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FOREWORD

The four independent reviewers estimated each paper and recommended 78 articles for publishing at the proceedings consisted of 2 volumes, which started life as presentations at the Annual 20th International Scientific Conference "Research for Rural Development 2014" held at the Latvia University of Agriculture, in Jelgava, on 21 to 23 May 2014.

In the retrospect of four months later, we can count the Conference as a great success. The theme – Research for Rural Development - attracted participation more than 150 researchers with very different backgrounds. There were 123 presentations from different universities of Lithuania, Estonia, Italy, Poland, Iran, Ukraine, Hungary, Czech Republic, Kazakhstan and Latvia.

Thank you for your participation! I'm sure that you have learned from the presentations and discussions during the conference and you can use the outcomes in the future.

The cross disciplinary proceedings of the Annual 20th International Scientific Conference "Research for Rural Development 2014" (2 volume since 2010) are intended for academics, students and professionals. The subjects covered by those issues are crop production, animal breeding, agricultural engineering, agrarian and regional economics, food sciences, veterinary medicine, forestry, wood processing, water management, environmental engineering, landscape architecture, information and communication technologies. The papers are grouped according to the sessions in which they have been presented.

Finally, I wish to thank Organizing and Scientific Committee and the sponsors for their great support to the conference and proceedings.

On behalf of the Organizing Committee

of Annual 20th International Scientific Conference

"Research for Rural Development 2014"

Ausma Markevica

Latvia University of Agriculture

CONTENTS

AGRICULTURAL SCIENCES (CROP SCIENCES, ANIMAL SCIENCES)

DEMAND FOR APPLIED RESEARCH OF AGRICULTURAL ENGINEERING AND CROP MANAGEMENT IN LITHUANIA

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Abstract
Great emphasis has been placed on agriculture by the European Union (EU), considering that agricultural production provides a framework for development of the processing industry and ensures food provision. Europe 2020 Strategy presents coordinated objectives of support for the rural development for the years 2014–2020. It has been emphasized that each Member State needs to set its national headline targets. Implementation of strategic provisions of the EU and Lithuania requires adequate identification of the priorities of agricultural scientific research and experimental development. It may significantly contribute to securing competitiveness, economic and social progress of the agricultural sector. The aim of the research was to prepare proposals for the Lithuanian Programme that sets the scientific research and experimental development priorities, long- and short-term research programmes for the period until 2020 in the view of demands of economy. Individual Working Groups (WG) have been formed of specialists of Lithuanian research and educational institutions as well as the advisory services. WG have proposed prospective applied research to develop within different areas of agriculture in 2014–2020. This served as the basis for the survey aimed at identifying a general demand for applied scientific research and potential problem areas in relation to agriculture, food economy and rural development that could be addressed by prospective applied scientific research. An anonymous survey was used across the country and summary results have been presented for two areas: crop management and agricultural engineering.
Key words: applied research, experimental development, priority areas, crop management, agricultural engineering, strategy.

Introduction

The 21st century is the century of a very rapid technological, economic and climate change. This requires a prompt response to the changes, seizing all opportunities and adapting to the changes. Life and economy of the society we create must be based on the principles enabling us to courageously face new challenges. Europe 2020 Strategy prepared by the European Commission aims for smart, sustainable and inclusive growth. Five headline targets have been formulated in the Strategy: growth of jobs with the aim to increase employment; development of innovations by scientific research and experimental development funding; an efficient development of energy from renewable sources; tackling the issues of climate change with the aim to reduce emissions of greenhouse gases; educational improvements and poverty reduction among the socially excluded (Europe 2020..., 2010a). The EU is determined to achieve these targets by 2020. It has been emphasized in the document that each Member State must determine its own national headline targets. In line with the Europe 2020 Strategy, these broad objectives of support for rural development 2014 – 2020 are defined in more detail by the following EU-wide priorities: fostering knowledge transfer and innovation in agriculture, forestry and rural areas; enhancing competitiveness of all types of agricultural activity and farm viability; promotion of food chain organisation and risk management in agriculture; promotion of resource efficiency and supporting the shift towards low-carbon and climate resilient economy in the agriculture and food sectors, supporting a balanced territorial development of rural areas (Support for Rural Development..., 2011). A long-term perspective is needed in order to follow the scientific research and experimental development guidelines until 2020. Implementation of these targets depends heavily on focused cooperation between national business, research institutions and public authorities.

The Europe 2020 Strategy points out that besides other campaigns, the priority topic 'Innovation Union' needs to be promoted, i.e. better conditions for implementation and funding of scientific research and innovations are required to ensure that innovative ideas turn into goods and services that foster growth and job creation (The European Innovation Partnership..., 2012). The EU initiative 'Innovation Union' has set out the following objectives: strengthening the European knowledge base and reducing fragmentation to promote excellence in education and skills development; getting new ideas to market; eliminating social and territorial differences by adopting smart specialisation approach and increasing social benefit; pooling forces to achieve breakthroughs (Europe 2020 Flagship Initiative..., 2010b). By implementing these tasks, the benefits will be significant: according to recent estimates, achieving EU target of spending

3% of EU gross domestic product (GDP) on scientific research and experimental development by 2020 could create 3.7 million jobs and increase annual GDP by close to €800 billion by 2025 (Zagamé, 2010).

The EU project 'Facing sustainability: new relationships between rural areas and agriculture in Europe' (RURAGRI) has announced the Strategic Research Agenda defining scientific priorities with the focus on ecosystems, land use, farming practices, development of new, innovative, high added value products (RURAGRI..., 2012; Levidow, 2012). The need for sustainable planning and use of natural resources has been emphasized by the Agenda 21 Action Plan for Sustainable Development adopted by the Food and Agriculture Organization (FAO) of the United Nations at the United Nations Conference on Environment and Development in Rio de Janerio (Agenda 21..., 1992).

Horizon 2020 – the EU's new Framework Programme for Research and Innovation sets out the following main objectives: strengthening Europe's global position as the leader in science, eliminating obstacles for bringing innovations to market, and fundamentally changing cooperation between public and private sectors by introduction of innovations (Horizon 2020..., 2011). The Programme emphasizes novel and challenging ideas that can lead to scientific breakthrough and, in particular, interdisciplinarity – both local and regional – that could promote mobility, comprehensive use of available scientific resources, and creation of common European Research Area.

Lithuania cannot avoid the effects of global changes. Lithuania's Progress Strategy 'Lithuania 2030' points out that the country's wellbeing and development are based on national security, which must be ensured to achieve sustainable national progress (The State Progress Strategy 'Lithuania 2030'..., 2012). The Strategy further states that we need to develop technologies that would minimize adverse environmental impact and secure resource-friendly sustainable growth. Education of environmentally benign business culture and promotion of development of green economy, introduction of advanced technologies for reduction of pollution and climate change are needed.

Lithuanian Rural Development Programme 2014 – 2020 guidelines outline the main objectives to improve competitiveness of agriculture by promoting innovation, cooperation and restructuring and by creating conditions for more efficient use of resources in agricultural sector (Lietuvos kaimo plėtros 2014 – 2020 metų politikos gairės..., 2011). Sustainable territorial development in all rural areas must be secured by providing more opportunities for local residents, increasing production capacities and improving local conditions and links between rural and urban areas. All these objectives require support from science in order to ensure that added value is created not only by resource development, but also by their rational use, as well as structured and adopted scientific novelties. One of the key objectives for rural development in the period 2014 – 2020 is fostering the efficient use of resources and supporting the shift to climate resilient low-carbon technology economy in agricultural sector (Lietuvos kaimo plėtros 2014 – 2020 metų programa…, 2014; Proposal for a Regulation of the European Parliament and of the Council on support for rural development…, 2013). The analysis of strengths of agriculture and rural development usually stresses the wide network of research institutions, while the analysis of weaknesses tends to focus on a lack of scientific research and experimental development, an insufficient level of innovations and inefficient application of innovations in practice (Lietuvos žemės ūkio ir kaimo plėtros po 2013 metų strateginės kryptys..., 2012; Lietuvos kaimo plėtros 2014 – 2020 metų programa…, 2014).

