheat - sugar beet - maize. The total area of the field with leguminous crops was 70 ha. The field was divided into two equal parts for the cultivation of pea and lentil (3,220 m^2 each). In the crop rotation, the place of leguminous crops did not change; therefore, the effect of preceding crops can be neglected.

Soil conditions

The field experiment was established in deep medium-loamy chernozem with the humus content (by the Tyurin and Kononova method) of 3.9%, nitrate nitrogen of 16.4 mg kg^{-1}, ammonium nitrogen of 38.7 mg kg^{-1}, mobile phosphates (by the Chirikov method) of 83 mg kg^{-1} and exchangeable potassium (by the Chirikov method) of 103 mg kg^{-1}. The reaction of the soil solution was slightly acidic, close to neutral. The availability of mineral nitrogen (nitrate + ammonium) was medium, phosphorus low and exchangeable potassium high.

Weather conditions

In April−July 2015–2020, the air temperature was higher compared to the average long-term data, and precipitation was uneven. May of 2020 was colder by 2.6 °C compared to long-term (20 years) data (Fig. 1). The hottest vegetation seasons were in 2015, 2016, 2018 and 2019. The lack of precipitation was observed in April and July in all years of the experiment. The driest years were 2015 and 2017, while 2019 and 2020 had alternate dry and rainy periods. Therefore, it can be concluded that weather conditions were favourable for the purpose of studying drought stress in crops.

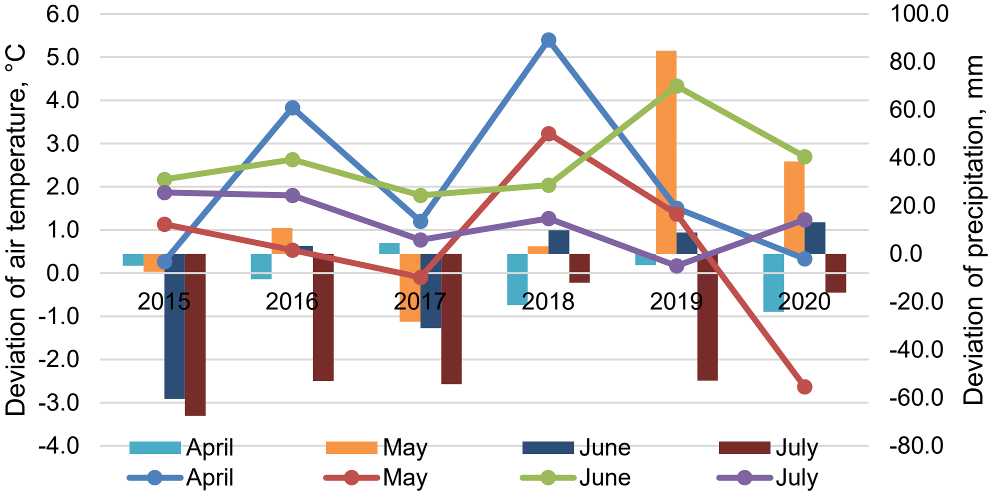


Figure 1. Deviation of monthly average temperature and precipitation from long-term average data.

Experimental design and treatments

Field experiments were carried out in four replications at the randomized block design. The area of each assessed plot was 35 m^2. Total area of lentil was 3,220 m^2 and pea 3,220 m^2.

Some treatments were the same for both pea and lentil - application of hydrogel Aquasorb (200 kg ha^{-1}), seed treatment with Kelpac SC and application of mycorrhizal bio formulation Mycofriend, while micro fertilisers were different due to the different needs of crops. Specifically, in lentil sowings, Reakom-SP-Legumes (3 L ha^{-1}, BBCH 14) and Quantum Legumes (1.0 L ha^{-1}, BBCH 14) fertilisers were used, while in pea sowings, Biovit (7 L ha^{-1}, BBCH 14) and Freya-Aqua Legumes (1.5 L ha^{-1}, BBCH 14) were used. The complete set of experimental treatments is presented in Table 1.

Hydrogel Aquasorb was incorporated into the soil in the process of early spring tillage using Amazone ZA-TS 3200 spreader. Mycorrhizal bio formulation Mycofriend (1 L ha^{-1}) was applied before soil cultivation with a hinged field sprayer Amazone UF at a rate of 200 L ha^{-1}.

Measurement of chlorophyll fluorescence

We used a portable fluorometer Floratest (developed at the Institute of Cybernetics National Academy of Sciences and the Institute of Bioenergy Crops and Sugar Beet National Academy of Agrarian Sciences of Ukraine). The device operates by generally recognized algorithms for the determination of the fluorescence intensity of chlorophyll and F_v/F_m of the photosystem (Maxwell & Johnson, 2000; Prysiazhniuk et al., 2017).

Plant chlorophylls absorb light energy in the photosynthetic active radiation range from 390 to 730 nm with the maxima in the ranges from 400 to 500 nm and 600 to 700 nm (Buschmann, 2007; Buschmann, 2008). Some design features of the fluorometer sensors impose some restrictions on determining the activity of the photosystem of plants with small leaves and tendrils (such as lentil and pea) (Cavender-Bares & Fakhri, 2004). For accurate determination of chlorophyll fluorescence in leaves of such plants we used a method described by Gitelson et al. (1999) which allows analysing the most reliable range of chlorophyll measurement by narrowing the measurement diapason to 700–735 nm. Limiting the spectrum range of red radiation made it possible to significantly increase the accuracy of the chlorophyll measurements compared to the methods commonly used to measure stress in plants (George et al., 2006). It was only necessary to change the settings of the microprocessor program of the device (Prysiazhniuk et al., 2017).

Table 1. Design of the lentil and pea experiments on the agronomic practices to increase drought tolerance at early stages of plant growth and development

Moisture-retainer	Mycorrhizal bio formulation	Growth regulator (seed treatment)	Micro fertilisers lentil	Micro fertilisers pea	Treatment No
Control	Control	Control	Control	Control	1
			Reakom-SP-Legumes	Biovit	2
			Quantum Legumes	Freya-Aqua Legumes	3
		Kelpak SC	Control	Control	4
			Reakom-SP-Legumes	Biovit	5
			Quantum Legumes	Freya-Aqua Legumes	6
	Mycofriend	Control	Control	Control	7
			Reakom-SP-Legumes	Biovit	8
			Quantum Legumes	Freya-Aqua Legumes	9
		Kelpak SC	Control	Control	10
			Reakom-SP-Legumes	Biovit	11
			Quantum Legumes	Freya-Aqua Legumes	12
Aquasorb	Control	Control	Control	Control	13
			Reakom-SP-Legumes	Biovit	14
			Quantum Legumes	Freya-Aqua Legumes	15
		Kelpak SC	Control	Control	16
			Reakom-SP-Legumes	Biovit	17
			Quantum Legumes	Freya-Aqua Legumes	18
	Mycofriend	Control	Control	Control	19
			Reakom-SP-Legumes	Biovit	20
			Quantum Legumes	Freya-Aqua Legumes	21
		Kelpak SC	Control	Control	22
			Reakom-SP-Legumes	Biovit	23
			Quantum Legumes	Freya-Aqua Legumes	24

Measurements and statistical analysis

The measurement of plant biometric parameters was carried out by sampling 50 plants per replication. The yield was determined in a plot-by-plot manner, and the grain moisture was adjusted accordingly.

The free proline content was determined by the method of colorimetric analysis using ninhydrin. To this end, plant material was homogenized. Extraction was carried out with a solution of ethanol and water in a ratio of 70:30. After that, a reaction mixture (1% ninhydrin in acetic acid with ethanol) was added, and the resulting mixture was incubated in a water bath at 95 °C for 30 min. Then, the tubes were cooled down and centrifuged. After that, the optical density of the ninhydrin-proline solution was determined using a spectrophotometer at a wavelength of 520 nm. The calibration graph was plotted using L-proline (Carillo & Gibon, 2011).

Statistical processing of the experimental data was performed using the analysis of variations (ANOVA) and correlation-regression analysis (Marques de Sá, 2007) using the software Statistica 12 (Rumsey, 2016). MS Excel 2019 was used for the visualization of the regression equations, obtained and verified in Statistica 12.

RESULTS AND DISCUSSION

Field experiments were carried out in the conditions of the unstable water content of soil; therefore, it was important to assess the water content available to plants in the 0−20 cm layer. In 2015, at the time of sowing, water content was 35 mm, while with the use of the hydrogel Aquasorb, it increased to 38 mm (satisfactory). Similarly, water content was assessed as satisfactory in 2016, 2019, and 2020 and good in 2017, and 2018. In 2015, at the time of flowering, water content decreased to 4–7 mm (unsatisfactory). Similarly unsatisfactory water content of the soil was also in 2017, while in 2016, 2018, 2019, and 2020 years it was satisfactory (Tables 2 and 3). Application of hydrogel provided an additional 3 mm of water available to plants in the 0–20 cm layer, as granules of hydrogel trap condensed water (dew) and capillary water in the upper soil layer.

Table 2. The water content of soil (mm) in pea sowings under the application of hydrogel Aquasorb at an application rate of 200 kg ha^{-1} (2015–2019)

Treatment	Stage of measurement					
	BBCH 01		BBCH 61		BBCH 91	
	Soil layer (cm)					
	0–20	0–100	0–20	0–100	0–20	0–100
2015						
Without moisture retainer	34	199	3	59	5	36
With moisture retainer	37	202	6	62	8	39
2016						
Without moisture retainer	32	198	21	151	25	88
With moisture retainer	35	201	24	154	28	91
2017						
Without moisture retainer	49	245	9	42	0	22
With moisture retainer	52	248	12	45	3	25
2018						
Without moisture retainer	41	203	38	138	34	112
With moisture retainer	44	206	41	141	37	115
2019						
Without moisture retainer	27	164	23	101	10	83
With moisture retainer	30	167	25	104	13	86

Plants undergo drought stress when the water content of the soil is limited or when transpiration is intensive (Sperdouli & Moustakas, 2014). In the literature, we found that a decrease in the water content of the soil to 70% of the soil capacity had a negative effect on the growth and development of pea (Moisa et al., 2019), while a decrease to 80% caused a decrease in the concentration of chlorophylls *a* and *b* and the maximum quantum efficiency of the photosystem II (Fv/Fm) in pea and lentil (Arafa et al., 2021; Suprasanna et al., 2016) along with an increase in the proline content (Meena et al., 2019).

Table 3. The water content of soil (mm) in lentil sowings under the application of hydrogel Aquasorb at a dose of 200 kg ha^{-1} (2015–2020)

Treatment	Stage of measurement					
	BBCH 01		BBCH 61		BBCH 91	
	Soil layer (cm)					
	0–20	0–100	0–20	0–100	0–20	0–100
2015						
Without moisture retainer	35	200	4	60	5	37
With moisture retainer	38	203	7	63	8	40
2016						
Without moisture retainer	33	198	20	150	24	87
With moisture retainer	36	201	23	153	27	90
2017						
Without moisture retainer	52	243	10	44	1	23
With moisture retainer	55	246	13	47	4	26
2018						
Without moisture retainer	42	205	37	137	34	112
With moisture retainer	45	208	40	140	37	115
2019						
Without moisture retainer	29	166	24	101	11	83
With moisture retainer	32	169	27	104	14	86
2020						
Without moisture retainer	26	140	20	94	7	48
With moisture retainer	29	143	23	97	10	51

In our research, we chose the ratio F_v/F_m as an indicator of drought stress state in pea (Table 4) and lentil plants (Table 5). The data on the pea photosystem efficiency (Table 4) show that in the control treatment, the plants were largely affected by drought at the budding stage (BBCH 51). This does not mean that they died since the drought in the years of research was not so severe but the indicator F_v/F_m was low - 0.33, while with the application of hydrogel, it was 0.43.

In our experiments, pea responded well to the application of all agronomic practices that increased the efficiency of the photosynthetic apparatus. The most efficient was the combined application of all practices, i.e., incorporation of hydrogel and mycorrhizal bio formulation, seed treatment with Kelpak SC, and foliar application of micronutrients Biovit or Freya-Aqua Legumes (BBCH 14). F_v/F_m values in such treatments were at the level of 0.81–0.82. A strong correlation was found between the pea yield and F_v/F_m of the photosystem at the budding stage (BBCH 51), with *r* = 0.99. The regression dependence is shown in Fig. 2.

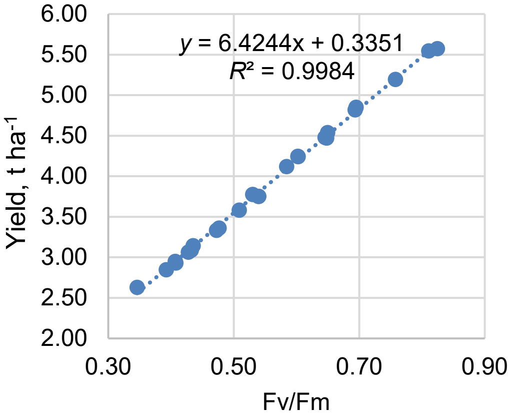


Figure 2. Regression between pea yield and F_v/F_m.

Table 4. F_v/F_m of pea at the budding stage (BBCH 51)

Treatment No	2015	2016	2017	2018	2019
1	0.33	0.37	0.34	0.36	0.33
2	0.38	0.43	0.40	0.42	0.39
3	0.36	0.41	0.36	0.43	0.40
4	0.42	0.45	0.42	0.45	0.43
5	0.45	0.49	0.47	0.50	0.48
6	0.43	0.47	0.45	0.52	0.49
7	0.40	0.42	0.39	0.42	0.41
8	0.42	0.46	0.42	0.44	0.42
9	0.41	0.45	0.40	0.44	0.43
10	0.46	0.53	0.51	0.50	0.54
11	0.51	0.57	0.54	0.52	0.57
12	0.50	0.55	0.53	0.53	0.59
13	0.56	0.53	0.46	0.59	0.51
14	0.63	0.61	0.52	0.66	0.59
15	0.60	0.58	0.48	0.67	0.60
16	0.70	0.63	0.56	0.71	0.64
17	0.70	0.69	0.63	0.76	0.70
18	0.69	0.66	0.60	0.80	0.71
19	0.65	0.58	0.52	0.67	0.60
20	0.67	0.64	0.58	0.71	0.62
21	0.69	0.63	0.55	0.72	0.64
22	0.79	0.74	0.67	0.80	0.79
23	0.79	0.82	0.73	0.87	0.85
24	0.83	0.79	0.72	0.91	0.88
$LSD_{0.05}$	0.04	0.05	0.03	0.04	0.03

Table 5. F_v/F_m of lentil at the flowering stage (BBCH 61)

Treatment No	2015	2016	2017	2018	2019	2020
1	0.29	0.37	0.39	0.30	0.31	0.33
2	0.30	0.38	0.39	0.30	0.33	0.35
3	0.32	0.38	0.39	0.31	0.32	0.36
4	0.37	0.39	0.41	0.32	0.33	0.35
5	0.41	0.39	0.40	0.32	0.33	0.34
6	0.40	0.39	0.40	0.32	0.34	0.34
7	0.36	0.39	0.40	0.35	0.35	0.38
8	0.39	0.39	0.40	0.34	0.33	0.38
9	0.41	0.38	0.40	0.35	0.35	0.39
10	0.42	0.39	0.40	0.36	0.34	0.37
11	0.45	0.41	0.42	0.37	0.37	0.39
12	0.46	0.41	0.43	0.38	0.36	0.39
13	0.39	0.40	0.41	0.35	0.36	0.36
14	0.43	0.40	0.42	0.38	0.37	0.37
15	0.46	0.41	0.42	0.39	0.37	0.38
16	0.50	0.43	0.43	0.39	0.35	0.37
17	0.55	0.44	0.45	0.42	0.38	0.39
18	0.56	0.44	0.45	0.42	0.39	0.39
19	0.51	0.42	0.42	0.39	0.35	0.36
20	0.55	0.42	0.43	0.40	0.37	0.38
21	0.56	0.42	0.44	0.41	0.37	0.40
22	0.56	0.49	0.45	0.45	0.42	0.43
23	0.61	0.52	0.48	0.47	0.45	0.45
24	0.64	0.52	0.49	0.49	0.44	0.45
$LSD_{0.05}$	0.03	0.03	0.04	0.03	0.02	0.03

In lentil, similar to pea, on average over the research years, the lowest ratio of Fv/Fm was observed in the control treatment, which means high exposure to drought at the flowering stage (BBCH 61). The use of hydrogel significantly improved plant condition. In the treatment with hydrogel, the ratio Fv/Fm was 0.67, while in the control it was 0.45. The most effective treatment that contributed to the maximum Fv/Fm values (0.81−0.82) was the cone with the application of hydrogel and mycorrhizal bio formulation, seed treatment and foliar application of micronutrients Reakom-SR-Legumes or Quantum Legumes (BBCH 14). Similar to pea, there was a strong correlation between yield and photosystem indicators at the flowering stage (BBCH 61), $r = 0.97$. The regression dependence is shown in Fig. 3. It should be noted that the

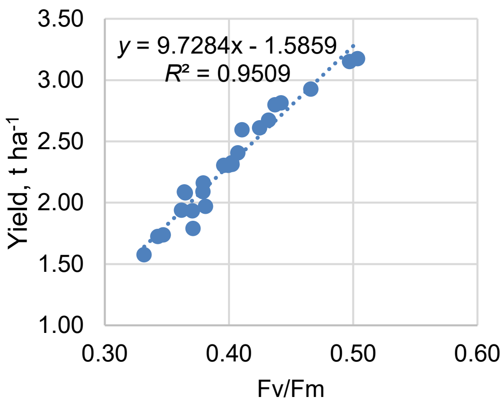


Figure 3. Regression between lentil yield and F_v/F_m.

efficiency of all agronomic practices increases with the optimization of plant provision with water (Larouk et al., 2021; Valcke, 2021).

When analysing the experimental data, we found species-specific values of the content of free proline in the photosynthetic plant organs. On average, the content of (crude) proline was 0.86 µmol g^{-1} in pea and 0.78 µmol g^{-1} in lentil (Table 6). In the control treatments, at the critical stages of growth and development, the maximum concentration of proline was determined as the plants were affected by drought. On the contrary, the application of hydrogel and additional agronomic practices contributed to reducing drought stress in plants as evidenced by the content of free proline in the photosynthetic plant organs.

Table 6. The content of free (crude) proline in plant photosynthetic organs (µmol g^{-1}) and yield (t ha^{-1}), average over the years of research

	Pea		Lentil	
Treatment No	content of free (crude) proline	yield	content of free (crude) proline	yield
1	1.10	2.63	1.40	1.58
2	1.09	2.95	1.38	1.73
3	1.09	2.85	1.36	1.74
4	1.05	3.14	1.29	1.94
5	1.01	3.36	1.23	2.08
6	1.02	3.33	1.22	2.09
7	1.06	2.93	1.11	1.79
8	1.04	3.09	1.00	1.93
9	1.04	3.07	0.98	1.97
10	0.97	3.58	0.78	2.16
11	0.95	3.75	0.71	2.30
12	0.95	3.75	0.72	2.31
13	0.84	3.78	0.65	2.09
14	0.80	4.25	0.56	2.30
15	0.82	4.12	0.55	2.32
16	0.68	4.54	0.48	2.59
17	0.65	4.85	0.46	2.80
18	0.66	4.82	0.46	2.81
19	0.75	4.24	0.45	2.41
20	0.72	4.48	0.44	2.61
21	0.73	4.47	0.43	2.67
22	0.58	5.20	0.39	2.93
23	0.56	5.55	0.36	3.15
24	0.56	5.57	0.36	3.17
$LSD_{0.05}$	0.05	0.21	0.04	0.16

In the condition of osmotic stress, the content of free proline in pea can increase 100 times (Dar et al., 2016). Other scientists (Lahuta et al., 2022) reported that a 5-day drought led to a fivefold increase in the free proline content in pea and after another 5 days, the content of proline increased 50 times. In our research, we recorded two times higher (in pea) and four times higher (in lentil) free proline content in the control plots.