Foresight of the Lithuanian Research and Higher Education System 'Learning Lithuania 2030' states that for natural and technical sciences particularly important are the areas where the country has been accumulating its potential for decades, i.e. biomedical research, biotechnologies, nanotechnologies, and agricultural research (Foresight on the Lithuanian research..., 2012). The Lithuanian Innovation Strategy for the year 2010 – 2020 states that the basis of the Lithuanian economy is production of high added value products and services (Lithuanian innovation strategy..., 2010). As for agriculture, the document specifically notes the need for production of ecological agricultural and food products and cooperation with business, as well as active participation in creation of the European Research Area.

Strategic documents of the EU and Lithuania show that research supporting a sustainable production of agricultural and food products and cleaner environment is needed to achieve the set targets and challenges. Besides global fundamental research, there is a particular need for applied research and innovations. Leading research knowledge and technologies must be made available to farmers, agricultural enterprises and advisory services. The aim of the research was to prepare proposals for the Programme that determine the scientific research and experimental development priorities, long- and short-term research programmes to 2020 in the view of demands of economy.

Materials and Methods

Individual WG by agriculture and food areas have been formed of specialists from Lithuanian research and educational institutions as well as the advisory services on the basis of the above review of strategic

documents and analysis of previously applied research. These WG have proposed prospective applied research to develop within different areas of agriculture in 2014 – 2020. This served as the basis for the survey aimed at identifying general demand for applied scientific research and potential problem areas in relation to agriculture, food economy and rural development that could be addressed by prospective applied scientific research. Another aim of the survey was to include potential consumers of the research results into decision making process, thus encouraging them for closer cooperation in future.

The survey was anonymous and was carried out during the period from April 11, 2013 to May 19, 2013 across the country. The questionnaire of the survey comprised two sections: a general section – data about the respondents, and special section – questions on individual aspects of applied research.

863 respondents participated in the survey; however, a share of questionnaires was rejected (as incomplete, non-validated). 489 respondents, 393 of whom were engaged in an agricultural activity, filled out the questionnaire properly. Summary analysis was carried out based on the information provided by the respondents.

Representatives of the Chamber of Agriculture of the Republic of Lithuania and agriculture- and rural development-related associations (33), consultants at the Lithuanian Agricultural Advisory Service, and farmers were chosen as respondents for the survey.

Due to the diversity of areas in applied scientific research, a single article cannot accommodate all aspects. For this reason, summary results of the survey have been presented for two areas: crop management and agricultural engineering. Special section questions on crop management were answered by 317 respondents, agricultural engineering – 155 respondents.

Distribution of respondents by the activities they were engaged in was determined by analysis of the general section of respondents' questionnaires (Figure 1). The majority of the respondents (78%) were engaged in farming, 61% of whom were farmers on their own farm.

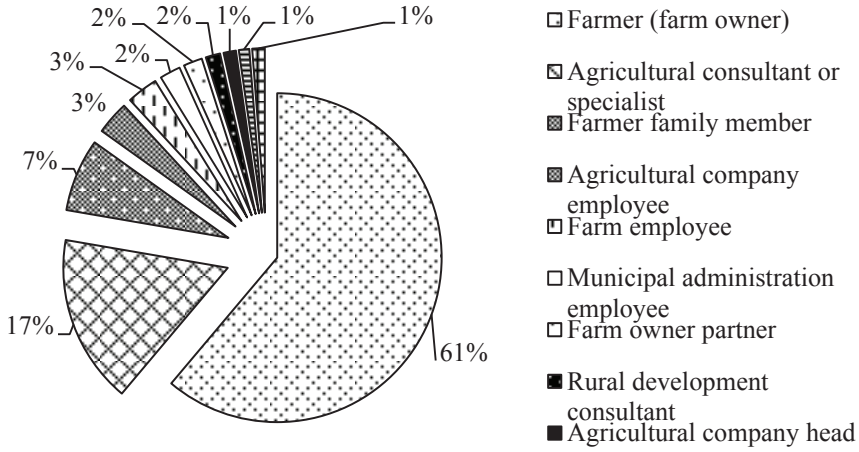

Figure 1. Distribution of respondents by the activities.

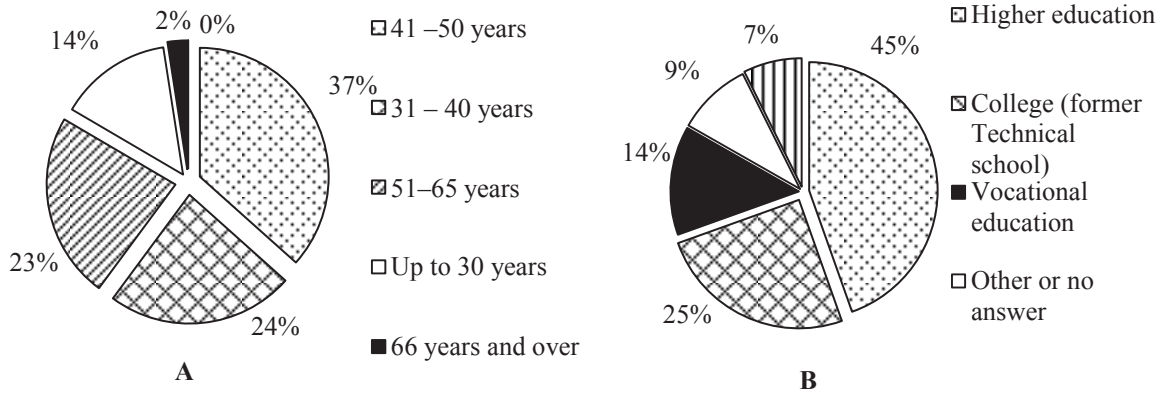

Figure 2. Distribution of respondents by age (A) and education (B).

Distribution of respondents by age and education is presented in Figure 2. The majority of the respondents were at the age of 41 to 50. About 23 – 24% of the respondents were at the age of 31 to 40 and 51 to 65.

With modernisation of agriculture, introduction of new crop management and smart agricultural engineering technologies, farmers' educational background has become particularly important. Most of the respondents (45%) indicated that they had a completed higher education. 25% of the respondents had a completed college education. 9% of the respondents neither clearly identified their educational background from the list of available options, nor responded to the question, or chose 'Other' and provided details (e.g. an incomplete higher education, basic education etc.). All responses by the survey respondents indicated that 54% of the respondents were males, 38% – females, while 8% of the respondents did not indicate their gender.

Results and Discussion

The EU has put great emphasis on agriculture in the time period until 2020, including crop management, as crop production by agricultural enterprises form the basis for processing industry and ensures national supply of food products. In light of the current situation in national agricultural sector of crop management, the anticipated development of production, internal market and export, crop structure could be expected to remain similar to the current structure regarding the time period until 2020. Farms are expected to focus on wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), rape (*Brassica napus* L.) and sugar beet (*Beta vulgaris* L.) cultivation. With the growing awareness among population of the country and prospective export regions on healthy nutrition, oat (*Avena sativa* L.) and protein crops consumption will exceed current levels, and such non-traditional crops as amaranths (*Amaranthus spp.*), lentils (*Lens esculenta* Moench) and less popular buckwheat (*Fagopyrum esculentum* Moench) might become in greater demand. The demand for precise parameters for raw material as well as wheat, barley and potatoes (*Solanum tuberosum* L.) cultivated for processing will be growing. Greater importance will be placed on qualitative characteristics of raw material both for food and feed or unprocessed food purposes. Cutting of production costs, higher crop yield, precision farming technologies will be needed as the farms will continue to concentrate and modernize. Considering the increasing health and environmental requirements, natural resistance of crops to harmful organisms and maximum consumption safety meaning minimal use of chemical substances will be of greater concern. Among other aspects, the notion of food safety also involves stability of national annual crop production by avoiding losses caused by adverse growing conditions. Prudent use of natural resource, possibility for crop growers and the country to receive greater benefit using fewer resources in light of growing potential biocapacity and by increasing potential biocapacity, as well as national development of competitive plant species will always remain on the agenda.

The WG have identified prospective fields of applied research by 2020 in the following areas of crop management: agrotechnology (soil preparation, crop care, identification and improvement of soil quality, crop rotation and other issues); crop protection (developmental biology of plant diseases and pests, use of chemicals and other measures, and other issues relevant to crop protection); crop nutrition (specifics of plant nutrition using macro- and micronutrients, optimization of use of mineral and organic fertilisers, nutritional balance); grassland management (use of grass plants for feed production, bio-energy, planting, establishment and use of grazing and pastures, perennial grass crop growing for seeds); seed production and plant breeding (development of crop species adapted to local conditions and special market demands, research on improvement of seed matter); plant raw material quality (research and improvement of nutritional and feed value of plants, determination of chemical composition and technological parameters of plants).