A study of the content of proline in wheat (Song et al., 2005) showed that a drought-tolerant genotype demonstrated a higher accumulation of proline compared to a susceptible one, with the proline content increasing along with the increasing intensity of drought. Similarly, in maize grown in conditions of water deficit, the content of proline was higher (Anjum et al., 2011; Koskeroglu & Tuna, 2010). The same is true for some other crops (Bartels & Sunkar, 2005; Chaves et al., 2009; Conde et al., 2011; Qin et al., 2011; Roy et al., 2009).

We also determined the type and strength of the correlation between the content of free proline and F_v/F_m in pea and lentil (Figs 4 and 5).

Our results let us assume that there is a strong correlation between the concentration of free proline and Fv/Fm in the studied crops, as correlation coefficients for pea (r = -0.97) and lentil (r = -0.86) corresponds to a very strong level of correlation. Some studies do not fully agree on the effectiveness of using the Fv/Fm indicator for the identification of stress in plants. Thus, when studying the reaction of plants to treatment with cadmium in doses of Cd20 and Cd25, it was found that the proline concentration in bean leaves increased 2.5 and 1.3 times, while Fv/Fm changed differently (Alle et al., 2019).

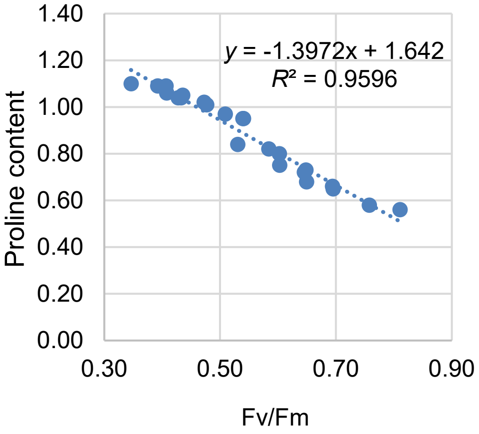


Figure 4. Regression between the content of proline and F_v/F_m in pea.

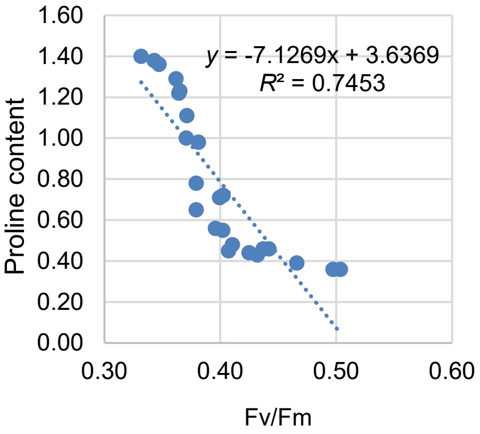
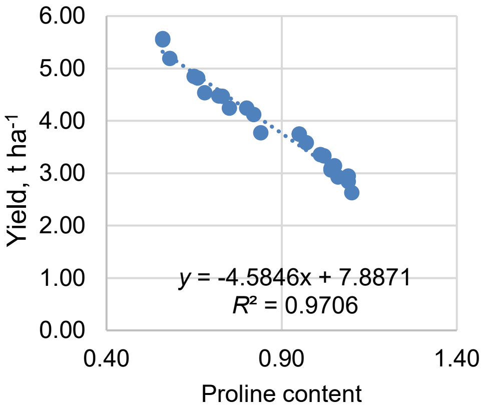


Figure 5. Regression between the content of proline and F_v/F_m in lentil.

Quite interesting is the dynamics of free proline concentration in the photosynthetic organs of lentil (leaves and tendrils) in comparison with the efficiency of the plant photosystem. In our opinion, the low level of correlation of the studied signs is due to the fact that lentil slows down its growth under the influence of stress and recovers in the event of favourable conditions. Consequently, with an increase in water deficit in lentil, other mechanisms for regulating plant stress are most likely involved. Thus, according to other researchers, water deficit during flowering reduces plant height, leaf area and dry matter accumulation, which leads to a decrease in the dry matter content in the biomass and seeds (Shrestha et al., 2005). Foti et al. (2021) showed the general metabolic disturbance in the lentil metabolism in response to drought stress. The metabolic response included the accumulation of D-fructose, a-trehalose, myoinositol and L-tryptophan, which indicates their crucial role in the response to drought and their potential to be used as biomarkers for the effective selection of drought-resistant germplasm. This corresponds to our assumptions about a more complex reaction to water deficit in lentil.

We also identified regression relationships between the content of proline in the studied crops and their yield (Figs 6, 7). The obtained patterns show that a high concentration of free proline might be an indicator of plant stress caused by water deficit, which is associated with crop productivity. Thus, we obtained correlation coefficients for pea $r = -0.98$ and lentil $r = -0.88$, which correspond to a very strong level of correlation. The obtained data positively correlates with recent publications of other authors (Baldocchi et al., 2020; Guo, et al., 2022; Larouk et al., 2021).



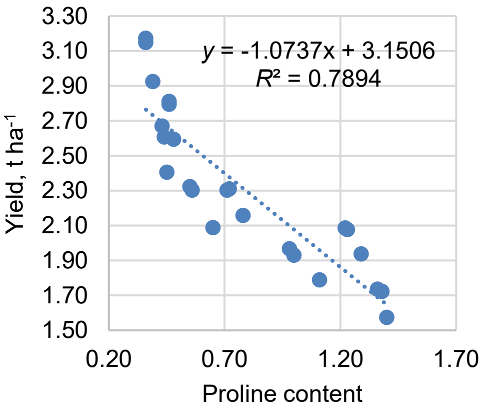


Figure 6. Regression between the content of proline and yield in pea.

Figure 7. Regression between proline content and seed yield in lentil

Regarding the influence of the studied agronomic practices, the application of hydrogel contributed to better conditions for water supply; therefore, pea yield was 1.15 t ha^{-1} higher than in the control. Application of hydrogel in lentil also ensured an increase in yield by 0.51 t ha^{-1} (Table 6) as it provided a smooth course of the critical for lentil stages - BBCH 30–30 and 61–69 (Coyne et al., 2020). In our experiments, the application of hydrogel interacted quite well with other experimental factors, especially with seed treatment and application of mycorrhiza-forming bio formulation ensuring pea yield increase of 2.57 t ha^{-1} compared to the control, while seed treatment and mycorrhiza-forming bio formulation ensured a 1.91 t ha^{-1} yield increase and seed treatment alone increased yield by only 0.51 t ha^{-1}. Lentil demonstrated a similar yield pattern, with a yield increase of 0.36 t ha^{-1}, 1.02 t ha^{-1} and 1.35 t ha^{-1}, respectively.

Foliar application of micro fertilisers in pea had a rather strong effect on the plants in the absence of other studied practices. Whereas in lentil, micro fertilisers showed maximum efficiency under the combination of all studied practices. This is consistent with the findings of other researchers, who found that lentil, compared to pea, had a better-developed root system, and therefore, provided its needs for nutrients through absorption from the soil (Khodanitska, 2019). Under the favourable conditions for biomass formation ensured by the application of other agronomic measures plants respond better to the application of micro fertilisers (Hospodarenko & Musiyenko, 2020).

In the studies of Le et al. (2018) and Akhtar et al. (2020) rhizobacteria formulations applied to soil worked especially effectively in dry periods as they increased the water use efficiency of plants and improved crop productivity (Backer et al., 2018). Growth regulators positively affected plant conditions in the study of Zeroual et al. (2023). However, Lamaoui et al. (2018) noted that the contribution of growth regulators to the alleviation of drought stress in plants is rather supplementary and they work well when combined with other agronomic practices. The contribution of hydrogel is more obvious as it helps plans obtain additional water every day, thereby contributing to better growth and development.

The effectiveness of the chlorophyll fluorescence method as a criterion for assessing the optimality of the agrocenosis of field crops has been proven against the background of various fertilisation options in the studies of Herritt et al. (2021) and Guo et al. (2022). Therefore, the interaction of the experimental factors obtained by us, especially the influence of the foliar application of micro fertilisers, should be further investigated in deep as a separate experimental factor.

CONCLUSIONS

1. Measuring the ratio of the variable to the maximum fluorescence F_v/F_m of the plant photosystem is demonstrated to be an efficient way of early detection of drought stress in pea and lentil. A very strong correlation was found between F_v/F_m and proline content in plant vegetative organs of pea ($r = -0.97$) and lentil ($r = -0.86$). A high concentration of proline in the photosynthetic plant organs indicates drought stress in plants leading to lower crop yield. The correlation between yield and proline content was also very strong, with $r = -0.98$ in pea and $r = -0.88$ in lentil.

2. All studied practices were effective for the alleviation of drought stress in plants; the most efficient for pea was the application of all studied agronomic practices i.e., incorporation of hydrogel (Aquasorb) and mycorrhizal bio formulation (Mycofriend) to the soil, seed treatment (Kelpak SC), and foliar application of micro fertilisers (Biovit or Freya-Aqua Legumes) at BBCH 14, which resulted in F_v/F_m values of 0.81−0.82 and ensured a yield increase of 2.92 (with Biovit) and 2.62 t ha^{-1} (with Freya-Aqua Legumes) compared to control. Similar to pea, lentil showed the maximum value of Fv/Fm (0.67) under the application of all studied agronomic practices, with the correlation coefficient between yield and Fv/Fm at the flowering stage (BBCH 61) $r = 0.97$ and yield increase of 1.57 (with Biovit) and 1.60 t ha^{-1} (with Freya-Aqua Legumes) compared to control

3. Further research should clarify the interaction between the proposed agronomic practices. Also, the effect of the foliar application of micro fertilisers should be further investigated in the context of the development of protocols for their application aimed at alleviating drought stress in crops.

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Carbon and nitrogen uptake in above- and below-ground biomass of cereal crops in the integrated farming system

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Abstract. A significant reduction in greenhouse gas (GHG) emissions, as well as technologies that ensure removal of CO_2 from the atmosphere, are necessary to achieve the set goals for the transition to carbon neutrality. During the crop growth cycle, a significant amount of biomass is produced, and carbon (C) and nitrogen (N) are captured both by the harvested crop removed from the field and by residues left on the field. The trials were conducted to find out patterns between crop and residues while trying to figure out the amount of captured C and N. In this study data of the most widely grown cereal crops in Latvia are summarized. The data are representative, obtained in different agroclimatic conditions, they vary both by species and variety, by year and fertilizers applied. The mean amount of biomass from cereal crops left on the field was 1,070.9 g m^{-2} DM, besides, 906.7 g m^{-2} of that was made up of above-ground (AG) residues and 164.2 g m^{-2} of below-ground (BG) residues. On average, 471.8 g m^{-2} C and 14.3 g m^{-2} N were captured, including: 411.2 g m^{-2} C and 12.9 g m^{-2} N by AG residues; 60.7 g m^{-2} C and 1.4 g m^{-2} N by BG residues. Regularities between grain yield and residues were found, however, they were not very strong. The dataset should be enlarged to reduce uncertainty. As the data calculated from crop have a greater uncertainty, the GHG inventory should be calculated according to the average AG and BG biomass, which provide more accurate data.

Key words: cereal crops, crop residues, harvest index, shoot/root ratio.

INTRODUCTION

To achieve the goals of the Paris Agreement adopted by the 21st Conference of Parties (COP21) of the United Nations Framework Convention on Climate Change

(UNFCCC)[1], which foresees limiting global warming well below 2 °C, major greenhouse gas (GHG) emission reductions are needed together with technologies for removal of CO_2 from the atmosphere.

Soil and its management practices has an essential role in a global C cycle, while C cycle together with changes of GHG concentration in atmosphere can have a significant impact on global biochemical cycle (Heikkinen et al., 2013). Soils are the largest terrestrial reservoir and may provide the best way to remove carbon from the atmosphere (FAO, 2004). Management practices that capture the atmospheric CO_2 and enhance carbon (C) sequestration in the soil are needed. The input of organic matter from plant residues contribute to carbon storage and sequestration in soil and may help to mitigate greenhouse gas emissions (Powlson et al., 2011). Organic matter storage in soil is directly related to the amount of C input through residue retention, below-ground root biomass, and rhizodepositions (Pasricha, 2017). Carbon storage in the soil mostly is the balance between the input of complicated mixture of dead plant material, soil fauna, root exudates, microbial residues and losses from decomposition and mineralization processes. Soil organic C stocks are altered by biotic activities of plants which are the main source of C through litter and root system, microorganisms (fungi and bacteria) and 'ecosystem engineers' (earthworms, termites, ants) (Dignac et al., 2017).

Depending on the land use, management activities and environmental conditions agricultural mineral soils can be either source or sink of carbon (FAO, 2004; Paustian et al., 2007; Eglin et al., 2010; Bardule et al., 2017). Terrestrial ecosystems could increase C sequestration readily by restoring vegetation and incorporating organic soil amendments (Fang et al., 2018). Carbon capture by crops can make a significant contribution in the reduction of the anthropogenic carbon dioxide (CO_2) emissions into the atmosphere, therefore concerted effort to reduce CO_2 emissions and increase C sequestration have to be provided.

Today, the agricultural sector has a significant carbon (C) footprint and accounts for > 25% of worldwide anthropogenic GHG emissions (Jat et al., 2022). At the same time agricultural soils have a significant CO_2 sink capacity. Plants, including cultivated crops, during their growing cycle accumulate from atmosphere large amount of C and N both by yield and by below-ground and above-ground residues. Crop residue is defined as the portion of plant biological yield left in the field after harvesting the grain (Chintala et al., 2014). Crop yields vary with time and space due to the spatial and temporal heterogeneity of environmental and management factors (Bakker et al., 2005; Williams et al., 2008). One of the most important variables determining the projections of crop response at regional scale models is climate (Challinor, 2003; Bakker et al., 2005). However, crop species, variety, soil conditions, fertilizer and other factors can also play a role. Crop residues improve the agronomic productivity through nutrient cycling and improved soil quality.

Understanding the processes that govern N fluxes, particularly N uptake and distribution in crops, is of major importance with respect to both environmental concerns and the quality of crop products. Nitrogen uptake and accumulation in crops represent two major components of the N cycle in the agrosystem. Nitrate ions not taken up by a

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[1] The Paris Agreement https://unfccc.int/process-and-meetings/the-paris-agreement/the-paris-agreement

crop, may potentially be leached to underground water. Modelling N uptake is, therefore, key in quantifying and preventing nitrate leaching (Gastal & Lemaire, 2002).

One of the most important and cultivated cereal crops in the world and in Latvia is wheat, especially winter wheat. According to the data of the Central Statistical Bureau of Latvia, in 2021, the area sown with cereals in Latvia accounted for 776.4 thousand ha or almost 60% of the total sown area, of which 426.4 thousand ha, or almost 55%, was sown with winter wheat. The second most widely grown crop was spring wheat - an area of 113.4 thousand ha or 14.6% was sown with spring wheat. The third most widely grown crop was oats - area sown with outs accounted for 90.1 thousand ha or 1.6% of total sown area (FAOSTAT, 2018; Central Statistical Bureau, 2020; Official statistics..., 2022).

To find out the possible amount of C and N accumulated in the biomass during growing cycle the trials of different agricultural crop species were established. The aim of these studies was to find out the amount of cereal crop residues: i.e., above-ground and below-ground residues left on the field and assess the extent to which it was affected by different factors such as species, variety, fertilizer, soil and meteorological conditions. As well amount of C and N captured and left on the field, and the possible regularities between the yield and the amount of C and N bound in the residues, were assessed.

MATERIALS AND METHODS

Experimental design and background

The conventionally grown cereals - winter crops: wheat (WW), rye (WR), and triticale (WT), as well as following summer crops: wheat (SW), barley (SB), and oats (OA), were included in the field experiment. Each crop was represented in field trials by two biologically/ morphologically different varieties (V1 and V2), and they were grown using a two-level cultivation technology, which is widely used by farmers in Latvia and differs according to the rate of nitrogen fertilization (F1 and F2). The field trials were set up in two locations (L1 and L2) with different soil and agroclimatic characteristics: in Dizstende 57. 1867 N, 22.5477 E and in Priekuli 57. 3152 N, 25.3376 E. The soil type was sod-podzolic which is typical soil in Latvia. The characteristics of experimental fields are summarized in Table 1.

Field experiments were carried out from 2018 to 2020 using a block design with four replicates, size of each plot - 20 m^2. The soil was ploughed in the autumn and each crop was sown in its optimal sowing period. The sowing rate used was 450–500 germinating seeds per m^2, row spacing was 12.5 cm. Complex mineral fertilizer at the rate of 330 kg ha^{-1} was applied for winter crop (33 kg ha^{-1} N, 85 kg ha^{-1} P, 85 kg ha^{-1} K) and at the rate of 350 kg^{-1} for spring crop (30 kg ha^{-1} N, 72 kg ha^{-1} P, 72 kg ha^{-1} K). The fertilizer was applied to the soil before sowing. In the spring, additional nitrogen fertilizer was applied to the winter cereals immediately after re-vegetation and to the summer cereals in the tillering stage (Table 2). During the growing season, plant protection products were applied according to the needs of the species. Plant biomass samples were collected at the stage of yellow ripeness of cereals (Zadoks Growth Stage GS84-89). The total grain mass was harvested with a small-size grain harvester *Wintersteiger Delta* at the stage of grain full ripening (GS95-99).

Table 1. The characteristics of soil and pre-crops in the experimental fields

Trial Year	Location	pH $_{KCl}$	Organic matter, %	K_2O mg kg^{-1}	P_2O_5 mg kg^{-1}	Soil type	Pre-crop
Winter crops							
2018	L1	5.6–5.8	1.8–2.0	201–218	161–192	light loam	winter oilseed rape
	L2	5.5–5.6	1.5–2.1	144–165	147–150	clay sand	green manure - buckwheat
2019	L1	5.9–6.3	2.2–2.6	239–322	159–197	heavy loam	green manure - buckwheat
	L2	5.1–5.6	1.5.–1.9	115–145	187–202	clay sand	spring barley
2020	L1	6.3–6.7	3.3–3.4	158–160	122–144	light loam	winter oilseed rape
	L2	5.1–5.6	1.5–1.9	115–145	187–202	clay sand	spring barley
Spring crops							
2018	L1	5.1–5.8	1.8–2.0	189–204	160–206	light loam	field bean
	L2	5.5– 6.2	1.7–2.1	183–202	177–203	clay sand	potatoes
2019	L1	5.0–5.6	1.8–2.0	201–232	150–186	light loam	potatoes
	L2	5.5–5.8	1.7–2.1	149–183	174–196	clay sand	potatoes
2020	L1	5.3–5.9	1.9–2.3	218–240	161–193	light loam	potatoes
	L2	5.5–5.6	1.5–2.1	144–165	147–150	clay sand	potatoes

Table 2. Information about the cereal varieties and fertilization rates

Cereal species	Varieties (origin, short description)		N fertilizer rate used in spring (kg ha^{-1} N)	
	V1	V2	F1	F2
Winter crops				
Winter wheat (WW)	*Fredis* (LV) early, short stem	*Brencis* (LV) semi early, long stem	33 + 75	33 + 135
Winter rye (WR)	*Su Nasri* (DE) hybrid, early, short stem	*Kaupo* (LV) semi early, long stem	33 + 75	33 + 115
Winter triticale (WT)	*Ruja* (LV) semi late, long stem	*Ramico* (DE) semi early, short stem	33 + 75	33 + 135
Spring crop				
Spring wheat (SW)	*Taifun* (DE) semi late, short stem	*Uffo* (LV) semi early, long stem	100	140
Spring barley (SB)	*Ansis* (LV) semi late, short stem	*Kristaps* (LV) semi early, long stem	100	140
Spring oat (OA)	*Symphony* (DE) semi late, long stem	*Laima* (LV) semi early, long stem	80	100

Description of meteorological conditions and plant development

Overall, the growing seasons of 2018, 2019 and 2020 were characterised by average monthly temperatures slightly above long-term averages (Fig. 1). Some short periods of extreme drought were observed during all vegetation periods. In 2018, hot and dry conditions were observed during the first days of May and at the end of June. During the autumn and winter months, the average daily air temperatures were a little higher than the long-term averages and, in general, wintering conditions were favourable. Also in April, in all three seasons, the air temperature was higher than that of long- term average, which contributed to the earlier recovery of the vegetation of winter cereals. Overall, the growing seasons of 2018, 2019 and 2020 were characterised by average temperature slightly above long-term averages. Moist conditions in July were favourable for plant development.