Considering the perspectives of agriculture in Europe and Lithuania, modernisation of agricultural engineering systems, robotisation of agricultural equipment in crop management, husbandry, horticultural and food processing sectors, new demands for engineering and research in agriculture will emerge. One of the possible research programmes is environmentally friendly and resource-conserving innovations in agricultural engineering. Such a programme would be aimed at developing and introducing engineering solutions into agriculture that would minimize the use of natural resources, mitigate adverse effects on humans, animals, environment and climate change, ensure efficient and safe production, processing, storage and use of agricultural products. Renewable energy innovations in agriculture could be included into another research programme.

The WG on agricultural engineering has estimated that in the coming funding period 2014 – 2020, it would be reasonable to develop applied research in the following areas: crop production engineering (engineering issues related to tillage, sowing, planting, crop care, harvesting and other machinery, cutting man-hour and energy costs); feed preparation engineering (cutting, milling, pressing, loading, transportation, drying, storage and other engineering issues, cutting man-hour and energy costs); husbandry engineering (low-waste production, cutting pollution and

energy costs, organic waste collection and recovery, automation/robotisation, creation of favourable conditions for livestock, control and management of technological processes, and other engineering issues); precision farming engineering (automated vehicle driving; installation of the satellite system in tillage, sowing, fertilisation, spraying, harvesting machinery; ISOBUS system (international standard for electronic communication between implements, tractors and computers); soil, fertilization and spraying maps and other related issues); engineering of biomass preparation for energy production (energy crops growing, harvesting, milling, drying, storage, pelletizing, briquetting, transportation, combustion, and other engineering issues); operation and servicing of agricultural machinery (cutting operating costs, increasing service life, development and installation of lubrication systems, mitigating adverse effects by tractors, combine harvesters and other machinery on humans and environment).

Survey respondents were asked questions related to agriculture and applied research. In their responses to the question 'Do you take interest in information related to agriculture?', 71% of the respondents answered that they took regular interest (Figure 3). In case of specific question 'Do you take interest in applied research?', 23% of the respondents answered that they took regular interest in applied research. However, more respondents answered that their interest in applied research was of general nature, i.e. they took occasional interest or when needed only.

32% of the respondents indicated that innovations were introduced into their farms, i.e. new modern agricultural equipment was purchased; buildings were reconstructed; agricultural technologies or their parts were replaced and improved; new species of crops and livestock were purchased; new crop protection and fertilisation products were used; innovative management, disposal of the production and other methods were applied.

Respondents were asked what innovations were introduced into the farm and what obstacles to introduction of innovations were faced (Figure 4). Innovations were classified under four headings: product innovations – development, production or use of new goods and/or services; technological innovations – development or application of new technologies in various areas of economic activity;

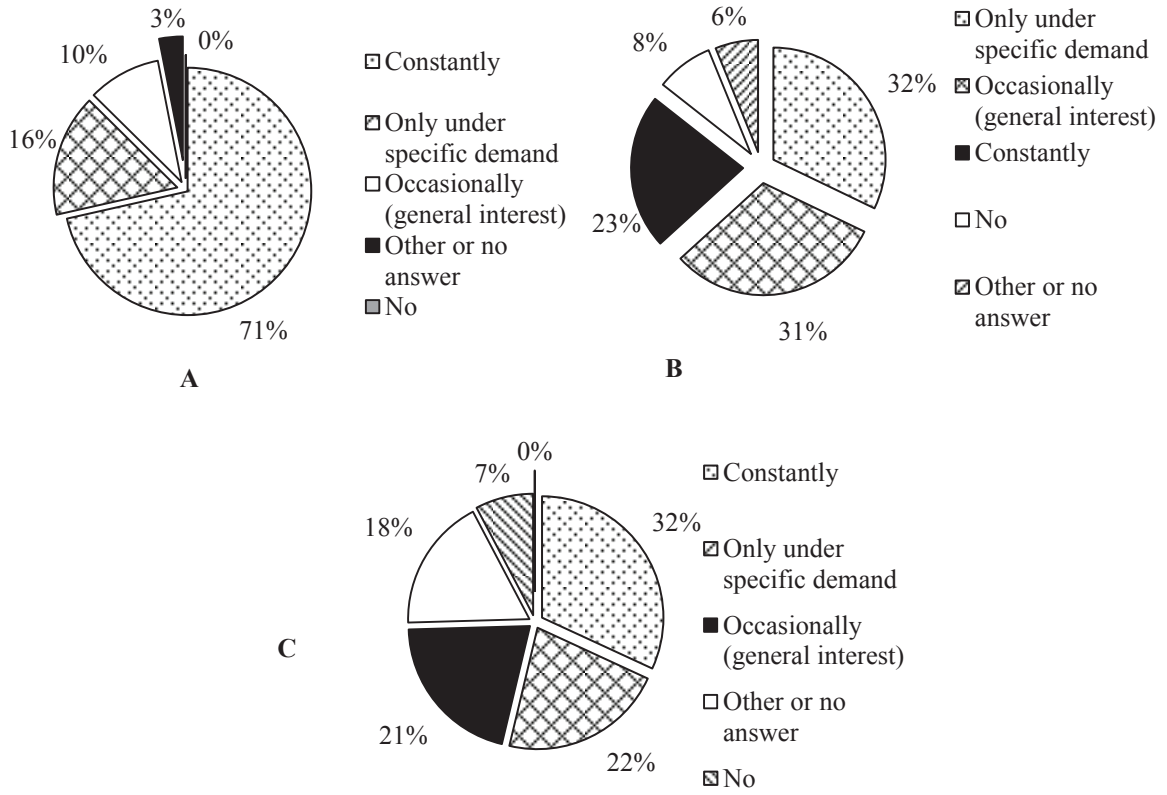

Figure 3. Respondents' answers to questions 'Do you take interest in information related to agriculture?' (A); 'Do you take interest in applied research?' (B) and 'Does your farm, agricultural company (enterprise) apply novelties (innovations)?' (C).

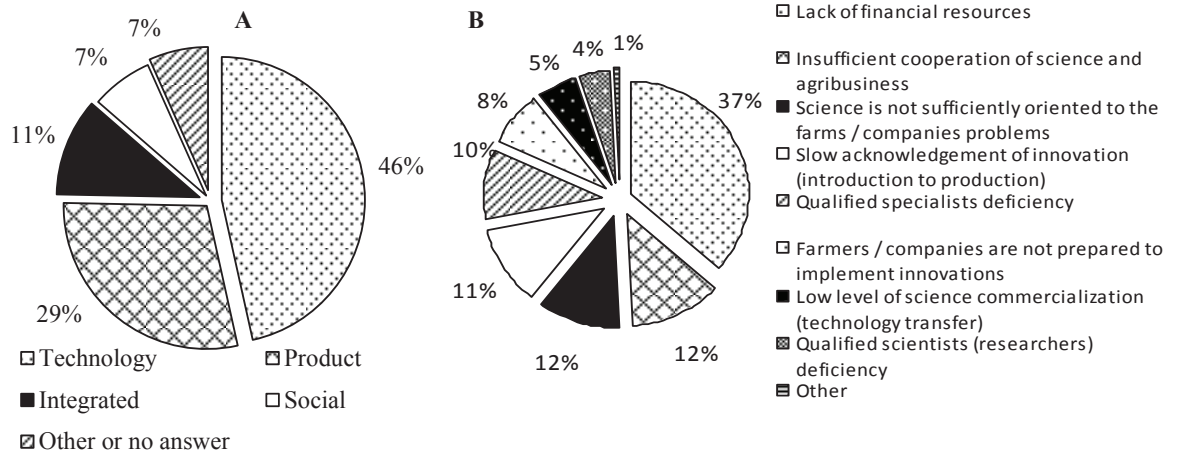

Figure 4. Introduction of innovations into respondents' farms (A) and obstacles to introduction of innovations (B) in a farm or rural area.

social innovations – development and introduction of new management, organisational and other structures and forms in various areas of economic activity; integrated innovations (a set of product, technological and social innovations) – a complex of product, technological and social innovations. Most of the respondents (46%) indicated that their farms adopted technological innovations, 29% – product innovations.

The survey demonstrated that the main obstacle (37%) to introduction of innovations into a farm or rural area was the lack of financial resources. Some of the respondents indicated insufficient cooperation between science and agricultural business (12%), insufficient focus of science on solution of specific problems of farms and enterprises (12%), slow acknowledgement (introduction to production) of innovations (11%), and lack of qualified specialists (10%). 8% of the respondents indicated that farmers/ enterprises were not ready for innovations.

The results of the survey (Table 1) support the demand for applied research and innovations in crop management and agricultural engineering. 68 – 85% of the respondents agreed fully and 11 – 21% agreed partially to the idea that applied research was needed

Table 1

Summary of results supporting the demand for applied research and innovations in crop management and agricultural engineering

Areas and topics	Agree		Partly agree		Disagree		Total number of respondents
	num-ber	%	num-ber	%	num-ber	%	
CROP MANAGEMENT							
Crop protection	263	85	33	11	13	4	309
Agrotechnology	254	81	44	14	15	5	313
Crop nutrition	239	78	53	18	13	4	305
Seed production and plant breeding	222	74	51	17	29	9	302
Plant raw material quality	201	68	63	21	34	11	298
Grassland management	201	68	55	19	42	13	298
AGRICULTURAL ENGINEERING							
Crop production engineering	120	79	21	14	10	7	151
Operation and servicing of agricultural machinery	108	75	26	18	10	7	144
Feed preparation engineering	102	75	21	16	13	9	136
Precision farming engineering	107	74	27	19	10	7	144
Husbandry engineering	99	72	26	19	13	9	138
Engineering of biomass preparation for energy production	96	69	26	19	16	12	138

in separate areas of crop management, whereas 4 – 13% of the respondents did not see any necessity in such research. The majority of respondents (over 80%) indicated that the areas of crop protection and agrotechnology needed applied research most.

The survey showed that 69 – 79% of the respondents agreed that applied research in various areas of agricultural engineering was needed, while 14 – 19% of the respondents agreed partially, and 7 – 12% did not agree to the idea. The majority of the respondents (79%) agreed that applied research was needed in the area of crop production engineering. Only a minor share of the respondents (7%) believed that research in precision farming engineering was not necessary.

Conclusions

Implementation of strategic provisions of the EU and Lithuania requires adequate identification of the priorities of agricultural scientific research and experimental development. It heavily depends on focused cooperation between national business, research institutions and public authorities. Besides global fundamental research, there is a particular need for applied research and innovations that would be adopted by agricultural enterprises and farms.

Based on the review of strategic documents and analysis and synthesis of previously applied research and survey were representatives of the Chamber of Agriculture of the Republic of Lithuania and agriculture- and rural development-related associations, consultants at the Lithuanian Agricultural Advisory Service, and farmers were chosen as respondents, prospective fields of applied research for the period until 2020 in Lithuania in the following areas of crop management and agricultural engineering have been identified.

Topics identified in crop management: agrotechnology, crop protection, crop nutrition, grassland management, seed production and plant breeding, plant raw material quality. 68 – 85% of the respondents agreed to the need for applied research in individual areas of crop management. The majority of the respondents (over 80%) indicated that the areas of crop protection and agrotechnology needed applied research most.

Topics identified in agricultural engineering: crop production engineering, feed preparation engineering, husbandry engineering, precision farming engineering, engineering of biomass preparation for energy production, operation and servicing of agricultural machinery. The survey showed that 69 – 79% of the respondents agreed that applied research in various areas of agricultural engineering was needed. The majority of the respondents (79%) agreed that applied research was needed in the area of crop production engineering.

Acknowledgements

The paper presents research findings, which have been funded by The Ministry of Agriculture of the Republic of Lithuania under the project ‘Scientific Recommendations on the Agricultural, Fisheries and Rural Development Applied Research and Innovation Development for 2014 – 2020’.

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INFLUENCE OF INTERCROP ON PLANT GROWTH AND YIELD

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Abstract
The demand for healthy and reasonably cheap food is growing and governments are expanding policies to preserve soil fertility and nature. In addition, climatic conditions are changing. Arable lands are decreasing. Due to all of these changes food growers are looking for new growing technologies. A monographic method to tackle these problems has been used in this article. Intercropping is one of growing systems how to reduce negative climatic aspects and meet other demands. Intercrop is convenient for growers who grow plants in rows, and a companion plant can be sown or planted between rows. In this case farmers can get two yields from one plot. Thus, growers do not need two plots for growing different plants. The intercrop diminishes spreading of pests and diseases, suppresses weed growth, and reduces need for pesticides. Legume (*Leguminosae*) intercrop gives extra nitrogen to companion plants. Those are aspects that we know intercrop can give, but we do not know how significant the influence is, what kind of influence on nature intercrops give in long term, what kind of influence they have on incomes, yield and its quality. This article shows that there are many intercropping systems to reduce some negative aspects and increase beneficial ones. Intercropping can reduce some pests and diseases, but promote other problems. Intercropping suppresses weeds. For some systems it gives bigger yield, for some smaller, but in total it gives bigger protein yield. There are still many unanswered questions and completely unexplained points.
Key words: companion plant, legume, growing system.

Introduction

It is well known that fertilizers are the most expensive compound in forming of crop prime cost. Nowadays fertilizer prices are increasing. Growers are interested in creating such technologies, which allow reducing of fertilizer usage. Consumers are more prone to choose healthier food. More and more pesticides are restricted from the usage. Due to these tendencies, growers are more open minded to nature friendly growing systems (LaMondia et al., 2002). Climatic conditions are also changing, and we are in need of new growing technologies that are more adaptable and sustainable. Intercropping is the cultivation of two or more crops simultaneously on the same field. It also means the growing of two or more crops on the same field with the planting of the second crop after the first one has completed its development. The rationale behind intercropping is that the different crops planted are unlikely to share the same insect pests and diseases-causing pathogens (infection agents (*Colloquially*)) and to conserve the soil (http://www.oisat.org/control_methods/ cultural__practices/ intercropping.html: found 13.03.2014). It can be one of the solutions to some of these problems. It is known that legumes can fixate atmospheric nitrogen. That could reduce nitrogen fertilizer usage. As mentioned in the definition of intercrop, it can reduce pests and diseases. These kinds of growing systems have been known for centuries, but they are not totally explored. In the beginning of 20th century, the most popular intercropping systems included legumes. As synthetic mineral fertilizers became more popular and cheaper, plantations with legume intercropping decreased severely. Only organic farmers used it (Jensen et al., 2010). Nowadays it again becomes more popular due to increasing costs of mineral fertilizers (Mahieu et al., 2009) and the government policy to preserve soil fertility. There are still a lot of uncertainties regarding intercrop. For example, it is not clearly known what kind of influence on pests and diseases intercrop exactly makes.

The aim of this paper is to explore literature about intercropping systems in general and see what has been done to explore their benefits and reduce negative sides.

Materials and Methods

Monographic method has been used for this article. Information all around the world from journals published by Elsevier, like – Field Crop Research, Agricultural and Forest Meteorology, Agriculture, Ecosystems and Environment, Crop Protection, Agricultural systems, Acta Agriculturae Scandinavica, Section B – Soil and Plant Science, European Journal of Agronomy, Soil Biology and Biochemistry, Journal of Agricultural Science, Ecological Engineering was collected.

Results and Discussion

The most widespread intercropping systems

The most widely spread intercropping system is where cereals (*Poaceae*) and legumes are grown. Mainly used for increased protein content in yield, nitrogen fixation is used to reduce fertilizer usage. As one of the main profits for intercropping with legumes, is their capability to give fixated atmospheric

nitrogen to component plant in system and to post crop (Jensen et al., 2010). However, the assessment tool for calculating fixated nitrogen is complex and expensive (Reining, 2005). It is still unexplored field how exactly legumes influence the fertilizer usage. Cereal growers, orchard farms and every single other farmer who is interested in preserving their money and nature are using these systems in hope that they are beneficial.