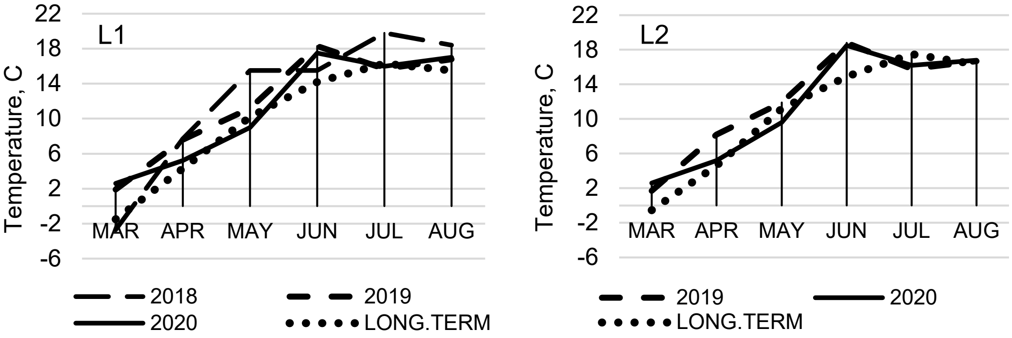

Figure 1. Temperature by month in both locations (L1, L2) during the growing season, over the three trial years compared to the long-term averages.

For spring crops an optimal air temperature and precipitation are important during the period of tillering and stem elongation in May and June. In 2018, only 14 mm of precipitation fell in location L1 in May and the first half of June. During this period, the average daily air temperature was also higher, which contributed to the rapid development of plants, while the moisture deficit prevented the creation of optimal plant biomass.

Agroclimatic conditions in the post-flowering period are also important for biomass formation. The average daily air temperature and the amount of precipitation per month at both experimental sites were optimal in July 2019 and 2020, but in 2018, in location L1, there was significant decrease in the precipitation compared to the long-term averages (Fig. 2). Over the three years, while the experiment was carried out, more precipitation was observed at the end of July and August, which corresponds to the long-term observations. During this period, some days were characterized by heavy rains and thunder alternating with sunny and dry days. Precipitation in August did not significantly influence the formation of biomass of the crops, because winter crops reach their maturity stage in the first decade of August, and spring crops - by August 20.

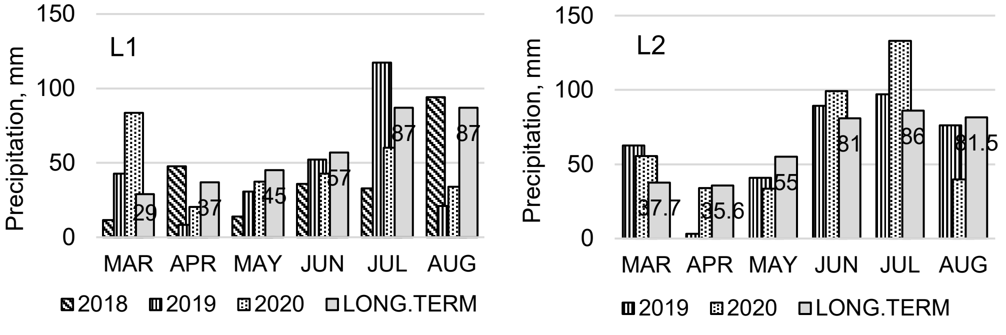

Figure 2. Precipitation by month in both locations (L1, L2) during the growing season over the three trial years compared to the long-term averages.

Collection and analysis of samples

The cereal biomass samples were taken from area of 0.125 m^2 in 2 places in each repetition of trial variant. The plant roots were dug out from the soil at a depth of 20 cm in the area of above-ground biomass recording, rinsed on a sieve (mesh size 1.0×1.0 mm), and collected. The roots and above-ground biomass samples were air-dried and weighed separately, using a laboratory balance (having readability of 0.01 g). The dry matter of each sample was determined (ISO 6496:1999) in the Laboratory of Cereal Technology and Agricultural Chemistry of the Institute of Agricultural Resources and Economics. The following methods (according to the LVS ISO standard) for determination of carbon content in biomass were used: total carbon (C) - using elemental analyser (dry combustion) LECO CR-12 (LVS ISO 106940; total nitrogen (N) - Kjeldahl procedure (LVS ISO 11261).

Data analyses

Descriptive statistics and Pearson correlation of experimental data were performed using Microsoft Excel 2019 for Windows (© Microsoft 2023) and SPSS. Normal distribution was tested using Kurtosis and Skewness values. Regression and variance analyses (ANOVA) were perfomed with R Studio for Windows(© 2009-2022 RStudio, PBC). Multifactorial analyse (MANOVA) were performed to evaluate significance of various factors on the amount of residues and C and N uptake. The following factors and their interactions were evaluated: variety, nitrogen fertilization level, location, year.

RESULTS AND DISCUSSION

Biomass from cereals

The total biomass of cereals consisted of grain yield removed from the field (GY); roots or below-ground (BG) residues; and above-ground (AG) residues (straws, leaves and stubbles) left on the field. The amount of biomass was strongly influenced by the species, variety, amount of N fertilizer applied, year, and location or agrochemical properties of the soil. The significance of individual factors and their interaction effects by species are summarized in the Table 3. It can be seen, that the effect of year was significant (p-value < 0.001) for all species. The studies conducted elsewhere have also concluded that meteorological conditions have a very significant impact on the biomass formation. Generally, the interannual variation in yields there was larger than the variation between regional mean yields (Palosuo et al., 2015).

Table 3. The significance (significance codes) of the effects of various factors and their mutual interaction by species

Cereal species[1]	Factors				Interaction of factors				
	Year (Y)	Variety (V)	N-fert (Nf)	Location (L)	Y×V	Y×Nf	V×Nf	V×L	Nf×L
WW	***	***	*	***	-	-	-	*	-
SW	***	***	***	-	-	-	-	-	-
OA	***	***	***	***	***	-	**	*	***
WT	***	***	**	***	-	*	-	-	**
WR	***	***	***	***	-	-	-	-	-
SB	***	-	***	***	-	-	-	-	**

[1] Cereal species: WW – winter wheat; SW – spring wheat; OA – oats; WT – winter triticale; WR – winter rye; SB – spring barley. Transcript of statistically significant difference codes: *** p-value < 0.001; ** p-value < 0.01; * p-value 0.05.

Mean total biomass of all cereal species was 1,628.8 g m^{-2} of dry matter (DM). Higher biomass was formed by winter cereal species, which is explicable given their longer growth cycle. The highest total biomass formed winter triticale (WT) - 2272.3 g m^{-2} DM, only slightly smaller biomass formed winter rye (WR), accounting for 2137.0 g m^{-2} DM. The amount of total biomass produced by spring cereals was almost half lower, it ranged from 1,165.5 g m^{-2} for spring barley (SB) to 1,386.8 g m^{-2} for oats (OA) (Table 4).

Table 4. The distribution of the total biomass accumulated during the growth cycle for different cereal species (DM, g m^{-2})

Cereal species[1]	Dry matter of different fractions, g m^{-2}			Total biomass, g m^{-2}
	BG residues	AG residues	Grain yield	
WW	142.8 ± 86.82*	821.6 ± 196.56	615.3 ± 210.52	1,579.7 ± 310.22
SW	128.9 ± 58.35	614.0 ± 174.89	488.6 ± 131.08	1,231.5 ± 219.92
OA	155.7 ± 64.06	700.7 ± 206.69	530.4 ± 113.75	1,386.8 ± 239.24
WT	232.6 ± 141.02	1,382.3 ± 760.93	657.4 ± 244.72	2,272.3 ± 608.10
WR	239.6 ± 125.33	1,315.6 ± 557.00	581.9 ± 221.50	2,137.0 ± 645.85
SB	85.6 ± 15.94	606.0 ± 198.08	473.9 ± 143.89	1,165.5 ± 249.84
On average	164.2	906.7	557.9	1,628.8

[1]Cereal species: WW – winter wheat; SW – spring wheat; OA – oats; WT – winter triticale; WR – winter rye; SB – spring barley; * – standard deviation.

The largest proportion – more than half of the total biomass was calculated for above-ground residues. Percentage distribution of cereal biomass was as follows: BG residues 9–11.2%; AG residues; 49.9–61.6%; grain yield 27.2–40.7% (Fig. 3).

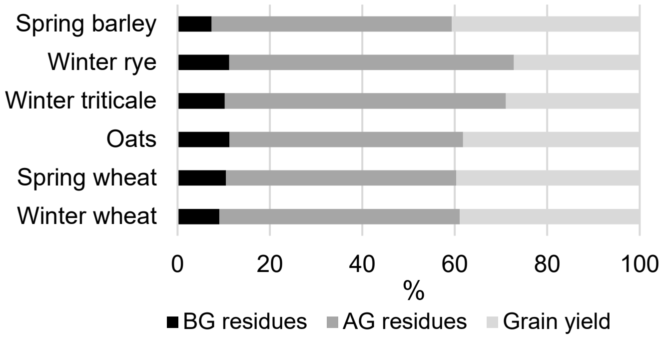

Figure 3. Percentage distribution of cereal biomass, %.

Distribution of average weight by cereal species was as follows: 164.2 g m^{-2} BG residues; 906.7 g m^{-2} AB residues; 557.9 g m^{-2} GY and (Table 4). Among species, AG residues varied greatly from 606.0 g m^{-2} (SB) to 1,382.3 g m^{-2} (WT). Grain yield varied from 473.9 g m^{-2} to 657.4 g m^{-2}, the highest yield produced winter triticale and winter wheat - 657.4 g m^{-2} DM and 615.3 g m^{-2} DM, respectively. Data regarding BG residues varied greatly from 85.6 g m^{-2} DM (SB) to 239.6 g m^{-2} (WR), and 232.6 g m^{-2} (WT). Published data from the research carried out in Denmark (Chirinda et al., 2012; Hu et al., 2018) shows that the average root dry matter in conventional farming systems at the depth 0–25 cm was 142 g m^{-2} DM (ranged 92–194 g m^{-2}) for wheat and 129 g m^{-2} DM (ranged 108–133 g m^{-2}) for barley. It can be concluded, that mentioned data of winter wheat root biomass are very close to our data (142.8 g m^{-2}). Trends by species are also consistent, in the mentioned experiment it was similarly concluded that the biomass of barley roots is lower if compared to the biomass of wheat. Mean DM of WW shoot generally was higher, ranged around 1,509 g m^{-2} DM (1,175–1,907 g m^{-2}). It could be greatly influenced by the variety as well as agrometeorological conditions. Williams et al (2013) reported significant variation for WW AG residues ranging from 537 g m^{-2} to 1,096 g m^{-2} depending on year, site and management.

Captured carbon and nitrogen

Carbon (C) and nitrogen (N) content was determined in below-ground and above-ground residues. The content of both C and N was significantly higher in AG residues. An average carbon (C) content in BG residues of cereal crops was 373.1 g kg^{-1} C; it varied from 307.7 g kg^{-1} C (WR) to 427.1 g kg^{-1} C (SB). An average carbon (C) content in AG residues was 447.2 g kg^{-1} C for all cereal crops. Here, among species it varied relatively less: from 439.5 g kg^{-1} C (SB) to 453.3 g kg^{-1} C (WR) (Table 5). An average nitrogen (N) content was 8.1 g kg^{-1} N in BG residues: and 14.3 g kg^{-1} N in AG residues. Nitrogen content varied within the following limits: from 7.2 g kg^{-1} N (WT) to 10.2 g kg^{-1} N (SB) in BG residues and from 11.6 g kg^{-1} N (WW) to 16.6 g kg^{-1} N (SB) in AG residues.

Table 5. Carbon (C) and nitrogen (N) content in above-ground (AB) and below-ground (BG) residues (g kg^{-1}) of cereal crops

Cereal species[1]	N	C, g kg^{-1}		N, g m^{-2}	
		in BG residues	in AG residues	in BG residues	in AG residues
WW	112	391.2 ± 29.71*	441.5 ± 13.36	7.5 ± 1.30	11.6 ± 2.20
SW	161	399.3 ± 55.22	451.0 ± 16.10	8.5 ± 2.33	16.3 ± 2.75
OA	157	390.1 ± 56.93	449.1 ± 28.87	8.0 ± 2.25	15.2 ± 3.44
WT	96	323.4 ± 68.39	448.7 ± 7.42	7.2 ± 1.81	13.8 ± 3.04
WR	96	307.7 ± 79.94	453.3 ± 10.91	7.4 ± 2.72	12.4 ± 2.55
SB	128	427.1 ± 29.35	439.5 ± 13.28	10.2 ± 2.09	16.6 ± 2.51
On average		373.1	447.2	8.1	14.3

[1] Cereal species: WW – winter wheat; SW – spring wheat; OA – oats; WT – winter triticale; WR – winter rye; SB – spring barley; *– standard deviation.

The amount of sequestered carbon and nitrogen per unit area was calculated (g m^{-2}). Like the data of total biomass distribution, a significantly greater amount of C and N was captured by AG residues: on average 6–7 times more C and about 9 times more N per m^{2}. Such ~~a~~ distribution was determined by both the amount of dry matter produced and a relatively higher C and N content in the AG residues (Table 6).

Average amount of sequestered C in the AG residues was 411.2 g m^{-2} C, among species it ranged from 266.3 g m^{-2} (SB) to 636.9 g m^{-2} (WR). Average amount of sequestered carbon in BG residues was 60.7 g m^{-2} C. Among cereal species it ranged from 36.6 g m^{-2} (SB) to 90.0 g m^{-2} (WR) proving once again that during growth, rye forms a very extensive root system (Table 5). Often the inputs of C from both above-ground and below-ground residues are generally calculated from plant biomass by multiplying with specific transfer coefficients (Kätterer et al., 2011; Chirinda et al., 2012). Such method is chosen due to the fact, that unlike above-ground plant biomass, root biomass and thus the amount of C captured by roots is difficult to sample and quantify. However, the C captured in roots can represent an important source for soil C storage (Warembourg & Paul, 1977), because it may contribute to more stable soil organic C pools than aboveground inputs (Kätterer et al., 2011). This is why simple estimation methods have been proposed for estimating belowground C inputs (Keel et al., 2017; Hu et al., 2018).

The amount of captured N was much lower than that of C, furthermore, it varied relatively less among individual cereal species: from 0.9 g m^{-2} to 2.3 g m^{-2} in below-ground residues; and from 9.6 g m^{-2} to 19.1 g m^{-2} in above-ground residues (Table 5).

Table 6. The amount of carbon (C) and nitrogen (N) accumulated in the above-ground (AG) and below-ground (BG) residues of cereal crops (g m^{-2})

Cereal species[1]	C, g m^{-2}		N, g m^{-2}	
	in BG residues	in AG residues	in BG residues	in AG residues
WW	55.2 ± 32.1*	362.7 ± 88.5	1.1 ± 0.8	9.6 ± 3.6
SW	45.3 ± 15.1	274.9 ± 74.7	1.0 ± 0.4	10.0 ± 3.5
OA	54.1 ± 15	323.6 ± 95.7	1.1 ± 0.3	11.0 ± 4.3
WT	82.8 ± 51.1	602.6 ± 320.7	1.9 ± 1.3	19.1 ± 12.9
WR	90.0 ± 52.4	636.9 ± 267.5	2.3 ± 1.5	17.5 ± 8.3
SB	36.6 ± 7.2	0.9 ± 0.2	0.9 ± 0.2	10.1 ± 3.7
On average	60.7	411.2	1.38	12.88

[1] Cereal species: WW – winter wheat; SW – spring wheat; OA – oats; WT – winter triticale; WR – winter rye; SB – spring barley; * – standard deviation.

The box plot figures (Fig. 4) show very different distribution of accumulated amount of carbon by different cereal crops. The highest content of carbon was established in winter rye and winter triticale both in the above-ground and in the below-ground residues. In turn, for spring cereals the level of captured carbon was generally lower, and the dispersion of the data here was much smaller. The amount of C accumulated in the BG residues was far lower than that in the AG residues for all cereal species.

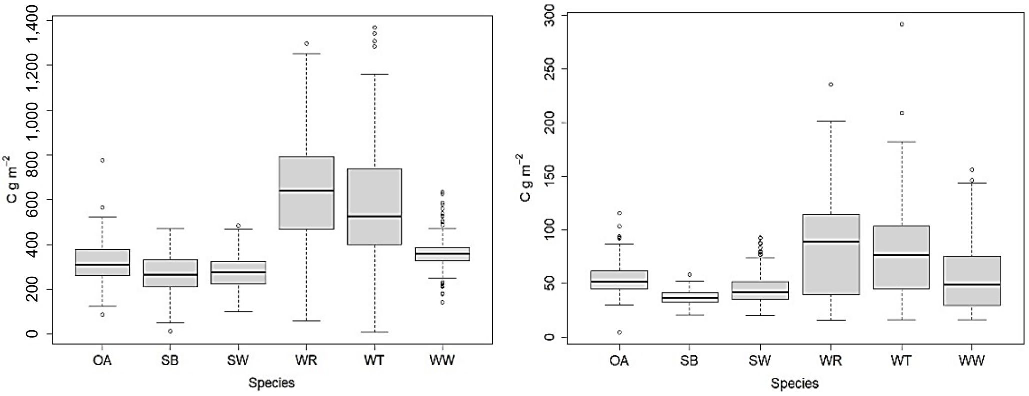

Figure 4. Accumulated amount of carbon (C) by different cereal crops: in the above-ground residues (picture at the left side); and in the below-ground residues (picture at the right side), C g m^2: OA – oats; SB – spring barley; SW – spring wheat; WR – winter rye; WT – winter triticale; WW – winter wheat.

Similar trend was observed for the distribution of the accumulated amount of nitrogen both in the above-ground and in the below-ground residues (Fig. 5). The highest content of nitrogen was established in winter rye and winter triticale both in the above-ground and in the below-ground residues. In general, it can be concluded - if the arrangement of medians and boxes shows the conditional similarity of the accumulated C and N between winter wheat and spring cereal species - wheat, barley and oats, then this is not applicable to winter rye and triticale.

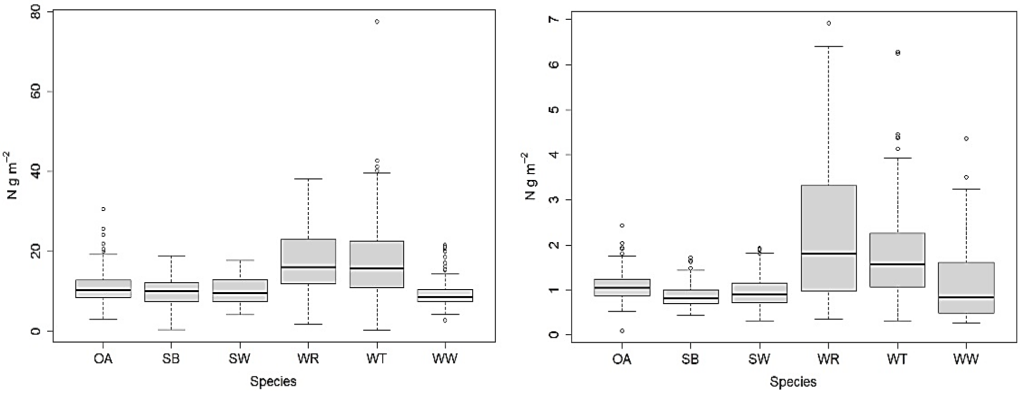

Figure 5. Accumulated amount of nitrogen (N) by different cereal crops: in the above-ground residues (picture at the left side); and in the below-ground residues (picture at the right side), N g m^2: OA – oats; SB – spring barley; SW – spring wheat; WR – winter rye; WT – winter triticale; WW – winter wheat.

C input

Conservation of crop residues in fields should be mandatory to maintain soil quality and offset agricultural C emissions (Stella et al., 2019). Carbon input often is calculated using allometric equations that describe the amount of C returned to the soil relative to yield. A set of crop-specific coefficients allows to estimate of C input to soil for individual species or groups of crops, such as cereals. Typically, allometric equations include a conversion from dry matter to C units and a factor that relates yield to the amount of above- and below-ground residues remaining in the field (e.g. straw, roots and rhizodeposition) (Keel et al., 2017). In order to increase the accuracy of calculations, it is necessary to collect and analyze the widest possible set of data specific to a particular region.

Carbon (C) input from above-ground residues can be calculated using C amount of harvested product and harvest index (HI) which is the ratio of harvested product (grain yield) to total above-ground biomass (grain yield and AG residues). Carbon input from the root biomass (BG residues) of cereal crops can be calculated using crop annual root biomass C, harvest index (HI) and SR - the ratio of shoot (AG residue) and root (BG residue) biomass of crop (Palosuo et al., 2015). Mean SR ratio of cereal crops studied was 5.6. However, by species it varied within quite wide limits - from 4.5 (OA) to 7.1 (SB). For winter cereal species, SR ratio fluctuated closely around the mean: 5.5 (WR); 5.8 (WW) and 5.9 (WT) (Table 7). Previous studies have concluded that the

SR ratio of 5.6 can be used as a constant in calculations for all cereals (Palosuo et al., 2015).

Harvest indexes fluctuated from 0.31 (WR) and 0.32 (WT) to 0.43–0.44 for other cereal crops. Previously published data of HI (Palosuo et al., 2015) agree very well with the data obtained in our trials for WW and SW (0.42), and oats (0.46). For the other species, the differences could have been influenced by specificities of the varieties and meteorological conditions.

Table 7. Parameter values used for converting statistical yield data to carbon input from crops to soil

Parameter	Winter wheat	Spring wheat	Oats	Winter triticale	Winter rye	Spring barley
SR (shoot/root ratio)	5.8	4.8	4.5	5.9	5.5	7.1
HI (harvest index)	0.43	0.44	0.43	0.32	0.31	0.44

Using different allometric equations the annual C inputs to 0–1 m depth from plant residues averaged accross all crop types and treatments ranged from 2.1 Mg C m^{-2} $year^{-1}$ to 5.3 Mg C m^{-2} $year^{-1}$ (Bolinder, 2007). It was considered that the upper 0.20 m of soil contained about 33% of soil organic content (SOC) in the 0–1 m profile (Fliessbach et al., 1999).

Correlations with yield

Winter wheat. The analysis of variance shows that the total biomass (B_{tot}) of winter wheat varied significantly by year, variety, N fertilizer, soil agrochemical parameters, and it was significantly dependent on the interaction of the mentioned factors ($p < 0.05$). The average biomass ($n = 112$) of the variety 'Brencis' 1526.1 g m^{-2} was significantly higher compared to that of variety 'Fredis' 1347.6 g m^{-2} with a reliable probability (> 95%, p-value = 0.002). The linear equation between grain yield harvested (GY) and total biomass was found: $B_{tot} = 729.51 + 1.15 \times GY$. With an increase in yield by 1 g m^{-2}, the total biomass increased by 1.15 g m^{-2} with almost 100% confidence. The determination coefficient ($R^2 = 0.61$) shows that 61% of the biomass data dispersion can be explained by the grain yield (Table 8). In turn, no correlation was found between grain yield and above-ground residues (AG_{resid}). The regression equation $AG_{resid} = 729.5 + 0.15$ GY shows that with the yield increase by 1 g m^{-2}, the above-ground residues increased by 0.15 g m^{-2} with a rather weak, only 90.9%, reliability ($R^2 = 0.03$). Similarly, the linear equation of the analysis of variance does not statistically significantly explain the dispersion of the above-ground residues value depending on the grain yield factor.

A close positive correlation ($r = 0.82$) was found between WW grain yield and below-ground residues (BG_{resid}). Regression analysis resulted in the equation $BG_{resid} = -64.7 + 0.34 \times GY$. With the GY increase by 1 g m^{-2}, amount of the below-ground residues increased by 0.34 g m^{-2} with almost 100% reliability ($R^2 = 0.67$). Total residues (Resid), i.e., above-ground and below-ground biomass remaining on the field and in the soil, depending on the GY, can be calculated with almost 100% reliability (p-value almost 0, $R^2 = 0.18$) using the following equation: $Resid = 664.77 + 0.49 \times GY$. The close positive correlation (0.81) between grain yield and both C and N captured by BG residues (g m^{-2}) was established and such linear equations were developed: $C_{BG} = -20.94 + 0.12 \times GY$, ($R^2 = 0.66$); $N_{BG} = -0.76 + 0.003 \times GY$, ($R^2 = 0.67$).

Spring wheat. The grain yield of spring wheat varied significantly ($p < 0.05$) by year, variety and N fertilizer rate, but the interaction of these factors was insignificant. A weak correlation was found between grain yield and: BG residues (0.4), C_{BG} (0.48), and N_{BG} (0.56).

Table 8. Regression equations between grain yield of cereals and residues; and captured carbon and nitrogen

Equation	r/R^2	Equation	r/R^2
	Winter wheat (WW)		
$B_{tot} = 729.51 + 1.15 \times GY$	0.78/0.61	$Resid = 664.77 + 0.49 \times GY$	0.49/0.18
$AG_{resid} = 729.5 + 0.15 \times GY$	0.16/0.03	$C_{BG} = -20.94 + 0.12 \times GY$	0.81/0.66
$BG_{resid} = -64.7 + 0.34 \times GY$	0.82/0.67	$N_{BG} = -0.76 + 0.003 \times GY$	0.81/0.67
	Oats (OA)		
$B_{tot} = 667.1 + 1.1 \times GY$	0.51/0.18	$C_{AG} = -97.8 + 0.34 \times B_{tot}$	0.88/0.78
$C_{AG} = 133.1 + 17.33 \times N_{AG}$	0.78/0.61	$N_{AG} = -1.8 + 0.01 \times B_{tot}$	0.60/0.35
	Winter triticale (WT)		
$AG_{resid} = 2859.3 - 2.2 \times GY$	0.72/0.52	$C_{AG} = 1277.26 - 0.95 \times GY$	0.69/0.48

r/R^2 – correlation coefficient/ determination coefficient.

Oats. Oat grain yield was significantly influenced by all factors: year, variety, N fertilizer rate, and location. Significant was also the interaction of mentioned factors (Table 3). There was found a weak linear correlation (0.51) between the grain yield and total amount of biomass: $B_{tot} = 667.1 + 1.1 \times GY$ ($R^2 = 0.18$). Strong correlations between total biomass and C amount captured by above-ground residues as well as between N and C captured by aboveground residues were found: $C_{AG} = -97.8 + 0.34 \times B_{tot}$ ($r = 0.88$; $R^2 = 0.78$); $C_{AG} = 133.1 + 17.33 \times N_{AG}$ ($r = 0.78$; $R^2 = 0.61$).

Winter triticale. Grain yield and biomass of winter triticale differed significantly by year, variety, N fertilizer rate, and location or soil properties. The interactions of mentioned factors were also significant (Table 3). Grain yield correlated with the above-ground residues, and linear equation was developed: $AG_{resid} = 2{,}859.3 - 2.2 \times GY$. With the yield increase by 1 g m^{-2}, the AG residues decreased by 2.2 g m^{-2}, $R^2 = 0.52$. Grain yield correlated (0.69) also with the accumulated carbon amount (C, g m^{-2}) in AG residues: $C_{AG} = 1{,}277.26 - 0.95 \times GY$. With the yield increase by 1 g m^{-2}, the amount of C_{AG} decreased by 0.95 g m^{-2} ($R^2 = 0.48$).

CONCLUSIONS

Biomass data on various cereal species collected are representative considering both the different soil and meteorological conditions during the three trial years, different varieties and rates of nitrogen fertilizers applied.

It can be concluded that cereal crops with above-ground and below-ground residues accumulate a significant amount of carbon and nitrogen. The amount of residues and, therefore, the amount of accumulated C and N are strongly influenced by various factors - species, variety, fertilizer, soil conditions. A very important role plays the year and interaction of the above-mentioned factors. It is important to understand the role of each factor and find options to drive them to increase C and N sequestration in the soil and reduce the environmental impact of intensive agriculture.

Average values of dry matter of cereal crop residues left on the field were 906.7 g m^{-2} DM of above-ground (AG) residues and 164.2 g m^{-2} of below-ground (BG) residues.

Average C amount captured by cereal crop residues was as following: 411.2 g m^{-2} C in AG residues and 60.7 g m^{-2} C in BG residues. An average amount of captured N was as following: 12.88 g m^{-2} N in AG residues and 1.38 g m^{-2} N in BG residues.

Regularities between grain yield and residues were found, however, they were not very strong. The closest were found for winter wheat whose data approached the average values of all cereal species. This study demonstrated a medium strong linear relationship between WW grain yield and: total biomass; BG residue; C_{BG}; $N_{BG.}$ The dataset should be enlarged to reduce uncertainty.

The obtained results allow us to conclude that the data calculated from the yield will have a greater uncertainty, therefore the GHG inventory should be calculated according to the average AG and BG biomass, which is more accurate.

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DATA AVAILABILITY STATEMENT: The data presented in this study are available on request from the corresponding author.

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Diversified cropping systems for promoting the beneficial insects - ground beetles (*Coleoptera: Carabidae*)

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Abstract. In agro-ecosystems ground beetles - carabids (*Coleoptera: Carabidae*) are important as generalist predators of invertebrate pests and weed seeds and as prey for larger animals. This way they contribute to biodiversity and influence the most important ecological processes. Impacts of crop management practices on the carabids are not well described. Carabids were studied in winter wheat which is one crop in the rotation experiment (barley undersown with clover-clover-winter wheat-pea-potato). Carabids were collected with pitfall trap during one week at the end of June 2022. In laboratory, their species was identified. Trapping of carabids during the spiking phase of winter wheat has shown significant differences in carabids activity-density and diversity depending on five different cropping systems. In two conventional systems where pesticides were used the number of carabids was two times smaller in comparison with three organic systems. Activity-density and diversity of carabids was significantly higher in all organic systems and especially in Org II system where winter cover crops and composted manure where used for rotation diversification. The Shannon–Wiener index values, which takes into account the number of species and their relative abundance were 1.24–1.53 in conventional systems, but higher in diversified organic systems (1.60–1.78). Only in organic systems Org I and Org II there were very rare species present, like *Acupalpus meridianus* (Linnaeus) and *Microlestes minutulus* (Goeze). In diversified organic systems the higher activity-density and abundance of carabids could be explained by the diverse plant community as possible source for better food and microclimatic conditions.

Key words: organic cropping, winter cover crops, conventional cropping, pesticides, weed.

INTRODUCTION

Ground beetles - carabids are species rich and abundant in arable habitats all over the world. Because of their predatory polyphagous nutrition, they are potentially important natural pest-controlling agents. For sustainable agricultural systems, self-regulation of predatory arthropods is considered to be crucial in preventing insect pest outbreaks. At the same time many of carabids as seed predators have potential for weed control (Bàrberi et al., 2010). The species assemblage of carabids present in any particular crop is determined by multiple factors. Crop type affects the carabid assemblage indirectly through cultivation practices and microclimatic changes (Holland & Luff, 2000).

Besides arthropods and weed seeds carabids are also slug predators, but long-term repeated pesticides treatments affect the number and diversity of carabids in agricultural habitats (van Toor, 2006). Moreover, overall carabid activity-density and species richness were higher in the low input 4-year crop rotation compared with the conventionally managed 2-year crop rotation (O'Rourke et al., 2008). The activity-density and species richness of carabids increased in cover crop based reduced tillage systems (Rivers et al., 2017). But Bourassa et al. (2008) have found that crop type had a stronger effect than sustainable treatment on the species richness and abundance of carabids. Still, they observed lower activity-density in potato plots which were sprayed with insecticides. It has been found that carabids as generalist predators had a strong positive response to plant diversity, that is, their abundance increased as the plant diversity increased. Positive effects of plant diversity on generalist predators confirm that, at a local scale, plant diversification of agroecosystems is a credible and promising option for increasing the effect of pest control (Dassou & Tixier, 2016). Weeds as part of biodiversity of a agroecosystem facilitate the diversity of carabid beetles. Intermediate diversity of carabid beetles species (3–8) exhibited the highest weed seed predation by invertebrates (Schumacher et al., 2020).

The aim of present study was to explain the activity-density and diversity of carabids depending on the cropping systems and diversity weed species in winter wheat during the spiking phase.

MATERIALS AND METHODS

Carabids were studied in winter wheat, which is a crop in the long-term rotation experiment (barley undersown with clover–clover-winter wheat-pea-potato). All crops are cultivated each year in two conventional and three organic systems. The experiment is set up in a systematic block design with four replicates of each treatment and a plot size of 60 m^2. The organic and conventional plots were separated with an 18 m wide section of grass-clover to prevent the spread of synthetic plant protection products and mineral fertilizers. In both conventional systems, winter wheat plots were treated with pesticides: seed treatment before drilling was done by Lamardor 400 FS (propiconazole 250 g L^{-1} + tebuconazole 150 g L^{-1}) (0.2 L ha^{-1}), weed control (middle of May) with Secator OD (amidosulfuron 100 g L^{-1} + iodosulfuron-methyl-sodium 25 g L^{-1} + mefenpyrodiethyl 250 g L^{-1}) (150 ml ha^{-1}) and fungicide Zantara (biksafen 50 g L^{-1} + tebukonazole 166 g L^{-1}) (1.2 L ha^{-1}) was used against plant diseases (end of May). In Conv 0 no mineral fertilizers were used. In Conv II system in winter wheat plots mineral nitrogen fertilizer was applied (150 kg ha^{-1}) and phosphorus and potassium mineral fertilizers were added to the soil at the rate of 25 and 95 kg ha^{-1}, respectively. All organic system crops were cultivated without any pesticides and Org 0 system also without fertilizers. In Org I and Org II systems winter cover crops were used as green manures, after winter wheat, pea and potato in rotation. Winter cover crops were used: the mixture of turnip rape, winter rye and phacelia after the winter wheat, mixture of winter turnip rape and phacelia after the pea, and mixture of winter rye and phacelia after the potato. Cover crops were ploughed into the soil before the drilling of the main crop in spring. Clover as precrop for winter wheat was ploughed into the soil at the beginning of September. Winter wheat was drilled in middle of September. Before drilling, in Org II system winter wheat plots fully composted cattle manure (10 t ha^{-1}) was applied.

Carabids were collected in winter wheat plots at the spiking phase (BBCH 51-52) with pitfall traps during the last week (from 22 to 28) of June 2022. This period was dry and warm with average temperature of 21 °C. On each of the 20 winter wheat plots a trap was set in the middle of the plot. Pitfall traps were with 8.5 cm diameter and 10 cm deep and ¾ filled with saturated salt (NaCl) solution. From all the traps the material collected was stored in 70% ethanol. In laboratory the material was assorted and carabid species were identified under microscope according to identification keys (Haberman, 1968; Lompe, 2002). While carabids are also dependent on the plant diversity the study of the composition of weed species was carried out at the same time. Weeds were collected from four squares of 0.25 m^2 in each plot. All weeds were collected, counted by species and the total biomass was weighed.

The statistical analysis of collected data was performed with the software Statistica 13 (Quest Software Inc., Aliso Viejo, Ca, USA). The significance of differences between the data on cropping systems tested with the Tukey HSD (honest significant difference) *post hoc* test. For carabids and weeds the Shannon-Wiener Diversity Index (H'), which takes into account the number of species and their relative abundance, was calculated using the following equation:

$$H' = -\sum P_i(\ln P_i)$$

where P_i is the proportion of each species in the sample.

RESULTS AND DISCUSSION

In winter wheat plots 15 different carabid species were found but the number of species was different in systems. In organic systems 12 species, but in conventional systems only 7 species were found (Table 1). Such finding supports the results of a previous study by Döring and Kromp (2003), who found 34% more species on the organic than on the conventional fields. In all cropping systems the most numerous species were *Harpalus rufipes* (Degeer)*, Harpalus affinis* (Schrank) and *Bembidion properans* (Stephens) but these were more abundant in organic systems. These species are common in cereal fields (Kinnunen at al., 2001; Guseva & Koval, 2021).

Table 1. Presence (x) or absence (-) of ground beetle species in different cropping systems in winter wheat

Carabid species	Conv 0	Conv II	Org 0	Org I	Org II
Acupalpus meridianus	-	-	-	x	x
Amara fulva	-	-	-	x	x
Anchomenus dorsalis	-	-	-	x	x
Bembidion lampros (Herbst)	-	x	x	x	x
Bembidion properans (Stephens)	-	x	x	x	x
Bembidion quadrimaculatum (Linnaeus)	x	-	x	x	x
Harpalus affinis	x	x	x	x	x
Harpalus rufipes	x	x	x	x	x
Microlestes minutulus	-	-	-	x	x
Nebria brevicollis (Fabricius)	x	x	x	x	x
Poecilus cupreus (Linnaeus)	x	x	x	x	x
Pretostichus melanarius	-	-	-	-	x

Notes: Org 0 – without cover crops (CC), Org I – with CC, Org II – with CC and composted cattle manure Conv I – with pesticides, without fertilizers and Conv II – with mineral fertilizers and pesticides.

The rare species of *Acupalpus meridianus, Anchomenus dorsalis* (Pontoppidan), *Amara fulva* (Müller), *Microlestes minutulus* and *Pretostichus melanarius* (Illiger) appeared only in organic systems.