There are also other popular intercropping systems like wheat (*Triticum spp.*) and cotton (*Gossypium spp.*) in China (Zhang et al., 2008). In Sri Lanka the government explores policy to promote growers use intercropping system in growing of rubber trees (*Heveinae brasiliensis*) and tea (*Camellia sinensis*). In this system with rubber tree and tea, the need for labour is bigger and they need to be more skilled, that increases yield cost (Iqbal et al., 2006). There are many other intercrop systems in the world, and it is impossible to summarize all combinations. Some of intercrop systems and research done are shown in Table.

Intercrop influence on plant growth

As previously mentioned, the most popular intercropping system is grain with legume. It is stated by some scientists that this kind of system improves health of planting that enhances plant growth. According to research results, it reduces aphids for both plants and reduces spreading of diseases (Hooks and Johnson, 2003).

In systems where both plants are coalescent plants or trees and bushes, there can be problems with shading effect. It increases risk for mildews, rots and other diseases which have favourable growing conditions in increased moisture and decreased aridity. Shading can also lead to thinner stems, increased height which can lead to lodge (Yang et al., 2014).

Intercrop can suppress weeds (Oswald et al., 2002; Midega et al., 2013) that reduce the competition between cultivated plants and weeds for water and nutrition and succeed the growth of cultivated plants.

The availability of nutrients are playing very important role in plant development. Intercropping systems with legumes can supply with nitrogen not

Table 1

Intercropping systems evaluated and main aims of the research

Component crop 1	Component crop 2	Research purpose	Country	References
Triticum spp.	*Pisum sativum*	Intercrop functionality	France	Bedoussac, Justes, 2011
Vitis vinifera	Spring barley (*Hordeum vulgare* L.), *Festuca arundinacea* mixed with perennial *Lolium spp.*	Crop production, environmental impacts	France	Ripoche et al., 2010
Avena sativa	*Pisum sativum*	Optimal intercrop composition, competition, best model for evaluating of field data	Germany	Neumann et al., 2009
Zea mays	*Pisum sativum*	Yield advantage, water usage efficiency.	China	Mao et al., 2012
	Vigna unguiculata	Water and nutrition usage in acidic soils	France, Germany	de Barros et al., 2007
		Crop competition for minerals, intercrop advantages water usage, residual influence on the following crop	India	Aggarwal, Sidhu, 1988
	Arachis hypogaea	Radiation interception by intercropped plants, its usage in yield formation	Japan	Awal et al., 2006
	Vicia faba	Radiation interception and usage	South Africa	Tsubo, Walker, 2002
Pennisetum glaucum	*Arachis hypogaea*	Radiation interception and usage in growth	Great Britain, India	Marshall, Willey, 1983
Sorghum × *drummondi*	*Arachis hypogaea*	Yield advantages exposed to drought	Great Britain, India	Harris et al., 1987

only a companion plant but also post crop (Haynes et al., 1993; Kumar, Goh, 2000; Pappa et al., 2012). Intercropping is also used in orchards, where between rows there is grass with legumes, mostly clover (*Trifolium spp.*) grown (Jiao et al., 2013). This system gives not only nitrogen but also helps to maintain clean machinery. For this system it is important that grass is highly transport durable (García and Miñarro, 2014).

Intercrop influence on yield

Intercrop influence on yield has proven to give different effects. The decrease in yield for about 20% compared to solo crop in intercropping system with soybean (*Glycina max*), maize and sunflower (*Helianthus annuus*) (Coll et al., 2012) has been observed. However, the water usage efficiency and productivity, radiation use efficiency was increased in intercrop. On contradiction, in other experiment barley intercropped with clover showed significantly higher grain yield than growing alone (Pappa et al., 2012). In the experiment carried out in China by L. Mao et al. (2012), where intercropping of maize with pea was evaluated, the yields of both crops in intercropping system were smaller than in solo crops. However, the intercropping increased land and water use efficiency. With the usage of film covers over the field, where distance between maize and pea rows was 0.40 m, they gained almost the same maize yield as in integrated growing system, and additionally pea yield, which can increase farmer income. In experiment carried out in France in 15 institutions by E. Palzer et al. (2012), using intercropping system of peas with wheat gave almost the same yield as integrated growing of solo crop and additionally pea crop.

Intercropping that is used in orchards, where between rows there is grass (mostly clover) with legumes, grown, pollinator amount increases in orchards. Subsequently, there is better flower pollination and higher yield (García and Miñarro, 2014).

The most positive thing is that in intercropping system it is possible to get two separate yields form one plot and economically use the land resources, as, for example, in systems with rubber tree and tea bushes (Iqbal et al., 2006). Intercrop can also increase total protein yield if grown with cereals (Lithourgidis, 2011). Farmers can get two yields in one year, but it also increases growing costs and complicates cropping.

Some controversial aspects of intercropping

There are many opinions on how big benefits of intercropping there really are. There can be problems with shading effect. Competition for nutrition is also a problem (Fukai and Trenbath, 1993; Reynolds et al., 2007). It may take bigger investments in mineral fertilizers. To use all benefits of intercropping, crops need to be in similar needs of fertilizers, soil pH reaction. The yielding times need to be the same or with big gap between them, that harvesting of one doesn't bother harvesting of other plant or is harvested at the same time. For these kinds of systems special machinery and skilled labour is needed. These aspects increase cost of yields, but farmers are not interested in increase of costs. Scientists should find the most optimal growing system (Mucheru-Muna et al., 2010).

Conclusions

Intercropping is becoming more popular. There are many combinations practiced worldwide. Main reasons for using this system is to diversify agro system, suppress weeds, possibility to reduce diseases and pests, increase income, improve land use, improve water, radiation usage in order to get healthier food, succeed in environmentally friendly growing systems. Scientists are coming closer to create best intercropping systems for these days' demands and weather conditions, but there are still many issues on which they have to work. Further research on nitrogen cycling and legume influence on it is needed, relationship between companion plants and economic aspects of intercropping to make it more acceptable for growers should be explored.

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PSEUDOMONAS SYRINGAE AS IMPORTANT PATHOGEN OF FRUIT TREES WITH EMPHASIS ON PLUM AND CHERRY

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Abstract
The aim of this article was to provide an overview on the current status of fruit tree diseases caused by *Pseudomonas syringae*, their importance and distribution, epidemiology and control possibilities with emphasis on plums and cherry. The plant diseases caused by *Pseudomonas syringae* are economically important and occur worldwide on large diversity of plants. On stone fruits, diseases caused by different pathovars of *Pseudomonas syringae* are economically important in major fruit growing regions. The severity of damages and economic importance depends on the geographical region, host plant species and pathovar of *P. syringae* involved in the disease. Bacterial canker induced by *P. syringae* pv. *syringae* on all commercially grown stone fruit species and by pv. *morsprunorum* predominantly on cherries and plums is considered to be the most serious one. Bacterial decline caused by *P. syringae* pv. *persica*e is severe on nectarine and peach. Despite the wide spread and economic importance in the most stone fruit growing areas the diseases caused by *Pseudomonas syringae* in some areas, e.g. Baltic States, are poorly studied, and the data on distribution and pathovars involved in the diseases observed are still missing.
Key words: *Pseudomonas syringae* pv. *syringae*, pv. *morsprunorum*, stone fruits, pathovars, canker.

Introduction

The plant diseases caused by *Pseudomonas* bacteria are economically important and occur worldwide on large diversity of plants. Among plant pathogenic *Pseudomonas*, *P. syringae* can cause diseases in more than 180 plant species including annual and perennial plants, fruit trees, ornamentals and vegetables (Little et al., 1998; Agrios, 2005). The phytopatogenic pseudomonads cause plant diseases with different symptoms, including cankers, diebacks, blossom, twig, leaf or kernel blights, leaf spots (*P. syringae* different pathovars), soft and brown rots (*P. viridiflava*, *P. marginalis* different pathovars), tumors or galls (*P. savastanoi* pathovars), mushroom blights (*P. tolasii*, *P. agarici* pathovars) (Braun-Kiewnick and Sands, 2001). Among plant pathogenic *Pseudomonas* 14 species are well known, of which *Pseudomonas syringae* is the most economically important with many pathovars (Braun-Kiewnick and Sands, 2001). According to J.M. Young et al. (2001) definition, the term *pathovar* is used to refer to a strain or set of strains with the same or similar characteristics, differentiated at infrasubspecific level from other strains of the same species or subspecies on the basis of distinctive pathogenicity to one or more plant hosts whereas in other sources it is pointed out that pathotype or pathovar is a subdivision of a species distinguished by common characters of pathogenicity, particularly in relation to host range. In bacteriology, where pathovar is the preferred and more used term, pathotype is used to describe the type (or reference) culture of a pathovar (Waller et al., 2002). Further in this article we use the term 'pathovar'.