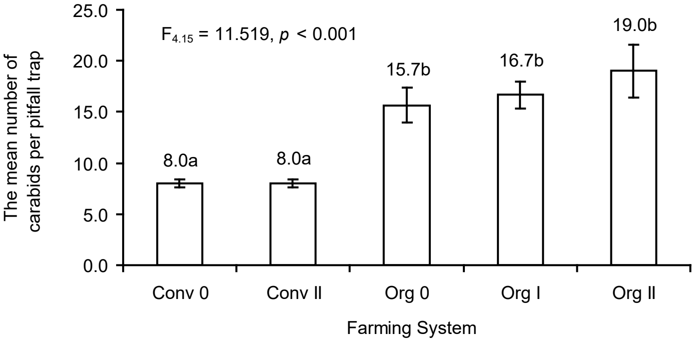

Figure 1. The mean activity-density of carabids per pitfall trap, depending on the cropping system. Org 0 – without cover crops (CC), Org I – with CC, Org II – with CC and composted cattle manure Conv I – with pesticides, without fertilizers and Conv II – with mineral fertilizers and pesticides. Means followed by a different letters indicate significant influence of crops (Tukey HSD post-hoc test, $p < 0.05$). Error bars denote the standard error of the means, $n = 4$.

The mean number of carabids per trap was significantly higher in all organic systems than in conventional ones (Fig. 1). In Org I and Org II systems the more abundant carabids was also more diverse (Fig. 2). The highest activity-density and diversity was observed in Org II system, where the crop rotation had been diversified with winter cover crop and composted manure (Figs 1, 2). The research of Adikhari and Menalled (2020) confirmed that the use of cover crops increased the populations of ground beetle not only in organic farming, but also in chemical-based conventional systems. Therefore, conventional producers should also use more cover crops in their crop rotation. Shannon–Wiener index is significantly higher in Org I system than in Conv 0. In organic systems less chemical disturbances occur and that is favoring the ground-dwelling insects (Kromp, 1999). In conventional systems chemical weed control decreases the density and diversity of weeds. It is possible that *Harpalus rufipes and Harpalus affinis* as first of all seed predators could not have had enough food. Holland & Luff (2000) confirmed that *Harpalus* sp has mixed diet, at the beginning of vegetation period when they use animal food, as later on they prefer seeds. Gallant et al. (2017) established that the activity-density of *Harpalus rufipes* was positively correlated with the mean seed predation.

Weed biodiversity facilitates the diversity of carabid beetle species (Schumacher et al., 2020). Weeds offer shelter, food and change in the microclimate. Our results also confirm that the occurrence of carabids was influenced by plant diversity. Significantly more diverse weed species' composition was seen in all organic systems (Fig. 3). The dominant weed species in the organic systems were *Matricaria inodora* L., *Viola arvensis* Murr., *Taraxacum officinale* and *Capsella bursa pastoris* L. Medicus. In the conventional systems *Elytrigia repens* was the dominant species. Also, the density and biomass of weeds were higher in organic systems, compared to conventional systems. In agroecosystems weeds provide also oviposition and mating sites. Therefore, the decline in the number of weed species affects also the higher trophic levels. Therefore, the conservation of diversity of weed species is also contributing to the conservation of higher order taxa (invertebrates and vertebrates) in the food web (Bàrberi et al., 2010).

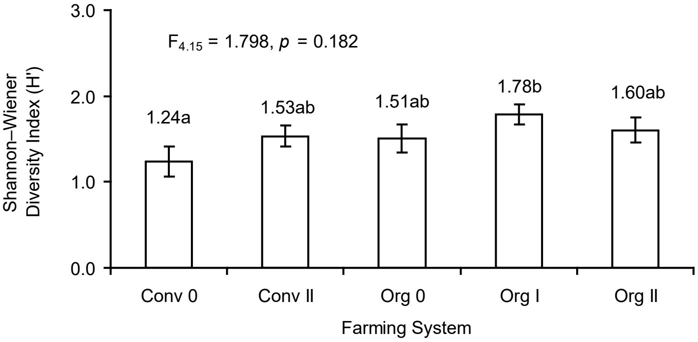

Figure 2. The Shannon-Wiener diversity index of carabids, depending on the cropping system (b). Org 0 – without cover crops (CC), Org I – with CC, Org II – with CC and composted cattle manure Conv I – with pesticides, without fertilizers and Conv II – with mineral fertilizers and pesticides. Indexes followed by a different letters indicate significant influence of crops (Tukey HSD post-hoc test, $p < 0.05$). Error bars denote the standard error of the means, $n = 4$.

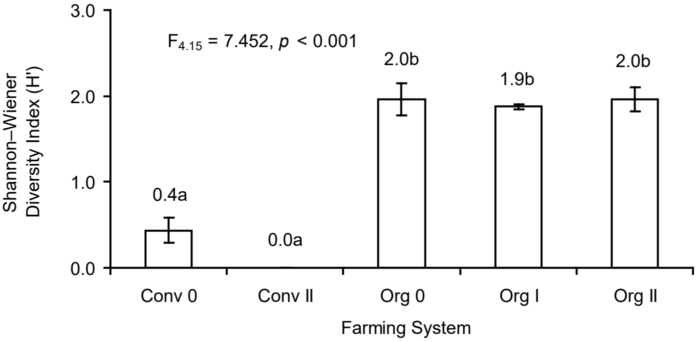

Figure 3. The Shannon-Wiener diversity index of weeds, depending on the cropping system. Org 0 – without cover crops (CC), Org I – with CC, Org II – with CC and composted cattle manure Conv I – with pesticides, without fertilizers and Conv II – with mineral fertilizers and pesticides. Indexes followed by a different letters indicate significant influence of crops (Tukey HSD post-hoc test, $p < 0.05$). Error bars denote the standard error of the means, $n = 4$.

CONCLUSIONS

Species richness, diversity, and community structure is significantly influenced by the farming systems. In organic cropping systems, where higher diversity of plant species occurs through the presence of weeds, also the activity-density and diversity of carabids are increased. Therefore the weedier cereal fields should also be tolerated in conventional management. The cultivation of winter cover crops in crop rotations improves the populations of ground beetles and therefore it is important to include the cover crops into the chemical-based conventional farming systems.

In this article the activity-density and diversity of carabids is analyzed in one of the crops in rotation. Further studies should include all rotational crops for better understanding and estimation of the role of carabids in agroecosystems.

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Comparison of sire rams of the Latvian Dark-Head breed according to feed efficiency indicators as the beginning of genomic breeding research

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Abstract. In sheep (*Ovis aries*) farming, feed costs are the largest variable cost component. Breeders are showing an increased interest in breeding sire rams with improved feed efficiency characteristics because of the possibility that the offspring will have a higher value of this indicator. The result shows that for one ram, the progeny indicators tend to be variable. Currently, no marker has successfully explained enough of the variability of feed efficiency that they were used as part of a routine improvement program. The aim is to analyze feed efficiency indicators for lambs of sire rams of Latvian Dark-Head (LT; Latvijas tumšgalve) to identify sire rams producing lambs with potentially higher feed efficiency. Fattening data of 48 lambs from 13 sire rams were analyzed to determine the correlation of feed efficiency parameters. The average weight of lambs at birth was 4.08 ± 0.56 kg, while the average weight gain reached 47.43 ± 3.17 kg with an average fattening period of 73.27 ± 8.90 days. A strong and very strong correlation between the studied indicators of feed efficiency was revealed. The correlation between these indicators and live weight gain over for 60 days indicates their economic importance in meat production. Certain phenotypic and genotypic factors cause the influence on their value. The phenotypic influence may consist of environmental and external signs, but the genotypic influence is at the DNA level, which requires further study.

Key words: feed efficiency, lambs, Latvian Dark–Head, selection, sire rams.

INTRODUCTION

Raising lambs for selection purposes and meat production is the daily routine of modern sheep farmers. Every day, in a growing economy, it is necessary to be able to keep low production costs, of which about 70% are animal feeding costs (Berry & Crowley, 2013; Lima et al., 2017). To reduce feeding costs, cheaper feeds can be used, which often means a lower economic yield of meat products, for which feeding must be complete. However, the best method is to maintain an efficient herd with good breeding material. (Lima et al., 2013). Accordingly, reducing the fattening time of lambs in meat production is only possible with improved feed intake in the breeding process or the use of lambs with high feed efficiency (Hu et al., 2022).

Feed efficiency indicators can be loosely described as (1) ratio traits: Feed efficiency (FE), Feed conversion ratio (FCR), Relative growth rate (RGR), Kleiber's ratio (KR), or (2) regression or residual traits: Residual feed intake (RFI), Residual weight gain (RWG) and Residual intake and body weight gain (RIG). RGR and KR may be classified as comparative growth traits (Berry & Crowley, 2013)

As of 2018, there were 49.5 thousand (K) ewes and 73 sire rams in Latvia from a flock of 134.29K sheep. In 2018, Latvian Dark-Head (Latvijas tumšgalve; LT) sheep were bred for the implementation of the sheep breeding program in 35 farms, which had a total of 3,752 ewes, while 13 farms had 43 breeding rams (LAAA, 2022a). According to the EUROSTAT data (Eurostat, 2021), the number of sheep in Latvia in 2018 was 107.29K, but in December 2021 was 90.34K.

The Latvian Dark-Head sheep breed was created by crossing local Latvian sheep with Shropshire and Oxfordshire rams imported from Sweden and England. The first pedigree or breeder's book for LT rams was issued in 1939 (Vecvagars & Kairisa, 2018). Currently live weight of ewes of the Latvian Dark-Head breed is about 55–65 kg, rams - 95–120 kg, wool cut 3.5–4.5 and 5.0–6.0 kg, respectively. The average prolificacy of ewes is 150–160% (LAAA, 2022b).

Breeding of Latvian Dark-Head sheep in Latvia is carried out according to phenotypic and bloodline data, without information about values of feed efficiency indicators or carrying out genetic breeding. Therefore, this study aimed to analyze the Latvian Dark-Head breeds rams according to lambs' feed efficiency values. Previous studies have analyzed the fattening indicators (Bārzdiņa & Kairiša, 2015a), the novelty of this study lies in the analysis of the feed efficiency parameters. Scientifically studying sire rams by the indicators of feed efficiency of their offspring, is possible to give recommendations to breeders so that they can select for meat production those sire rams whose offspring have higher indicators. That way, not only breeders get offspring that grow faster and eat less, but the breed improves with each generation.

MATERIALS AND METHODS

Animals of intensive fattening

Based on the requirements of the breeding program of the Latvian dark–headed breed (LAAA, 2022a), every year the offspring of sire ram, certified for breeding activity, are selected. Forty–eight rams' lambs from 13 purebred sire rams of the Latvian Dark-Head breed (3 or 4 lambs per sire ram) were fattened at the ram breeding control station 'Klimpas' in collaboration with the association 'Latvian Sheep Breeders Association' in the summer months of 2022.

The age of the animals at the beginning of the process was 87.02 ± 7.14 days with an interval of 73 to 102 days (2.5–3.4 months), start body weight was 25.12 ± 2.50 kg.

According to the fattening control protocol, all offspring from the same sire ram were fattened in the same pen with a size of approximately 4 m^2 and equipped with a loose silo for combined concentrate and a slatted silo for hay. Straw is used as bedding. After each batch of lambs, the pen is cleaned and disinfected. There is natural ventilation through ceiling slots and windows equipped with anti–insect nets.

Lambs were fattened after an adaptation period of 1 to 2 weeks. During the study, lambs were fed unlimited combined concentrate (869.5 g kg^{-1} of consumed dry matter with 96.6 g kg^{-1} crude protein and 9.72 MJ kg^{-1} metabolizable energy) and hay (Šenfelde et al., 2020); in addition, mineral feed and licks were ensured. Water was provided from automatic waterers without limit.

Before the start of fattening and at the end of the process, the lambs were weighed on an electronic scale with an accuracy of 0.01 kg and measured using ultrasound (US) equipment Mindray Dp–50 Vet along the longest back muscle *(Longissimus dorsi)*, muscle depth and fat thickness of the 13th rib.

The keeping of animals during the research met the animal welfare requirements.

Feed efficiency variables

Each indicator value for each sire ram was calculated as the average from offspring.

Delta of *Longissimus dorsi* muscle depth and fat thickness depth per 1 kg (ΔMD, and ΔFD per 1kg, accordingly) were calculated as the difference between the initial and final measurements of US per feeding time weight gain.

Based on the initial and final weight, the average daily weight gain (ADG) was calculated for each lamb and then the average for each ram from their offspring. Finally, the amount of dry matter intake (DMI) per day for lambs of one sire ram, kept in one pen, was calculated, taking into account that only 20% of hay was digested (Šenfelde et al., 2020). The result obtained per lamb is the average amount of dry matter consumed by 2–4 lambs during the fattening period.

Feed efficiency, Feed conversion ratio, Relative growth rate, Kleiber's ratio, and Residual indicators: Residual feed intake, Residual weight gain, Residual intake, and body weight gain as FE indicators were calculated by using formulas previously published (Berry & Crowley, 2013; Lima et al., 2017).

Statistical analyses

General and analytical statistics were performed with SPSS v.25 (IBM Corp., 2017), *Pearson* or *Spearman correlation* was calculated for lambs all together from measured and calculated values depending on normal distribution for data. In addition, the mean and standard error of the mean (*SEM*) were calculated for measurement data.

To calculate the difference between the parameters related to the FE in the experimental group of animals, the *ANOVA* test was used in the presence of a normal distribution and the *Kruskal–Wallis* test, in the absence of a normal distribution in at least one group. A significant result was determined if $P < 0.05$. The relationship between ram and lamb indicators was used to determine with association's coefficient *eta* (η), which ranges from 0, no relationship, to 1, excellent relationship.

RESULTS AND DISCUSSION

Description of rams according to offspring's fattening performance

The average body weight at birth of the offspring of 13 Latvian Dark-Head sire rams was 4.08 ± 0.08 kg with a difference from 2.80 to 5.40 kg. No statistically significant difference was found between the mean scores of average birth weight of the offspring of the rams (Fig. 1).

In early sources (Bārzdiņa & Kairiša, 2015b), it was found that the average weight of LT ram lambs born in summer was 3.70 ± 0.07 kg, which is about 300 g less than the stated values of the present study. Taking into account that the average weight of the offspring of the sire rams analyzed in our study was higher in almost all cases, except for LT_8, which had a similar weight, we can state an increase in the weight of lambs of Latvian breeds at birth over the past seven years.

According to the Latvian Breed Fattening Control Protocol, lambs from the same ram fed together are fattened to an average lamb weight in the range of 45 to 50 kg. In our study, LT lambs were fattened for an average of 73.27 ± 8.90 days with an interval of 60 to 94 days (at the end of fattening, lambs were around 5.21 months old). Considering that both the initial and final age of each lamb was different, the live weight correction was carried out on the 90th (beginning of fattening) and 150th (finishing of fattening) days (Fig. 1). The difference between the average weight of the offspring of LT sire rams on the 90th day of life is at the border of statistical significance (P = 0.056). However, on day 150th, the difference is statistically significant ($P = 7.10\times10^{-4}$). At both points of measurement, the relationship between the weight of the offspring and the ram is moderately close ($\eta > 0.60$). It can be assumed that the live weight of the offspring at particular moment (90th and 150th day) depends also on chosen sire ram.

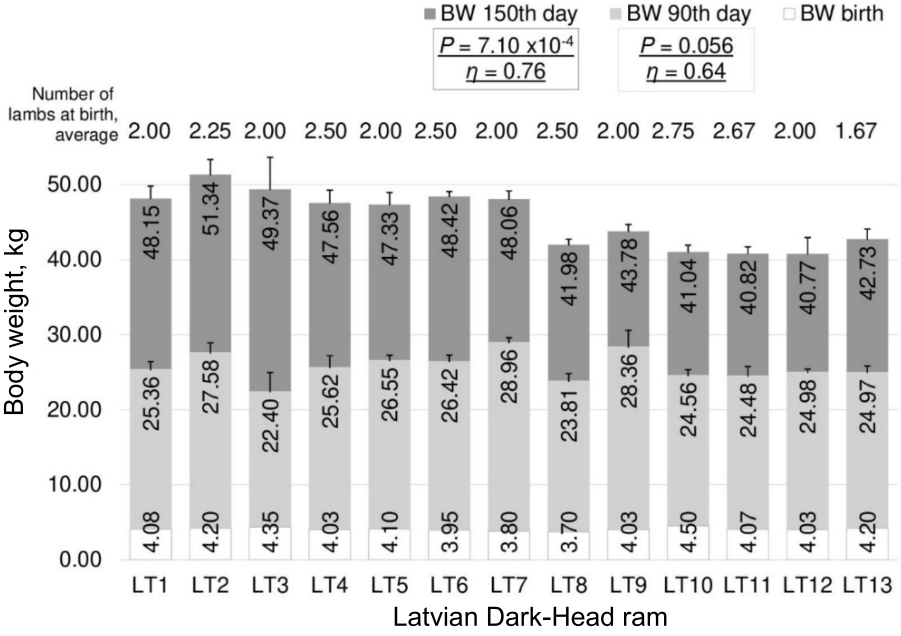


Figure 1. Mean of body weight (BW), kg, of lambs of Latvian Dark-Head rams at birth and corrected for day 90 and day 150. P – Statistical significance of mean with ANOVA; η – a measure of association.

Despite the same feeding conditions, there are rams whose offspring' average daily gain (ADG) prevails in relation to the rest of the lambs of the experimental cohort. On average, ADG in the experimental cohort of lambs was determined as 334.70 ± 9.96 g per day (Fig. 2) with a dry matter intake (DMI) of 1.65 ± 0.02 kg per day. According to the results obtained, a statistically significant difference was determined (*P* = 7.66×10–4) in the mean value of ADG in the offspring of sire rams. In the LT breed, the presence of rams with an average ADG of the offspring was also found to differ by almost 200 grams. Interestingly, in the offspring of five rams from the experimental cohort, the prevalence of the ADG value in relation to the average total value was determined. This revealed difference in ADG values in the offspring of rams from the experimental herd is also confirmed by the value of the relationship index *Eta* ($\eta = 0.83$) or close relationship. Thus, among the 13 analyzed sires, there are those whose offspring have a high level of ADG, and there are rams with offspring that have a low value.

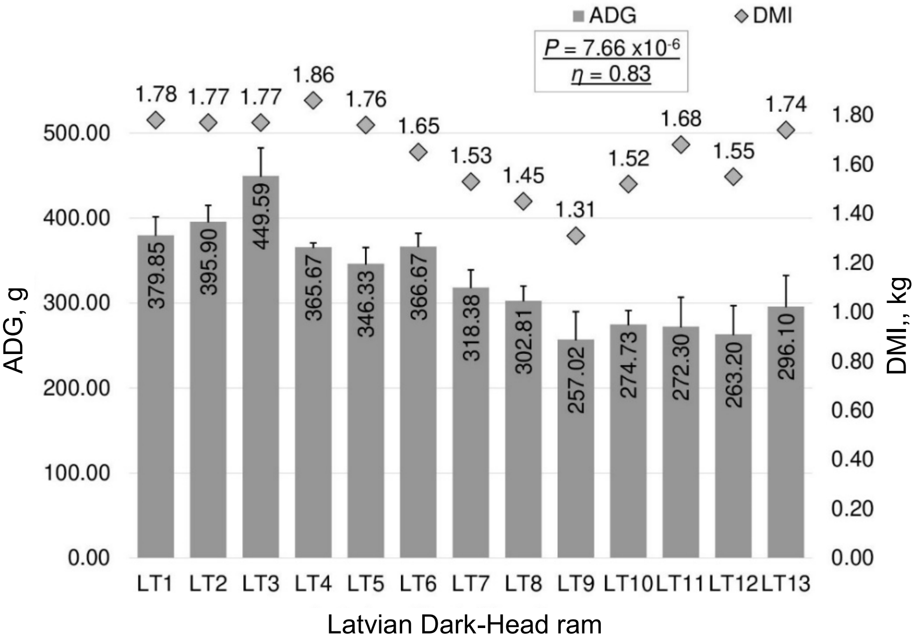


Figure 2. Mean of average daily gain (ADG), g, and dry matter intake (DMI), kg, per day in lambs from Latvian Dark-Head sire rams. *P* – Statistical significance of mean with *ANOVA*; η – a measure of association.

ADG values determined in the present and previous (Kairiša & Bārzdiņa, 2016) studies were found to be similar. However, in the experimental lambs' cohort of 2016, aged 81–99 days, the highest ADG value was found, which, however, turned out to be lower than the average value of this indicator from the present cohort for our three top rams (LT_1 – LT_3; Fig. 2).

According to the results of the study, LT lambs gained 20.08 ± 0.60 kg in weight over 60 days (ΔBW 60d) with an interval from 11.84 to 31.90 kg (Table 1). Thus, the difference between the lightest and the heaviest lamb was on average 20 kg. A statistically significant difference in ΔBW values, as well as a close relationship between the rams of the experimental cohort, was determined at the fattening period of 60 days. These data confirm our assumption that the offspring of rams from the experimental herd have potentially different values of feed efficiency, which have both phenotypic and genotypic effects.

The data obtained by measuring the depth of the *Longissimus dorsi* muscle (ΔMD) and adipose tissue at the level of the 13th rib using US were calculated per 1 kg of weight gain (Table 1). It was found that this average value of the growth of the long back muscle per 1 kg statistically significantly differs in the offspring of LT sire rams ($P = 1.26\times10^{-2}$), however, in relation to the growth in adipose tissue depth per 1 kg, no statistical difference was found. For the LT breed, ΔMD per kg is 0.46 ± 0.02 mm, with a final average MD value of 31.01 ± 0.21 mm. Among 13 sire rams, only six showed a prevailing value for offspring indicator in relation to the average value for the breed. Thus, it can be assumed that these sire rams can improve the breed in this indicator.

However, in our study, none of the sire rams was found with the prevailing average values of all previous indicators in relation to the rest of the rams. Thus, it is assumed that no animal from our experimental herd can provide the best quality indicators in the future, in relation to its offspring. For example, ram LT_10 is in the top three in terms of birth weight, as well as ΔMD and ΔFD, but has one of the lowest values of the average daily gain. Animal LT_3 has very good indicators regarding birth weight, ΔFD, and best ADG, but has a higher average DMI than the average for all rams.

Table 1. Mean of average of fattening traits in lambs from Latvian Dark-Head sire rams

Rams	Traits, mean ± SEM		
Nr	ΔBW in 60 days, kg	ΔFD, mm per 1 kg	ΔMD, mm per 1kg
All*	20.08 ± 0.60	0.044 ± 0.003	0.46 ± 0.02
	(11.84–31.90)	(0.006–0.084)	(0.25–0.80)
LT_1	**22.79 ± 1.30**	0.030 ± 0.007	0.35 ± 0.02
LT_2	**23.76 ± 1.14**	0.036 ± 0.013	0.43 ± 0.06
LT_3	**26.98 ± 1.97**	0.041 ± 0.004	**0.54 ± 0.09**
LT_4	21.94 ± 0.30	0.033 ± 0.009	0.37 ± 0.04
LT_5	20.78 ± 1.14	0.036 ± 0.009	0.38 ± 0.03
LT_6	22.00 ± 0.92	0.047 ± 0.008	0.47 ± 0.02
LT_7	19.10 ± 1.24	0.036 ± 0.016	0.47 ± 0.04
LT_8	18.17 ± 1.03	0.050 ± 0.007	0.36 ± 0.03
LT_9	15.42 ± 1.97	**0.062 ± 0.011**	0.46 ± 0.02
LT_10	16.48 ± 0.98	**0.062 ± 0.009**	**0.61 ± 0.05**
LT_11	16.34 ± 2.07	0.048 ± 0.003	**0.54 ± 0.07**
LT_12	15.79 ± 2.03	**0.067 ± 0.011**	**0.67 ± 0.07**
LT_13	17.77 ± 2.18	0.039 ± 0.004	0.46 ± 0.11
P	8.53×10^{-6}	0.15	1.26×10^{-2}
η	0.83	0.59	0.73

ΔMD – delta of *Longissimus dorsi* muscle depth per 1kg; ΔFD – delta of Fat thickness depth per 1kg; ΔBW 60 – change in body weight in 60–day period. All – all lambs with min and max values in brackets; *P* – the statistical significance of mean with ANOVA or Kruskal-Wallis (underlined) test; η – a measure of association; in bold the first three values and underline the last three.

Description of sire rams according to feed efficiency indicators

Feeding efficiency indicators were calculated from fattening performance: ratio traits: FE, FCR (Fig. 3) and RGR, KR (Fig. 4), and residual traits: RWG, RFI, and RIG (Table 2). A statistically significant difference was established between all indicators of the feeding efficiency trait in LT rams of the experimental herd. This effect may be due to differences in phenotypic characteristics, as well as in the genetic background associated with this tract, between animals of the same breed (Lima et al., 2017). We also assume the possibility of genetic and phenotypic correlations between the constituent feeding efficiency traits, the analysis of which is also presented in this study.

Feed efficiency and Feed conversion ratio

The first two traits are Feed efficiency (FE) and Feed conversion ratio (FCR), which are inverse to each other, or FCR = 1/FE (Lima et al., 2017). The values of the FCR (kg of feed dry matter intake per kg of live weight gain) for lambs vary from 4 to 5 (for FE grams from 200 to 250) on highly concentrated ratio, from 5 to 6 (FE, g, 166.67–200) falls on some good quality feeds and more than 6 (> 166.67 g) - on feeds on lower quality food (National Research Council, 2007).

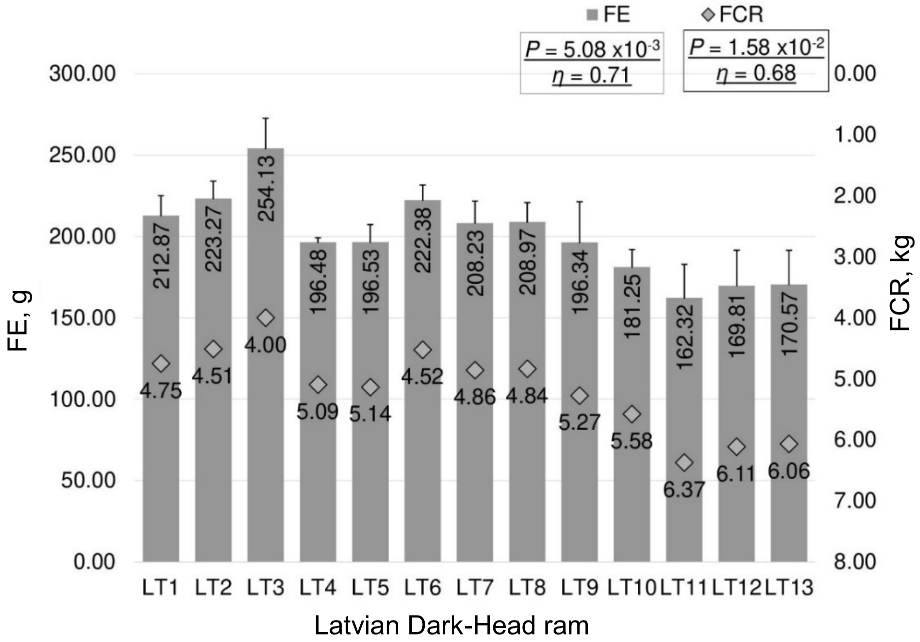


Figure 3. Mean of Feed efficiency (FE), g, and Feed conversion ratio (FCR), kg, per day for lambs of Latvian Dark-Head rams. *P* – Statistical significance of mean with *ANOVA*; *η* – a measure of association.

In the LT breed, the average FCR is 5.09 ± 0.15 kg with an interval from 3.33 to 7.92. That means that 1 kg of weight gain requires an average of 5.09 kg of dry matter. However, when comparing rams (Fig. 3), six has an average FCR of less than 5.00, and the rest rams range from 5 to 6. Thus, out of 13 sires, half produce lambs with a high FCR value. There must be some other factors, molecular or genetic, that may positively influence high FCR and improve breeding.

Relative growth rate and Kleiber's ratio

In this study, the Relative growth rate (RGR), which is also an indicator of feeding efficiency (Berry & Crowley. 2013), as well as the Kleiber ratio (KR), an indicator of growth efficiency regardless of body size (Köster et al., 1994), were also calculated. For LT lambs, the average RGR value was 0.43 ± 0.01. According to our data (Fig. 4), the sire ram (LT_3) with the lowest FCR value also has the highest average RGR score, indicating the high potential of this ram as a producer of lambs with a potentially high feeding efficiency value.

Based on the published data, rams have a higher average RGR than ewes (Kesbi & Tari, 2015). The highest value of this indicator is observed during the period when the ewe nursing the lamb, but after the end of nursing, it decreases. In our study, RGR values for the time periods in the period from weaning to 6–9 months were found to be similar or higher relative to other breeds (Kesbi & Tari, 2015; Lima et al., 2017; Ghafouri–Kesbi & Eskandarinasab, 2018; Ehsaninia, 2022). These data confirm the competitiveness of the LT breed. However, for its subsequent improvement, it seems necessary to use sire rams with the highest indicators related to the feeding efficiency tract.

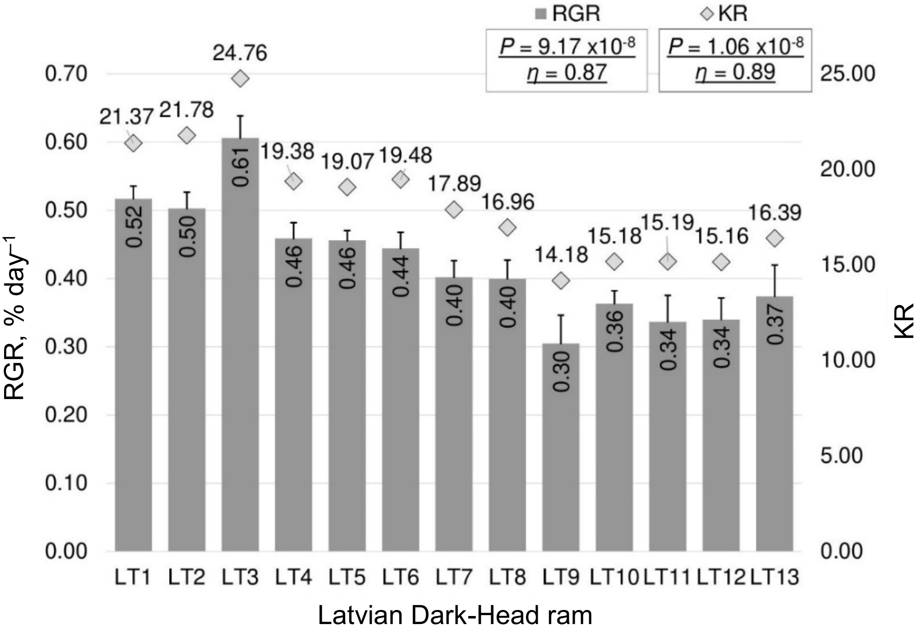


Figure 4. Mean of relative growth rate (RGR) and Kleiber's ratio (KR) for lambs of Latvian Dark-Head rams. *P* – Statistical significance of mean with *ANOVA*; *η* – a measure of association.

Based on our calculations, for six sire rams the KR average value from the experimental cohort turned out to be higher than the average for the breed (18.46 ± 0.49) which suggests that the potential for these animals as producers of offspring with improved feeding efficiency indicator (Fig. 4). The values of the KR obtained by us turned out to be significantly higher than in other breeds of sheep in the corresponding age (Talebi, 2012a; Kumar et al., 2017; Venkataramanan et al., 2019; Bansal et al., 2021; Ehsaninia, 2022) However, it should be taken into account that at the moment there is no clearly established norm for this feeding efficiency related parameter in lambs.

The high correlation between KR and FE indicators shows that animals with higher KR require less energy to maintain weight and growth (Bansal et al., 2021). In addition, the highest values of the Kleiber coefficient indicate an increase in body weight gain at the same metabolic weight ($BW^{0.75}$), which means that higher growth is achieved without an increase in the cost of energy for maintenance (Talebi, 2012b).

Residual feed intake and Residual weight gain

The expected DMI and ADG values were calculated using the BW$^{0.75}$ parameter for all LT lambs from the experimental cohort. Based on the obtained data, the Residual feed intake (RFI) and Residual weight gain (RWG) parameters (Table 2) and both standardized values for RIG were further calculated (Lima et al., 2017). As a result, all three residual values were, on average, equal to 0.

RFI measures the economic value of feed consumption (Berry & Crowley, 2013). The RFI values in the experimental cohort of lambs from LT breeds were determined in the range from –0.27 to 0.25 kg (Table 2). These values indicate the presence of animals consuming dry matter both 0.27 kg less than planned and 0.25 kg more than they are supposed to according to metabolic weight. According to the results of this study, a negative value of the RFI parameter was revealed for half of the sire rams. Thus, their offspring would be expected to consume less dry matter and have a lower DMI than would be expected from calculations based on ADG and metabolic body weight at the end of the fattening period. It was found a statistically significant difference between the average RFI for sire rams ($P = 1.27$ x10^{-13}), and the relationship between sire rams and offspring data is closely related ($\eta = 0.95$). Thus, it is assumed that among the 13 rams analyzed, there are those whose offspring need less dry matter to reach marketable weight.

Table 2. Feed efficiency residual traits of Latvian Dark-Head rams

Rams Nr	Feed efficiency residual traits. mean ± *SEM*		
	RFI, kg day^{-1}	RWG, g day^{-1}	RIG
All*	0.00 ± 0.02 (–0.27–0.25)	0.00 ± 6.56 (–96.83–203.62)	0.00 ± 0.26 (–4.17–3.38)
LT_1	0.05 ± 0.02	**28.48 ± 5.63**	0.25 ± 0.24
LT_2	0.03 ± 0.02	**30.90 ± 12.89**	0.46 ± 0.44
LT_3	–0.06 ± 0.03	**86.71 ± 15.26**	**2.41 ± 0.57**
LT_4	0.19 ± 0.02	–46.99 ± 17.94	–2.64 ± 0.56
LT_5	0.09 ± 0.01	–15.13 ± 4.66	–1.11 ± 0.18
LT_6	–0.02 ± 0.02	4.31 ± 13.16	0.27 ± 0.47
LT_7	**–0.11 ± 0.02**	21.85 ± 11.34	1.36 ± 0.43
LT_8	**–0.16 ± 0.02**	21.45 ± 12.75	**1.78 ± 0.46**
LT_9	**–0.22 ± 0.04**	–1.27 ± 22.55	**1.77 ± 0.83**
LT_10	–0.04 ± 0.01	–30.81 ± 8.01	–0.39 ± 0.28
LT_11	0.12 ± 0.03	–59.95 ± 13.12	–2.30 ± 0.55
LT_12	–0.02 ± 0.02	–18.13 ± 4.25	–0.23 ± 0.25
LT_13	0.15 ± 0.04	–55.00 ± 18.09	–2.43 ± 0.71
P	1.27 x10^{-13}	3.94×10^{-8}	3.03×10^{-9}
η	0.95	0.88	0.90

RFI – Residual feed intake; RWG – Residual weight gain; RIG – Residual intake and body weight gain; All – all lambs with min and max value in brackets; *P* – the statistical significance of mean with ANOVA test; the first three values are in bold and the last three are underlined.

According to published data (Tortereau et al., 2020), when selecting rams of the Romane breed according to the increased RFI, already in the first generation of their offspring, a decrease in the DMI required for feeding was observed and, thus, the cost of fattening was reduced.

A similar result is observed in the case of our study: a high statistically significant difference ($P = 3.94$ x10^{-8}) between rams in the experimental cohort, as well as a close correlation ($\eta = 0.88$), is observed between the sire rams and lambs' cohorts when calculating the RWG parameter (Table 2). For example, the calculated ADG for LT_3 ram was found to differ from expected by an average of 86.71 ± 15.26 g per day; in the case of LT_11, the difference was –59.95 ± 13.12 g per day, or weight gain was less than expected based on metabolic weight calculations and the DMI used.

Residual intake and weight gain

Using the Residual Intake and Growth (RIG) indicators, it is possible to identify fast–growing animals, with the highest ADG and the lowest DMI, consuming less feed than the population average, with no difference in BW. Thus, an increase in the value of the RGI parameter, in turn, leads to an improved ratio of ADG and DMI indicators (Lima et al., 2017). With a higher RIG value, the feeding efficiency increases correspondingly, which characterizes animals with higher growth rates and lower fat content, without affecting the quality of meat and carcass. In addition, if this characteristic is present in an animal, the time it is kept until it reaches its marketable weight is reduced (Arce–Recinos et al., 2021).

According to our data (Table 2), among certain sire rams from the experimental cohort, an average RIG significantly prevailed in relation to the other ($P = 3.03 \text{ x}10^{-9}$; $\eta = 0.90$) and reflected improved characteristics of these animals.

In the present study, in our experimental cohort, one sire ram (LT_3) was identified with prevailing values of all the above indicators (excluding the RIF index) associated with feed efficiency (Figs 3, 4 and Table 2). Thus, it is assumed that this animal has an increased potential for improving the breed.

However, in the context of the obtained results, it seems necessary to find out the features and differences at the level of the genetic background related to the feeding efficiency tract in sheep with high and low rates of the above indicators in order to determine genetic markers to improve selection in the LT breed for this trait.

Correlation of feed efficiency indicators

Strong and negative correlation (Fig. 5) in all analyzed LT lambs were identified between FCR and RGR (–0.74) and FCR and KR (–0.76), but strong and positive correlation – between FE and RGR (0.82) and KR (0.85). Very strong and positive correlations were identified between RGR and KR (0.99) traits in LT lambs. Considering that similar algorithms are used in the calculation of feed efficiency ratio indicators; the correlation between feed efficiency ratio traits and residual traits is more significant.

The residual traits: RFI, RWG and RIG, has a statistically significant correlations with all other feed efficiency indicators for LT lambs, except RIF hasn't correlation with RGR and KR.

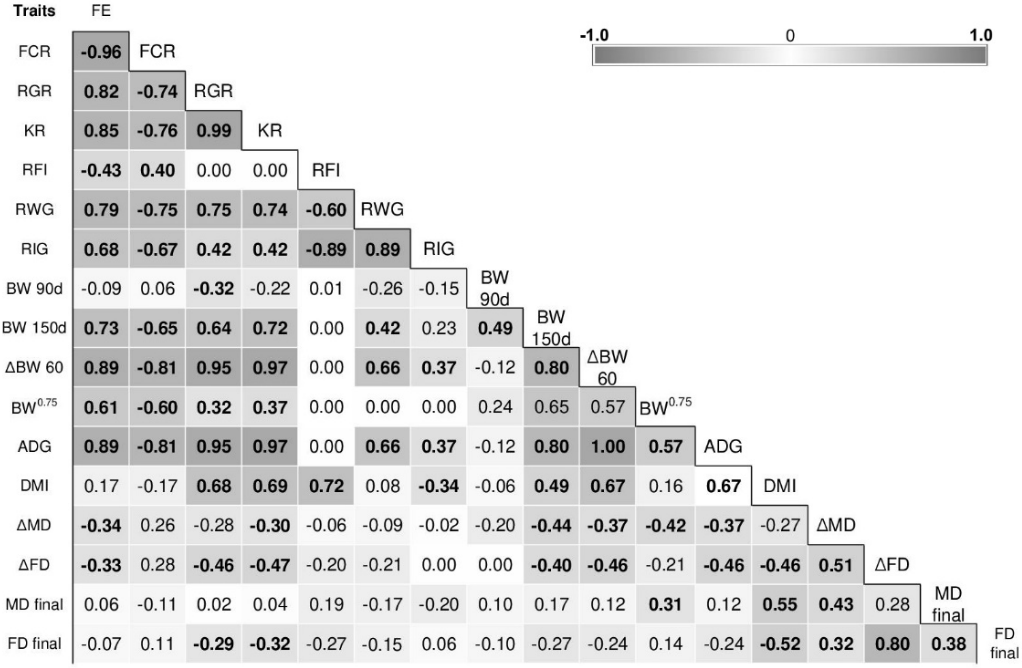
In case of RFI indicator have weak correlations with FE/FCR (–0.43/0.40), which indicates the possibility that using RFI and FE/FCR in breeding is possible to promote weight gain in LT lambs with lower feed intake than expected.