An exact number of the *Pseudomonas syringae* pathovars is not defined. In 1994, about 40 pathovars were recognized (Young and Triggs, 1994). Later, the number of defined pathovars was increased to more than 50 (Braun-Kiewnick and Sands, 2001; Höfte and De Vos, 2006; Young, 2010). Among fluorescent *Pseudomonas* species, *P. marginalis*, *P. savastanoi* and *P. syringae*, contain several pathovars, which are defined based on pathogenicity to host plant species and biochemical properties (Young et al., 1996; Braun-Kiewnick and Sands, 2001).

The aim of this article is to provide an overview on current status of fruit tree diseases caused by *Pseudomonas syringae*, their importance and distribution, epidemiology and control possibilities with emphasis on cherry and plums.

Materials and Methods

Monographic method has been used for this research. As the research on *Pseudomonas* pathogens and present situation in Latvia is quite new, available scientific literature from other countries, basically from Europe has been used.

Results and Discussion

The pathogens and pathovars

The studies on bacterial canker started Brzezinski (Bultreys and Kaluzna, 2010) in Poland. He determined that the gummosis and dieback of peach, plum, apricot and sweet cherry trees were of bacterial origin. At the same time in Holland, van Hall (1902) described the causal agent of lilac bacterial blight as *Pseudomonas syringae*. Several years later, Aderhold and Ruhland described *Bacillus spongiosus* as the causal agent of death of sweet cherry in Germany. Later Griffin proved that gummosis and cankers on sweet cherries in Oregon (USA) were caused by *Pseudomonas cerasi*

and scientist Barrs confirmed the pathogenicity of this organism on various organs. In England, H. Wormald described the bacterium *Pseudomonas morsprunorum* as the causal agent of bacterial canker of plum trees. Also, he found out that *Pseudomonas prunicola* was associated with bacterial canker of stone fruits and blossom blight of pear (Bultreys and Kaluzna, 2010).

As summarized by Schaad et al. (2000), the term 'pathovar' or 'pathotype' emerged to conserve the name of the pathogens and proposals to rename several pathovars of *Pseudomonas* and *Xanthomonas* as species have caused great confusion in the literature. Pathovars of *P. syringae* vary in their capacity for epiphytic survival, the nature and severity of the symptoms they cause and their host range (Kerkoud et al., 2000; Kerkoud et al., 2002; Lindeberg et al., 2006). *P. syringae* at the beginning was isolated from diseased lilac (*Syringa vulgaris* L.) and characterized by M. Beijerinck in 1899 (Hirano and Upper, 2000). Bacteria with similar characteristics were isolated from a variety of tissues and a vast number of host plants, and following this involved pathogens were classified as separate species (Hirano and Upper, 2000). When count of the hosts and bacteria became more than 40, all these bacteria were classified as one species *P. syringae* (Hirano and Upper, 2000).

Bacterial canker of stone fruits is caused by two *Pseudomonas syringae* pathovars *P. syringae* pv. *syringae* van Hall and *P. syringae* pv. *morsprunorum* (Wormald) Young. *P. syringae* pv. *syringae* can cause canker on any commercially grown fruit species while *P. syringae* pv. *morsprunorum* (Wormald) Young infect predominantly sweet and sour cherries and plums. The behaviour of both pathovars in host tissue is similar.

Some authors highlight that bacterial canker of stone fruits is caused mostly by *P. syringae* pv. *syringae* (predominantly on sour cherries) and *P. syringae* pv. *morsprunorum* (predominantly on sweet cherries and its subspecies) (Young, 1991; Gardan et al., 1999; Kałużna et al., 2009). In the fruit growing *P. syringae* is an important pathogen also on pears and causes pear blast that limit pear production throughout the world (Moragrega et al., 1998; Moragrega et al., 2003).

Diversity of the *P. syringae* pathovars is rather complicated. *P. syringae* pv. *morsprunorum* race 1 is characterized and separated in 4 strains, *P. syringae* pv. *morsprunorum* race 2 is characterized and separated in 7 strains (Endert and Ritchie, 1984; Gardan et al., 1999). Most of the *P. syringae* pv. *syringae* strains produce the toxic lipodepsipeptides syringomycins and syringopeptins and molecular tests based on this characteristic can be used for the diagnosis of the pathovar (Bereswill, 1994; Gilbert et al., 2009). *P. syringae* pv. *syringae* is genetically heterogeneous pathovar with a wide host range. This pathovar shows different virulence on the lilacs, sweet cherries and plums (Gilbert et al., 2009). Phytotoxins of the pathogen can be host-specific, but usually phytotoxins produced by *P. syringae* are nonhost specific and cause symptoms on many plant species, which cannot be infected by the toxin-producing pathogen (Bender, 1999).

P. syringae pv. *persicae* was proved to be pathogenic on nectarine and peach, and also as weak pathogen on Japanese and European plum, but it is not pathogenic on apricots and cherries (Young, 1987). Pathovar *P. syringae* pv. *persicae* (Prunier et al., 1985) is a quarantine organism in Europe (EPPO) and causes leaf spots, cankers, gummosis of fruits on peach in France and bacterial decline of peach, nectarine and Japanese plum in New Zealand (Hattingsh and Roos, 1995).

In France, 2003, it was found that causal agents of bacterial canker of stone fruits are not limited only to *P. syringae* pv. *morsprunorum* and *P. syringae* pv. *syringae*, and one more pathovar *P. syringae* pv. *avii* (pv. nov. Menard) should be distinguished, which causes bacterial canker on sweet cherries (Menard et al., 2003). However, this pathovar is not officially confirmed. As described by M. Menard et al. (2003) pv. *avii* is characterized by slow growth on King`s B medium producing colonies 1.0 – 1.5 mm in diameter after three days of incubation and not producing fluorescent pigment. Japanese scientists have also proposed to recognize one more pathovar of *P. syringae* – pv. *cerasicola* (pv. nov. Kamiunten), which induces galls on cherry and apricots, but it is not pathogenic on other stone fruit species (Kamiunten et al., 2000). So far this pathovar is not recorded outside Japan.

Each *P. syringae* pathovar usually has a strong biochemical, immunological and DNA relatedness with several other pathovars, including *P. syringae* pv. *syringae*, which can be isolated from many species of plants, e.g. apples, even if these are not pathogens for these plants (Kerkoud et al., 2002).

Distribution and importance

Bacterial canker of stone fruit occurs in all fruit growing areas in the world (Hattingsh and Roos, 1995). On sweet cherries, bacterial canker is widespread, and it is economically important disease in all regions in the world where sweet cherries are grown (Prunier and Cotta, 1985; Prunier et al., 1985; Bradbury, 1986). On the West coast of the USA, only *P. syringae* pv. *syringae* was detected on sweet cherry while in Michigan *P. syringae* pv. *syringae*, *P. syringae morsprunorum* race 1 and probably a third pathovar were responsible for disease outbreaks in sweet and sour cherries (Jones, 1971).

Bacterial canker on sweet cherries can be caused by *P. syringae* pv. *morsprunorum*, *P. viridiflava* or *P. syringae* pv. *syringae* (Prunier and Cotta, 1985; Menard et al., 2003). In the United Kingdom predominantly *P. syringae* pv. *morsprunorum* is considered to be a causal agent of bacterial canker while in the other European countries, South Africa and the USA both pathovars are considered to be a causal agent of canker on sweet and sour cherries (Lattore and Jones, 1979; Vicente and Roberts, 2003; Vicente et al., 2004). Only one of these pathovars is usually found as a causal agent of bacterial canker in the most of the countries, although distribution of both pathovars is reported in the some parts of the world (Wimalajeewa and Flett, 1985). *P. syringae* pv. *morsprunorum* has been recorded as the cause of bacterial canker in Europe, the USA, Canada, but in some countries, e.g., Chile, it has not been identified (Latorre et al., 2002).

Damages caused by pathovars of *P. syringae* vary depending on stone fruit growing region and host plants. Since 2005, a disease similar to bacterial canker of stone fruit trees was observed only in some areas of Iran while in several provinces in Turkey severe bacterial canker was observed on almost 80% of apricot trees in commercial orchards and home gardens (Kotan and Sahin, 2002; Karimi-Kurdistani and Harighi, 2008). Reports from France, the UK, Germany, Poland, New Zealand, Lithuania and other countries indicate bacterial canker occurrence over many years, including wild cherry plantations grown for wood production (Menard et al., 2003; Vasinauskiene et al., 2008). In Poland, due to the favourable climate, bacterial canker is observed every year. The last serious outbreak occurred in 2007, resulting in substantial economic losses, especially in sour cherry. Both pathovars and both races of *P. syringae* pv. *morsprunorum* have been isolated from infected trees (Bultreys and Kaluzna, 2010).

The available data on fruit tree bacterial diseases caused by *P. syringae* in Baltic States are limited. In Lithuania and similarly in Latvia, mostly fungal diseases have been considered important in orchards for a long time. Only recently surveys of stone fruit orchards for possible infection of *P. syringae* were started in Lithuania (Vasinauskiene et al., 2008). The research was focused on the occurrence of bacterial canker in stone fruit orchards, evaluation of disease symptoms and preliminary diagnostics of the causal agents. The disease was recorded only on single trees and did not have economic importance. Pathogenicity tests with strains isolated from stone fruits and identification of pathovars and studies of the genetic diversity are still in progress (Vasinauskiene et al., 2008). Records on bacterial canker and its occurrence in Estonia were not found. In Latvia, commercially grown stone fruit species are plums, sweet and sour cherry. Peaches and apricots are grown only in home gardens or in varietal collections. Research on bacterial diseases of stone fruits, their distribution and causal agents in Latvia started in 2008 within the frame of COST Action Nr.873 and national research programs. The bacterial canker was not widely spread in stone fruit orchards, and *P. syringae* pv. *syringae* was detected as a cause of bacterial canker in sweet cherry and plums (Moročko-Bičevska et al., 2010; Konavko, 2011).

Symptoms caused by P. syringae and epidemiology

Depending on the symptoms and pathogens involved, diseases caused by *P.syringae* are known under various names, such as bacterial canker, bacterial decline, gummosis, blossom blast, dieback, spur blight, twig blight, bud bacteriosis (Braun-Kiewnick and Sands, 2001; Bultreys and Kaluzna, 2010). The studies show that the most important stone fruit diseases caused by *P. syringae* are bacterial canker and bacterial decline or dieback (Hattingsh and Roos, 1995). Symptoms of bacterial canker on trunks and branches occur predominantly on pears and sweet cherries, but also sour cherries, apricots, peaches, plums and in some years also apple trees suffer from bacterial canker (Prunier and Cotta, 1985; Prunier et al., 1985; Bradbury, 1986). As summarized by A. Bultreys and M. Kaluzna (2010), *P. syringae* basically can damage all areal parts of stone fruits. It causes cankers and necroses on trunks, branches, around spurs and in branch junctions. On leaves symptoms occur as small, rounded, light brown spots in various sizes, which become necrotic and drop off resembling 'shot hole' symptom. Blossoms turn brown and often fall before full blooming. On immature fruits, predominantly in cherry, symptoms are either regular or irregular, brown to black in colour, necrotic spots. *P. syringae* can cause canker also on sea buckthorn, but this disease is poorly studied (Janick and Paul, 2008).

Bacterial decline or dieback caused by *P. syringae* pv. *persicae* is a quarantine disease and affects nectarine, peaches, Japanese plums and Myrobalan plum (Young, 1995). In nectarine and peaches, symptoms include shoot dieback, limb and root injury, tree death, leaf spots and fruit lesions (Luisetti et al., 1976; Young, 1995). Distinctive characteristics of decline are staining of wood in branches above necrosis and the absence of obvious boundary between morbid and healthy bark in the lower tree parts (Luisetti et al., 1976; Young, 1995).

Besides causing cankers on trunks and branches of stone fruit trees *P. syringae* pv. *syringae* and pv. *morsprunorum* also infects leaves, shoots, flowers and fruits. In Europe, the disease is more common

on cherries and plums (Ephinstone et al., 2008). As summarized by J. Ephistone et al. (2008), cankers, which are not perennial, are formed in the late autumn and winter, but do not increase much in size till next spring, when they enlarge rapidly and kill large areas of green bark. When blossom fall occurs, the progress of cankers is stopped, and populations of these bacteria within the canker decline and often die out. During this time leaf infection phase occurs. Leaves of the spurs tend to be resistant as they mature, whereas young leaves on extension shoots are infected. Bacteria as epiphytes on all leaves in the summer reside till the leaf fall, being the main source of new infection (Ephinstone et al., 2008).

The diseases caused by *P. syringae* pathovars have a different epidemiology. As summarized by J. Ephistone et al. (2008), for *P. syringae* pv. *syringae* and pv. *morsprunorum*, cankers caused by these pathovars may be perennial and the bacteria as small populations overwinter in them. Fast multiplications of bacterial populations occur in the spring, bacterial ooze is often produced, and bacteria spread to leaves by the splash of the rain. Unlike *P. syringae* pv. *morsprunorum*, *P. syringae* pv. *syringae* gain to woody tissue only via wounds in scars of leaf and bark, from which new cankers arise further.

Symptoms vary between different host species. Cherry trees of all ages are susceptible, and most of the cankers are found at sites of leaf scars on fruiting spurs. This results in dieback of spur, but it can occasionally spread to form a canker in the parent branches. Cankers may also be located on the branches, mostly on the crotch and angles between branches. On younger branches with thin bark cankers at the beginning they are visible in the spring as shallow, discoloured sunken lesions often showing the presence of gummy exudates. On plums cankers occur mostly on trunks frequently leading to death of trees. Cankers can extend in length on the trunk and often appear like dark and linear depressions in the bark. Gumming on plums is less common and less obvious as on cherry. Spots on the leaves are caused by both pathovars and usually are reddish brown, rounded, sometimes angular, often coalescing to form big, irregular necrotic spots, which can drop out as a 'shot hole' effect (Ephinstone et al., 2008).

In spring, the first symptoms appear after late frosts, and this period is the most dangerous. Young leaves are the most susceptible. When they mature, infected areas become dry and fall out; the leaves then have a shot hole appearance and when they become mature, these leaves are not infected anymore (Süle and Seemüller, 1987). As summarized by M. J. Hattingsh and I. M. M. Roos (1995), terminal shoots or twigs of cankered trees can die back. If girdled by a canker, infected lateral branch or trunk dies during several weeks. The root system of diseased trees usually remains healthy, and suckers grow in the crown area. Pathogens can be present in dormant leaves and buds of flowers. Infected dormant buds are often killed, but some of the invaded buds normally open in the spring and then collapse in early summer. Leaves from buds like this wilt and fruits tend to dry out. Leaves and flowers arising from other diseased buds may remain symptomless. Leaf infections, mostly on cherry appear as water-soaked spots and those after that become brown, dry. After that, shot holes may be seen. Symptoms on the leaves occur sporadically and are not always typical of the disease. Flat, superficial, dark brown spots develop on the infected fruits, but lesions can be depressed in the fruit flesh, especially if cherry fruits are infected (Hattingsh and Roos, 1995). Bacterial blossom blight is attributed to *P. syringae* pv. *syringae* and pv. *morsprunorum*, and in most countries this is regarded as the cause of bacterial canker (Crosse, 1966).

Pathogenic bacteria may survive in the buds of infected trees (Roos and Hattingh, 1986). Dangerous source of infection are branches with infection, where from *Pseudomonas* bacteria spread further by a wind and rain (Hirano and Upper, 2000). *P. syringae* does not survive in the soil for a long time (Hirano and Upper, 2000). Most of the *P. syringae* pathovars are known to be epiphytes on their hosts. According to C. Leben (Leben, 1965, cited by Luisetti, 1996), these pathogenic bacteria are able to survive on the aerial parts of the plants even if conditions are not favourable, like high temperature or low humidity. In these unfavourable conditions, they can multiply and survive until environmental conditions become favourable again (e.g. moisture after rain, dew) (Luisetti, 1996).