There is also moderately strong, negative correlation between RFI and RWG (–0.60), which shows, that there already are lambs with more than expect ADG and less than expected DMI. These lambs and their sire rams are necessity to improve meat production characteristics of the LT breed.

Correlations, but phenotypic and genetic, between RGR or KR and RIG has also been found in other sheep populations (Knott et al., 2003; Talebi, 2012b). In LT lambs also are correlation between this indicators: 0.42 in both cases. So LT lambs with a high RGR and/or KR will also have a high RIG. But RIG indicators have stronger correlation with FE/FCR (0.68/–0.67) in LT lambs.

The found correlation between feed efficiency indicators, except RFI, and live weight gain over a period of 60 days indicates their economic importance in meat production. Correlation ΔBW 60d with FE/FCR are 0.89/–0.81, but with RGR and

KR – 0.95 and 0.97, respectively, which means that improving feed efficiency improves weight gain in intensive fattening over a 60–day period.



Traits	FE	FCR	RGR	KR	RFI	RWG	RIG	BW 90d	BW 150d	ΔBW 60	$BW^{0.75}$	ADG	DMI	ΔMD	ΔFD	MD final	FD final
FCR	**-0.96**																
RGR	**0.82**	**-0.74**															
KR	**0.85**	**-0.76**	**0.99**														
RFI	**-0.43**	**0.40**	0.00	0.00													
RWG	**0.79**	**-0.75**	**0.75**	**0.74**	**-0.60**												
RIG	**0.68**	**-0.67**	**0.42**	**0.42**	**-0.89**	**0.89**											
BW 90d	-0.09	0.06	**-0.32**	-0.22	0.01	-0.26	-0.15										
BW 150d	**0.73**	**-0.65**	**0.64**	**0.72**	0.00	**0.42**	0.23	**0.49**									
ΔBW 60	**0.89**	**-0.81**	**0.95**	**0.97**	0.00	**0.66**	**0.37**	-0.12	**0.80**								
$BW^{0.75}$	**0.61**	**-0.60**	**0.32**	**0.37**	0.00	0.00	0.00	0.24	0.65	0.57							
ADG	**0.89**	**-0.81**	**0.95**	**0.97**	0.00	**0.66**	**0.37**	-0.12	**0.80**	**1.00**	**0.57**						
DMI	0.17	-0.17	**0.68**	**0.69**	**0.72**	0.08	**-0.34**	-0.06	**0.49**	**0.67**	0.16	**0.67**					
ΔMD	**-0.34**	0.26	-0.28	**-0.30**	-0.06	-0.09	-0.02	-0.20	**-0.44**	**-0.37**	**-0.42**	**-0.37**	-0.27				
ΔFD	**-0.33**	0.28	**-0.46**	**-0.47**	-0.20	-0.21	0.00	0.00	**-0.40**	**-0.46**	-0.21	**-0.46**	**-0.46**	**0.51**			
MD final	0.06	-0.11	0.02	0.04	0.19	-0.17	-0.20	0.10	0.17	0.12	**0.31**	0.12	**0.55**	**0.43**	0.28		
FD final	-0.07	0.11	**-0.29**	**-0.32**	-0.27	-0.15	0.06	-0.10	-0.27	-0.24	0.14	-0.24	**-0.52**	**0.32**	**0.80**	**0.38**	

Figure 5. Correlation of feed efficiency indicators for lambs. FCR – Feed conversion ratio; FE – Feed efficiency; RGR – Relative growth rate; KR – Kleiber's ratio; RFI – Residual feed intake. RWG – Residual weight gain; RIG – Residual intake and body weight gain; BW – body weight at 90th day (90d), 150th day (150d); ΔBW 60 – change in body weight in 60 day period; $BW^{0.75}$ – metabolic weight; ADG – average day gained weight; DMI – dry matter intake; ΔMD – Delta of *Longissimus dorsi* muscle depth per 1kg; ΔFD – fat thickness depth per 1 kg; MD final – *Longissimus dorsi* muscle depth at the end of fattening. FD final – fat thickness depth at the end of fattening Value with statistical significance $p < 0.05$ is highlighted in bold.

As a result of our studies, a correlation was established between DMI and RGR, KR, BW at day 150 and body weight gained at 60 days, which indicates an unknown factor, for example differences in DNA level, affecting feed intake of lambs, since feed was available all lambs without restrictions.

CONCLUSIONS

The feeding productivity of LT sheep was determined to be medium in our study. Obviously, this parameter should be improved, considering, however, the preservation of breed specificity. This problem can be solved by selecting animals for breeding with a clear phenotypic description, related to the current breed, and a deterministic genotype associated with high rates of feed efficiency indicators. Based on the results of the analysis of the above feed efficiency indicators, several sire rams from our experimental cohort can be selected for subsequent selection improvement.

Thus, additional studies are needed to clarify the quality of feed efficiency indicators when using other ewe genetic materials. It is desirable, however, to conduct an additional experimental trial for fattening a cohort of offspring from other ewes and rams with improved parameters, selected in the current study.

The results of our study indicate that the LT breed has sire rams whose offspring have a higher feed efficiency compared to the offspring of other sire rams. According to the most prevailing indicators of feed efficiency among the animals of the experimental herd, it is possible to provide recommendations to breeders for the best breeding selection using the identified sire rams, whose offspring have higher indicators. Such selection within a certain breed will improve the overall economic performance of the herd: breeders not only get offspring that grow faster and eat less, but also the effect of a systematic improvement of the breed with each generation is obtained.

Feed efficiency indicators have common effects in LT breed sire rams. Possibly, the phenotypic effects determine the living conditions or environment and external signs of the ram, but the genetic effect is at the DNA level, which requires further study. This possibility needs to be tested in future studies.

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Feed efficiency indicators and hormones related to nutrient metabolism in intensive fattened lambs of sire rams of different sheep breeds in Latvia

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Abstract. The feed efficiency increase of each sheep indicates its profitability. Production costs and the environmental impact of animal husbandry are reduced as feed efficiency improves. The gastrointestinal tract is a source of hormones and is important in regulating feed intake and nutrient utilization. The study analyses the relationship between feed efficiency indicators and hormone levels in Latvian sheep breeds. After control fattening, blood samples were taken from 76 lambs, representatives of six Latvian breeds, for seven hormonal analyzes and seven feed efficiency indicators. Feed efficiency, Feed conversion ratio (FCR), Relative growth rate, Kleiber ratio (KR), Residual feed intake (RFI), Residual weight gain, and Residual intake were calculated from daily weight gain and amount of dry matter. Interbreed differences and relationships between indicators/hormones were identified. The best scores of feed efficiency were found for the lamb rams of the Dorper breed; the Latvian black-head was the second according to these indicators. The mean FRC for lambs was determined to be 5.13 ± 0.13 kg with a range of 2.98–10.15 kg; the mean KR was 18.11 ± 0.39 with a range of 8.78–26.88; the mean RFI was in the range of -0.51 to 0.42 kg. A statistically significant difference was found between the breeds for all feed efficiency indicators. Biochemical parameters (IGF-1, insulin, and glucose) were found to be in correlation with feed efficiency indicators. Our results show that Latvian sheep breeds differ in fattening performance. To improve breeds without crossing them, subsequent genetic analysis of breed differences is necessary.

Key words: breeding, fattening, feed efficiency, hormones, Latvian sheep.

INTRODUCTION

In sheep breeding, 60–70% of the daily costs are for their fattening (Lima et al., 2017). Therefore, to reduce these costs, raising animals with a higher value or feed efficiency is necessary, which will contribute to the profitability of this production (Berry & Crowley, 2013; Lima et al., 2017).

Improved feed efficiencies indicators such as Feed efficiency (FE), Feed conversion ratio (FCR), Relative growth rate (RGR), Kleiber ratio (KR), Residual feed intake (RFI), Residual weight gain (RWG), and Residual intake and body weight gain (RIG), reduced the ratio of average weight gain and amount of feed required what in final reduce production costs (Berry & Crowley, 2013). In addition, this process also reduces the environmental impact of animal husbandry (Hu et al., 2022).

Many factors affect feed efficiency, including body composition, digestion, and metabolism of nutrients (Zhang et al., 2017). In addition, the endocrine system could affect feed efficiency by regulating feed intake and nutrient utilization (Richardson et al., 2004). The gastrointestinal tract with attached glands is the source of secretes hormones, which are important in regulating feed intake (insulin) and nutrient use (glucose, insulin, and IGF-1). Previous studies have reported that the systemic insulin concentration in low-feed efficiency steers is greater than in high-feed efficiency steers (Richardson et al., 2004). In addition, plasma concentrations of thyroxine T4 and adrenocorticotropic hormone (ACTH) were lower in high-feed efficiency than in low-feed efficiency cows (Walker et al., 2015).

The world's sheep stock rose to a new record high of 1.266 billion head in 2021 (IWTO, 2022), including approximately 1,400 breeds (Kawęcka, 2022), classified according to (1) type production - wool, meat, milk, fur and other; (2) phenotypic characteristics such as head colour (white head, dark head), tail length and ability to accumulate fat (short, long, fat), presence of horns (horned or dehorned); (3) by breeding place - Germany, Latvia, Estonia, Spain and other or by topographical habitat - lowlands, highlands (LAAA, 2023). The number of sheep in Latvia in 2018 was 107.29 thousand, having increased in December 2021 to 90.34 thousand, according to Eurostat data (Eurostat, 2021). There are currently ten sheep breeding programs in Latvia (LAAA, 2022), according to which Latvian breeds are divided into maternal and paternal breeds according to their characteristics.

Maternal breeds such as Latvian dark-head (Latvijas tumšgalve; LT) and Merinolandschaf (MLS), also known as Württemberger, have good fertility and the ability to rear lambs, while paternal breeds: Île de France (IF), Charollais (CH), Dorper (DOR) and Texel (TEX), have fattening abilities - quick monitoring, feed conversion, and carcass quality (LAAA, 2023).

The analyzed parameters of feed efficiency and hormone levels related to nutrient metabolism in single-litter lambs of different breeds are variable and affects meat quality (Zhang et al., 2017). Until now, there is no information on whether feed efficiency indicators and hormone levels, which also depend on the feed used, differ in different breeds of Latvian sheep reared under the same conditions.

The study aims to analyze the value of feed efficiency indicators and the level of hormones related to nutrient metabolism in Latvian sheep breeds. Scientifically based knowledge of breed differences and the relationship of feed efficiency indicators with specific hormones can be used as an economical and rapid breeding tool in the selection of animals within the same herd with the highest feed efficiency indicators. In this way, breeders will be able to systematically improve the breed of sheep with each generation.

MATERIALS AND METHODS

Animals of intensive fattening

Based on the requirements of the breeding program of the breeds (LAAA, 2022), every year, the offspring of the sire ram, certified for breeding activity, are selected and analyzed to estimate the sire rams. Seventy-six (76) lambs (63.16% LT) from 22 sire rams of six breeds: Latvian dark-head (48 lambs), Merinolandschaf (8 lambs), Île de France (6 lambs), Charollais (3 lambs), Dorper (5 lambs) and Texel (6 lambs), were included in controlled fattening from March to October 2022. All lambs were born as twins, triplets or quadruplets from different ewes and health status was assessed prior to inclusion in the study so that there were at least two lambs per sire ram from the breed. This study was carried out in cooperation with Latvian Sheep Breeders' Association at the ram breeding control station (Table 2). Lambs were fattened for 66.38 ± 1.27 days with an interval of 44 to 83 days.

According to the fattening control protocol, all offspring from the same sire ram were fattened in the same pen with a size of approximately 4 m^2 and equipped with a loose silo for combined concentrate and a slatted silo for hay. Straw is used as bedding. After each batch of lambs, the pen is cleaned and disinfected. There is natural ventilation through ceiling slots and windows equipped with anti-insect nets. The keeping of animals during the research met the animal welfare requirements.

Lambs were fattened with unlimited water from automatic drinkers, a combined concentrate (869.5 $g\,kg^{-1}$ of consumed dry matter with 96.6 $g\,kg^{-1}$ crude protein and 9.72 $MJ\,kg^{-1}$ metabolizable energy) and hay; in addition, mineral feed and licks were ensured. The dry matter and chemical composition of grass hay has been published previously by Šenfelde and colleagues (2020).

The live weight of lambs was determined before and after intensive fattening by weighing with calibrated and certified electronic scales (accuracy ± 0.01). As a result, the average initial body weight of the lambs was 25.12 ± 2.50 kg (approximate age 2.5–3.4 months) according to the controlled fattening process established in Latvia for each breed. The end of fattening was determined by the average final body weight of at least 45–50 kg for all offspring from one sire ram, fattened in one pen, according to the control fattening protocol.

Feed efficiency variables

The average daily gain (ADG) in grams for each lamb was calculated based on the initial and final weights, which were then used to calculate the breed average. The amount of dry matter consumed per day (DMI) in kilograms is calculated as the average amount per lamb from the total amount of feed eaten by one lamb in one pen (Šenfelde et al., 2020).

Feed efficiency, Feed conversion rate, Relative growth rate, Kleiber ratio, and residuals: Residual feed intake, Residual weight gain, and Residual intake and live weight gain were calculated (Table 1) using previously published formulas (Berry & Crowley, 2013; Lima et al., 2017). The indicator FE (the amount of weight gain per 1 kg of feed) and FCR (the amount of feed required for 1 kg of weight gain) are inverse to each other; FCR = 1/FE (Lima et al., 2017)

Biochemical analysis

At the end of the fattening or before 24 h fasting before slaughter, body weight measurements and blood samples from the jugular vein were taken from each lamb for hematological and biochemical analysis. Blood samples were taken in the morning before midday. In addition, tests for insulin-like growth factor 1 (IGF-1), insulin, total thyroxine (TT4), adrenocorticotropic hormone, hormone (ACHT), hematocrit (HCT), hemoglobin (Hb), and glucose were performed in a certified laboratory.

Statistiscal analyses

The mean and standard error *(SEM)* were calculated for the measurement data. Appropriate statistical tests *(ANOVA, Kruskal-Wallis, or Median test)* were used to determine the magnitude of the difference between the breed data depending on the normality of the data and/or the homogeneity of the variances. The post hoc tests between the two breeds were used. A significant result was defined as $P < 0.05$. To determine the relationship between the performance of lambs from different Latvian breeds, the relationship index *Eta* (η) was used with limits from 0 (no relationship) to 1 (ideal relationship). *Spearman correlation* was calculated for the total experimental cohort of lambs as several data weren't in the normal distribution. Analytical statistics were performed with SPSS v.25 (IBM Corp., 2017).

Table 1. Traits of Feed efficiency

Traits	Calculated
Feed efficiency, g	$\frac{ADG}{\mathrm{DMI}}$
Feed conversion ratio, kg	$\frac{\mathrm{DMI}}{\mathrm{ADG}/1000}$
Relative growth rate, % day^{-1}	$100 \times \frac{\log \mathrm{BWf} - \log \mathrm{BWi}}{\text{days of fattening}}$
Kleiber ratio	$100 \times \frac{\mathrm{ADG}}{\mathrm{BWf}^{0.75}}$
Residual feed intake, kg day^{-1}	$\mathrm{DMI} - ex\mathrm{DMI}$
Residual weight gain, g day^{-1}	$\mathrm{ADG} - ex\mathrm{ADG}$
Residual intake and live weight gain	$\mathrm{RWG} - \mathrm{RFI}$

ADG – average day gained weight; DMI – dry matter intake; BWi – initial body weight; BWf final body weight RFI – Residual feed intake; RWG – Residual weight gain.

RESULTS AND DISCUSSION

Description of fattening performance

The present study was carried out on an experimental cohort of 76 purebred lambs, the offspring of 22 purebred sire rams: 13 rams from the LT breed, one to three animals each from IF, DOR, MLS, TEX, and CH breeds. Thus, the offspring of sire rams involved in breeding were compared (Table 2).

The lowest rates of ADG were determined for TEX lambs, and the highest was for IF lambs; during the fattening process, all animals had unlimited access to food; however, DMI was statistically different ($P < 0.001$) between breeds. The difference ($P < 0.05$) between the CH breed, whose lambs consumed the least dry matter per day, and the IF breed, with the highest dry matter intake, is 0.67 kg per day.

The values of the average daily weight gain obtained in our study are similar to other studies. Thus, ADG in Belgian TEX lambs is 230 g per day up to the 130th day (Janssens, 2000). According to the study of colleagues from Latvia, the ADG value for the LT breed was 355.7 g (Kairiša & Bārzdiņa, 2016). Dorper lambs weaned at 2–3 months of age gained 0.230 kg per day (Cloete et al., 2000). The difference between the above data and the result of our study is about 100 g. However, it should be considered that different feeding conditions can affect the final gain values (Mahgoub et al., 2000).

Description of feeding efficiency indicators

Feeding efficiency indicators were calculated from AGD and DMI fattening indicators (Table 2). FCR values for lambs range from 4 to 5 kg (for FE in grams from 200 to 250) if a highly concentrated diet is used; from 5 to 6 kg (FE, g, 166.67–200) for a diet from good forage quality; and over 6 kg (> 166.67 g) using lower-quality feed (National Research Council, 2007). The lower the FCR, the less feed is required per 1 kg of weight gain. The lowest mean FCR was determined in our study in DO lambs: 4.21 ± 0.42 kg, and the highest in CH lambs: 5.91 ± 2.13 kg. The results between the breeds were similar when comparing the values of the FCR indicators, as well as FE: in DOR lambs, 1 kg of dry matter accounts for almost 250 g of live weight gain; in IF lambs, the gain was less than 190 g. The value of the FE indicator for DOR lambs was more than for the Doppler and Santa Ins (½:½) crossing breed in Brazil in 2017 (Lima et al., 2017) when it averaged 210 g.

Table 2. Traits of fattening of sheep of Latvian breeds

Traits	All lambs	Breed of sheep*					
		LT ^{a}	IF ^{b}	DOR ^{c}	MLS ^{d}	TE ^{e}	CH ^{f}
Lambs, No	76	48	6	5	8	6	3
ADG, g	330.3 ± 8.1	334.7 ± 10.0	373.5 ± 25.9	359.7 ± 26.00	319.7 ± 22.5	271.0 ± 15.9	272.4 ± 73.5
DMI, kg	1.63 ± 0.02	1.65 ± 0.02 ^{b,f}	1.97 ± 0.01 ^{a,c,d,e,f}	1.48 ± 0.11 ^{b}	1.61 ± 0.03^{b,f}	1.45 ± 0.08 ^{b}	1.30 ± 0.00 ^{a,b,d}
FE, g	203.1 ± 4.6	202.4 ± 4.9	189.6 ± 13.7	247.2 ± 25.2	197.7 ± 12.7	189.6 ± 14.7	209.8 ± 56.6
FCR, kg	5.13 ± 0.13	5.09 ± 0.14	5.42 ± 0.40	4.21 ± 0.42	5.18 ± 0.26	5.45 ± 0.45	5.91 ± 2.13
RGR, % day^{-1}	0.41 ± 0.01	0.43 ± 0.01	0.41 ± 0.03	0.47 ± 0.01	0.37 ± 0.02	0.32 ± 0.01	0.33 ± 0.07
KR	18.11 ± 0.39	18.46 ± 0.49	20.09 ± 1.13	20.40 ± 0.52	16.77 ± 0.88	14.80 ± 0.51	14.83 ± 3.06
RFI, kg day^{-1}	0.00 ± 0.02	0.01 ± 0.02 ^{b}	0.29 ± 0.04 ^{a,c,d,e,f}	-0.21 ± 0.10 ^{b}	0.02 ± 0.03 ^{b}	-0.09 ± 0.08 ^{b}	-0.25 ± 0.07 ^{b}
RWG, g day^{-1}	0.00 ± 5.7	5.1 ± 6.9 ^{c}	-16.00 ± 19.7 ^{c}	70.1 ± 14.8 ^{a,b,d,e}	-35.2 ± 13.8 ^{c}	-36.9 ± 14.7 ^{c}	2.1 ± 21.5
RIG	0.00 ± 0.20	0.05 ± 0.19 ^{b,c}	-2.00 ± 0.62 ^{a,c,f}	2.64 ± 0.89 ^{a,b,d,e}	-0.83 ± 0.41 ^{c}	-0.23 ± 0.76 ^{c}	1.52 ± 0.86 ^{b}

Breeds of sheep: LT – Latvian dark-head; IF – Île de France; DO – Dorper; MLS – Merinolandschaf; TE – Texel; CH – Charollais. ADG – average day gained weight; DMI – dry matter intake; FCR – Feed conversion ratio; FE – Feed efficiency; RGR – Relative growth rate; KR – Kleiber ratio; RFI – Residual feed intake; RWG – Residual weight gain; RIG – Residual intake and body weight gain; *subscript letters (shown by breed) indicate the variety with which there is a statistically significant difference of post hoc test of ANOVA or Kruskal-Wallis/Median.

There are few publications directly devoted to the feeding efficiency indicator. FCR indicator, the most commonly used in feeding efficiency trait analysis for our lambs, ranges from 3.79 kg for DOR lambs to 13.88 kg for SA lambs. A similar trend is observed when comparing the average values of the FCR of lambs from Latvian breeds with data from other breeds (Lima et al., 2017; Tortereau et al., 2020; Mupfiga et al., 2022). According to study of the Romane breed, the value of the FCR heritability index is 0.30. Thus, this breed has great potential for genetic improvement relative to this indicator (Tortereau et al., 2020).

The next two indicators of feeding efficiency are ratios indicators of growth efficiency: Relative growth rate (RGR) (Berry & Crowley, 2013) and the Kleiber ratio (KR), which provides a measure of growth efficiency independent of body size (Köster et al., 1994). According to our data, DOR lambs have the highest average RGR and KR values, while TEX lambs, in turn, have the lowest (Table 2). Thus, DOR lambs with the lowest body weight on the 90th day have the highest growth efficiency among the lambs from other breeds.

The RGR data of lambs, fattened in the current study, are similar to those reported for other breeds from weaning to six months of age (Kesbi & Tari, 2015; Lima et al., 2017; Ghafouri-Kesbi & Eskandarinasab, 2018; Ehsaninia, 2022), thus proving the competitiveness of Latvian sheep breeds.

The KR values calculated in this study for DOR, IF and LT breeds were significantly higher at the corresponding lambing age, relative to other sheep breeds (Talebi, 2012a; Kumar et al., 2017; Venkataramanan et al., 2019; Bansal et al., 2021; Bukhari et al., 2022; Ehsaninia, 2022) or were similar (Lima et al., 2017). Therefore, a higher KR value indicates greater weight gain for the same metabolic body weight ($BW^{0.75}$), i.e., without increasing energy consumption (Talebi, 2012b). On the contrary, the KR value was lower for the MSL, TEX, and CH breeds.

The FE scores are residuals for the feed efficiency tract (Table 2): Residual Feed Intake (RFI), Residual Gain (RWG), and their sum, or Residual Feed Intake and Body Weight Gain (RIG). The RFI is the most frequently analyzed quantity and is of great economic importance, allowing the detection of an animal consuming less than the planned amount of feed, thus reducing costs (Berry & Crowley, 2013). In turn, the RWG indicator shows which animal gained more / less weight than planned, taking into account the amount of food consumed; it allows to identify of fast-growing animals with the highest ADG and the lowest DMI, consuming less food than the average for the population, without differences in BW. The higher the RGI, the better the ratio between ADG and DMI (Lima et al., 2017).

The average calculated RFI statistically significantly ($P < 0.001$) differs between DOR and IF breeds, within -0.21 ± 0.10 kg day^{-1} for the DOR breed and 0.29 ± 0.04 kg day^{-1} for the IF, respectively. Thus, IF lambs consume, on average, more dry matter than DOR lambs.

Statistically significant differences between breeds in our study were also determined by calculating the values of the RWG indicator - a positive mean was found for the DOR, LT, and CH lambs, but negative for the other breeds. Thus, according to the results obtained, DOR, LT and CH breed lambs have a higher body weight at the end of fattening in accordance with the metabolic weight and DMI used, but for the other four breeds, the values of these indicators were lower.

Our results show that RIG is positive for LT, DOR, and CH breeds but negative - for IF, MLS, and TEX ($P < 0.001$; $\eta = 0.57$).

According to our data, lambs of the DOR breed have improved average values for all the above indicators of feeding efficiency trait; the second place is occupied by lambs of the LT breed regarding the quality of the studied parameters. The lowest result was presented in the analyzed group of CH and IF breeds lambs.

When analyzing the data obtained in the current study, we consider that the study was conducted in an experimental cohort of lambs. Thus, the obtained results and statistical conclusions from the data received can't be accepted for all Latvian breeds in general, because in the case of IF, DOR, MLS, TEX, and CH breeds offspring of 1 to 3 rams were used. Therefore, to create a permanent analysis of the breeds raised in Latvia, it would be necessary to analyze all breeds of lambs over several years.

Description of hematological and biochemical parameters

The level of hematological and biochemical parameters in the blood of lambs was determined at the end of fattening at about five months of age of animals, to identify individual characteristics for each of the studied breeds (Dias et al., 2010). According to early studies, hematological blood parameters: hematocrit and hemoglobin values are less variable over time (Ullrey et al., 1965), but biochemical parameters: IGF-1, insulin, TT4, ACTH, and glucose are more dependent on feed composition (Mahgoub et al., 2015).

A statistically significant difference between breeds grown in Latvia was found for three biochemical parameters (Table 3): IGF-1 ($P < 0.001$; $\eta = 0.56$), insulin ($P < 0.05$; $\eta = 0.22$) and glucose ($P < 0.001$; $\eta = 0.69$).

Table 3. Hematological and biochemical analysis of sheep of breeds of Latvia

Traits	Norm	All lambs	Breed of sheep *					
			LT ^{a}	IF ^{b}	DOR ^{c}	MLS ^{d}	TEX ^{e}	CH ^{f}
IGF-1, ng ml^{-1}	n.d.	198.68 ± 7.80	191.43 ± 9.99 ^{b,c,e}	254.17 ± 10.31 ^{a,e}	257.20 ± 11.36 ^{a,e}	238.00 ± 11.18 ^{e}	118.00 ± 8.39 ^{a,b,c,d}	125.00 ± 22.61
Insulin, mU l^{-1}	n.d.	0.75 ± 0.06	0.80 ± 0.09	0.92 ± 0.10	0.60 ± 0.04	0.69 ± 0.14	0.58 ± 0.04	0.40 ± 0.00
TT4, nmol l^{-1}	38.6 – 77.2$^{\$}$	67.90 ± 4.50	61.86 ± 5.38 ^{a}	93.33 ± 7.06 ^{b}	54.10 ± 21.00	70.49 ± 18.40	94.00 ± 14.14	72.67 ± 10.11
ACTH, pg ml^{-1}	50 – 100$^{\#}$	76.135 ± 8.50	70.45 ± 11.32	66.73 ± 14.21	112.06 ± 48.25	71.91 ± 15.02	102.22 ± 32.13	105.63 ± 33.20
HCT, %	27.0 – 45.0$^{\$}$	24.72 ± 0.79	23.44 ± 0.83	23.50 ± 1.82	22.80 ± 1.16	30.13 ± 2.82	26.33 ± 4.14	31.67 ± 7.26
Hb, g l^{-1}	90.0 – 150.0^	125.72 ± 1.24	127.28 ± 1.33	130.50 ± 4.33	121.00 ± 8.24	115.50 ± 3.47	127.83 ± 4.13	121.00 ± 4.58
Glucose, mmol l^{-1}	2,78 – 4,44$^{\$}$	4.14 ± 0.06	3.96 ± 0.06 ^{b}	5.23 ± 0.17 ^{a,c,d,e}	4.50 ± 0.26 ^{b}	4.29 ± 0.15 ^{b}	3.89 ± 0.12 ^{b}	4.03 ± 0.05 ^{b}

Breeds of sheep: LT – Latvian dark-head; IF – Île de France; DOR – Dorper; MLS – Merinolandschaf; TEX – Texel; CH – Charollais. IGF-1 – Insulin-like growth factor-1; TT4 – Total thyroxine; ACTH – Adrenocorticotropic hormone; HCT – hematocrit; Hb – hemoglobin; * subscript letters (shown by breed) indicate the variety with which there is statistically significant difference of post hoc test of ANOVA or Kruskal-Wallis/Median. $^{\#}$Amokrane-Ferrah et al., 2022; ^Latimer 2011; $^{\$}$Dias et al., 2010.

IGF-1 is a growth hormone-dependent peptide that, in its unbound form, has a very short - 4 min in sheep biological half-life (Bruce et al., 1991) and is involved in a variety of physiological processes: cell proliferation, embryogenesis, tissue repair, and the metabolism of carbohydrates, proteins, and lipids to stimulate bone and skeletal muscle pre- and post-natal growth as well as in lipolysis and mammary gland development (Flores-Encinas et al., 2021).

The average level of insulin-like growth factor-1 in lambs of three breeds LT, TEX, and CH was significantly lower compared to other Latvian breeds, as well as compared to the level of this hormone in DOR sheep (209.2/375.9 ng mg^{-1}; Flo; Tarazi et al., 2014). Interestingly, the average IGF-1 level in healthy Awassi sheep from this population was around 145 ng mL^{-1}, a value close to that we determined for LT, TEX, and CH lambs. The level of IGF-1 hormone depends on the breed's genomic differences and other intrinsic features, so its laboratory reference level is not established. Plasma levels of IGF-1 have also been found to differ in sheep with different alleles of the two SNPs in the IGF-1 gene promoter on the same diet (Flores-Encinas et al., 2021).

There is also no specific norm for the insulin level in sheep's blood. In the present study, differences were found in the average level of insulin in the blood of lambs of various Latvian breeds. Thus, LT and IF lambs have a higher average level of insulin, in turn, the lowest level was determined for lambs of the CH breed.

The hormones insulin and glucose analyzed in this study are jointly involved in energy metabolism; insulin promotes the breakdown or conversion of glucose into energy (Norton et al., 2022). Thus, we can assume a relationship between their levels, which was not observed in the present study: the lowest average insulin level was noted for CH lambs, and the lowest average glucose level for TEX lambs. In ruminants (Catunda et al., 2013), due to microbial activity in the rumen, the absorption of carbohydrates in the form of hexose sugar in the small intestine is insignificant or absent. For this reason, volatile fatty acids (propionate and butyrate) stimulate insulin secretion more effectively than glucose.

A laboratory standard for glucose is known: 2.78–4.44 nmol L^{-1}. When comparing the average values of this hormone in the blood of the analyzed IF and DOR lambs, it was determined to be higher than the established norm, considering the same feeding conditions. In addition, these breeds had the highest and lowest DMI, respectively. Thus, it can be assumed that in addition to the conditions and composition of feeding, another mechanism that affects insulin levels. The relationship between glucose levels with reduced and increased body weight in sheep breeds has been analyzed (Francis et al., 1999), but unequivocal conclusions have not been drawn.

No difference was found between the levels of other hematological and biochemical indicators between breeds, however, marked differences from their reference values were found, except for Hb. TT4 was above normal for IF and TEX breeds; the ACTH level had higher average values for DOR, TEX, and CH breeds.

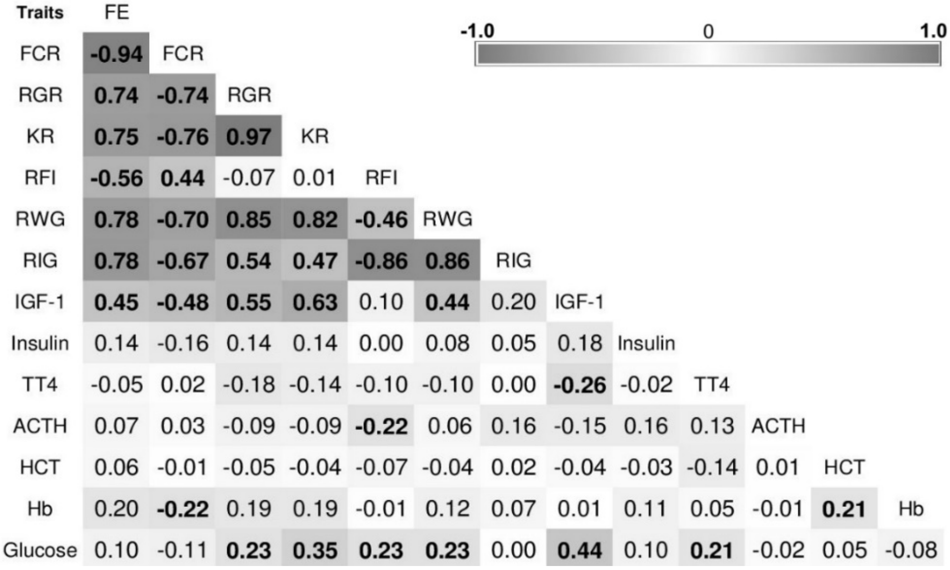
In the present study, hematocrit levels were found below the reference value of this hematological blood parameter in LT, IF, DOR, and TEX lambs. In addition, the HCT values outside (mostly below) the normal range have also been reported for different species; levels below 24 are considered in sheep as an indicator of susceptibility to anemia (Seixas et al., 2021); however, it should be taken into account that the reference values of blood parameters of different sheep breeds may differ significantly.

Correlation between indicators

In order to analyze the relationship between feed efficiency and hematological/ biochemical indicators, correlation analysis was carried out for all lambs from the experimental cohort (Fig. 1).

According to the results obtained, very tight or high correlations were found between all indicators of feed efficiency. Furthermore, according to studies in other sheep populations, Kermani lambs also showed a phenotypic correlation between RGR and KR indicators (Ehsaninia 2022); in the breeds INRA401 (Bibé et al., 2007), Romane

(Tortereau et al., 2020) and Hu (Zhang et al., 2017), correlations between FCR and RFI were determined; a negative correlation between FCR and KR was found in Lori-Bakhtiar lambs, as well as in various breeds of cows (Talebi, 2012b).



Traits	FE												
FCR	**-0.94**	FCR											
RGR	**0.74**	**-0.74**	RGR										
KR	**0.75**	**-0.76**	**0.97**	KR									
RFI	**-0.56**	**0.44**	-0.07	0.01	RFI								
RWG	**0.78**	**-0.70**	**0.85**	**0.82**	**-0.46**	RWG							
RIG	**0.78**	**-0.67**	**0.54**	**0.47**	**-0.86**	**0.86**	RIG						
IGF-1	**0.45**	**-0.48**	**0.55**	**0.63**	0.10	**0.44**	0.20	IGF-1					
Insulin	0.14	-0.16	0.14	0.14	0.00	0.08	0.05	0.18	Insulin				
TT4	-0.05	0.02	-0.18	-0.14	-0.10	-0.10	0.00	**-0.26**	-0.02	TT4			
ACTH	0.07	0.03	-0.09	-0.09	**-0.22**	0.06	0.16	-0.15	0.16	0.13	ACTH		
HCT	0.06	-0.01	-0.05	-0.04	-0.07	-0.04	0.02	-0.04	-0.03	-0.14	0.01	HCT	
Hb	0.20	**-0.22**	0.19	0.19	-0.01	0.12	0.07	0.01	0.11	0.05	-0.01	**0.21**	Hb
Glucose	0.10	-0.11	**0.23**	**0.35**	**0.23**	**0.23**	0.00	**0.44**	0.10	**0.21**	-0.02	0.05	-0.08

Figure 1. Correlation of feed efficiency and hematological/biochemical indicators for lambs of all breeds. FCR – Feed conversion ratio; FE – Feed efficiency; RGR – Relative growth rate; KR – Kleiber ratio; RFI – Residual feed intake. RWG – Residual weight gain; RIG – Residual intake and body weight gain; GF-1 – Insulin-like growth factor-1; TT4 – Total thyroxine; ACTH – Adrenocorticotropic hormone; HCT – hematocrit; Hb – hemoglobin. Value with statistical significance $P < 0.05$ is highlighted in bold.

As a result of the analysis of hematological and biochemical parameters in the blood of lambs, a medium direct correlation was determined between IGF-1, TT4, and glucose; a statistically significant correlation was also found between Hb and HCT.

Thus, one of the objectives of this study was to elucidate the relationship between the indicators of feed efficiency and hematological and biochemical parameters of the blood of lambs. According to the results obtained, IGF-1 and Glucose have a statistically significant correlation with feed efficiency indicators in a joint analysis of all lambs from the experimental cohort; the same level of correlation was also determined between ACTH and the RFI indicator.

According to published data, Hu lambs with low RFI values had lower TT4 and ACTH values and a positive correlation between these parameters; a positive correlation between FCR and TT4 indicators was also found in this breed (Zhang et al., 2017).

CONCLUSIONS

According to the results of our study, the breeds of sheep raised in Latvia differ in feed efficiency and hematological and biochemical parameters. The highest feed efficiency values were found in offspring from two sire rams of the DOR breed, and the lowest values were in offspring from two sire rams of the CH and IF breeds. Accordingly, it would be advisable in future studies to determine the genetic determinants of these differences since IF animals are superior in terms of body weight and ADG value.

Our results prove the differences between the studied Latvian sheep breeds regarding of the hematological and biochemical parameters levels. In the context of this study, the question arises whether these levels directly or indirectly affect indicators of feed efficiency. At the same time, it is necessary to continue research on reference norms in different breeds since our data showed that healthy lambs' hematological and biochemical parameters could be either higher or lower than laboratory-established reference norms. By obtaining information about the norms of breeds grown in Latvia, it is possible to improve sheep veterinary knowledge.

The correlation between the feed efficiency indicators and biochemical parameters of the blood of lambs shows the possibility of hormonal influence on feed efficiency traits in Latvian sheep breeds. Therefore, by using the acquired knowledge in fattening lambs, it is possible to improve feed efficiency and increase the amount of meat obtained by correcting hormonal levels.

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