Control possibilities

When fruit trees are already infected with *P. syringae* pathovars and express disease symptoms, it is impossible to treat them. Therefore, important control strategy is the use of preventive measures. The use of canker-free nursery stock is good and effective practice to reduce disease occurrence (Young, 1995). Selection of a suitable site and soil for the establishment of new orchards is an important step to prevent disease spread. Establishment of orchards under marginal soil and climatic conditions create a risk for development of the disease (Hattingsh and Roos, 1995; Young, 1995).

Other measures include selection of cultivars with partial resistance, avoidance from early winter pruning, application of fixed-copper sprays in autumn to reduce inoculum (Sundin et al., 1989; Young, 1995). Breeding for resistance is a slow process with woody trees, because of the time involved for tree growth

and the threat of *Pseudomonas syringae* to adapt genetically and infect the new germplasm. However, some successful control of *Pseudomonas syringae* has been realized using plant germplasm resistant to this pathogen (Moore, 1988). The cultivars of sweet cherry are not resistant (Moore, 1988). Cultivars differ in susceptibility, and some exhibit partial resistance, but some of them are immune (Luisetti et al., 1976; Young, 1995).

Copper sprays are aimed at reducing epiphytic populations of bacteria and are timed to correspond with 20% and 80% leaf drop and can be up to three late dormant applications (Wimalajeewa et al., 1991). Copper compounds are commonly used to minimize the distribution of disease in sweet cherry orchards, but these compounds have limited efficacy and may have also phytotoxic effects (Hibberd, 1988; cit. by Vicente et al., 2004). As noted by J. G. Vicente et al. (2004), the control of diseases caused by *P. syringae* pathovars is problematic in woodlands because it is not economical and practical to make sprays in woodland plantations. Therefore, the only practical approaches to control canker in woodlands are disease avoidance and use of resistant plants. However, both of these approaches are limited because of lack of sufficient amount of knowledge and understanding of the pathogens involved as well as lack of consistent methods for their detection and discrimination as also pointed by J. G. Vicente et al. (2004).

Conclusions

1. Bacterial diseases of stone fruits caused by different pathovars of *Pseudomonas syringae* are economically important in major fruit growing regions. The severity of damages and economic importance depends of the growing region, host species and pathovar involved.
2. Among stone fruit diseases caused by different *Pseudomonas syringae* pathovars as the most serious is considered bacterial canker caused by *P. syringae* pv. *syringae* on any stone fruit species and *P. syringae* pv. *morsprunorum* on predominantly sour and sweet cherries and plums, and bacterial decline caused by *P. syringae* pv. *persica*e on nectarine and peach.
3. Despite a wide spread and economic importance of diseases caused by *Pseudomonas syringae* in several stone fruit growing areas, in some areas, e.g. Baltic States, the data on distribution and *P. syringae* pathovars involved in the diseases observed are still missing.

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REVIEW OF THE PEAR SCAB CAUSED BY *VENTURIA PYRINA*

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Abstract
European pear scab (*Venturia pyrina* Aderh.) is common and economically important disease in commercial orchards in most of the pear (*Pyrus communis* L.) growing areas worldwide. Studies on pear scab pathogen *V. pyrina* diversity in general and among different geographical regions are scars at an early stage. In the limited number of studies reported so far, some attempts have been made to characterize and define races or biotypes of *V. pyrina* and new biotypes co-adaptated to specific cultivars have been recorded recently. Despite the long history, worldwide distribution and increasing economic significance of the European pear scab, the research on control methods, and pathogen biology and disease epidemiology that could aid to develop more effective and also alternative to fungicide application control methods are still limited. Mechanisms of European pear resistance to scab remains uncertain and currently only one major resistance gene *Rvp1* has been identified and characterised. Although the disease is important in many European countries, breeding programs of pear scab-resistant varieties are still under development. In this paper we provide an overview on *V. pyrina*, its significance and distribution, control methods and current scientific progress in understaning of the pathogen and the disease.
Overview of literature on *V. pyrina* characterization, ecology, biology and diseases epidemiology from Latvia and other countries has been used for the study.
Key words: *Pyrus communis*, races, fungal diseases, control, diversity.

Introduction

The European pear is one of the most widely grown pome fruit tree species with long cultivation history (Sánchez, 2005; Sharma et al., 2010). In Latvia, pear is a second common pome fruit crop grown commercially and in home gardens, although commercial pear plantations are limited in comparison to other pear producing countries (Skrīvele et al., 2008; Lācis et al., 2012). Emphasis on integrated and organic fruit production in Europe is increasing every year due to environmental and food safety concerns. The pear production, especially in the integrated and organic production systems, is constrained by diseases, which reduce the viability of plants, fruit development and quality. Changes in production technologies, introduction of new cultivars as well as climatic changes can alter the pathogen populations resulting in the development of new and more aggressive forms adapted to the changing environmental conditions. The European pear is infected with a range of fungal, bacterial and viral pathogens, whose significance varies depending on growing reagion and particular disease (Wood, 1997; Johnson, 2000). In Europe, and also in other parts of the world where European pear is cultivated, as most significant pear diseases are considered fire blight (*Erwinia amylovora*), pear scab (*Venturia pyrina*), blossom blight (*Pseudomonas syringae pv. syringae*), powdery mildew (*Podosphaera leucotricha*) and brown spot (*Stemphylium vesicularium*) (Shabi et al., 1981; Deckers and Schoofs, 2002; Deckers and Schoofs, 2005; Postman et al., 2005; Mizuno et al., 2010).

Scab on pear is economically important disease worldwide, especially in organic orhards (Shabi, 1990; Postman et al., 2005; Lespinasse et al., 2008; Spotts and Castagnoli, 2010; Bouvier et al., 2012). Severe damages can be caused in conditions conducive to the disease and on susceptible cultivars in all main growing regions (Stehmann et al., 2001). Despite the long history, worldwide distribution and increasing economic significance of *V. pyrina*, the research on control methods, and pathogen biology and disease epidemiology that could aid to develop more effective and also alternative to fungicide application control methods are still limited.

The aim of the article is to give an overview of *Venturia pyrina* significance and distribution, control methods and current scientific progress in understaning of the pathogen and the disease.

Materials and Methods

Monographic method has been used for this study. Available literature of pathogen characterization, ecology, biology and diseases epidemiology from Latvia and other countries has been used for the study.

Results and Discussion

Distribution and significance of the disease

European pear scab for a long time was not considered as a destructive disease and was controled efficently with fungicide applications and growing of tolerant cultivars; however, in some areas severe losses were noted already for more than 80 years ago that continued to increase over the time (Bearden et al., 1976; Spotts and Covey, 1990; Spotts and Cervantes, 1994). In Israel, the European pear scab for the first time was observed in the beginning of 1960[ies] and after

ten years due to pathogen resistance to fungicides and cultivar susceptibility (Shabi et al., 1972; Shabi and Katan, 1979; Shabi, 1989) severe losses already occurred. Nowadays, the European pear scab is common, and it is considered as an economically important disease in commercial orchards in most of the pear growing areas worldwide (Lattore et al., 1985; Shabi, 1990; Bakker, 1999; Pierantoni et al., 2007; Chevalier et al., 2008; Rossi et al., 2009; Spotts and Castagnoli, 2010; Bouvier et al., 2011). In Latvia, pear scab is considered as one of the most important fungal diseases affecting pear production (Eglītis et al., 1943; Lācis et al., 2012).

Pear scab is an especially serious problem in organic orchards causing severe crop losses (Timmermans et al., 2010; Sugar and Hilton, 2011). In the Netherlands, the scab incidence has increased only during the last few years in organic pear farms (Timmermans and Jansonius, 2012). Similarly, severe attacks of *V. pyrina* have been detected recently in French orchards overcoming resistance of pear cultivar 'Conference', which still is dominant cultivar in large areas in Western Europe (Chevalier et al., 2008).

The major losses caused by *V. pyrina* are due to scabbed fruits, which are not marketable. Because of the loss of commercial value, frequent use of fungicides is required (Bouvier et al., 2011). Sometimes due to the scab infection yield losses in pear orchards reached 40 – 80% (Kienholz, 1937; Liu et al., 2009). If appropriate control measures are not applied, in case of susceptible cultivars and condusive weather conditions yield losses can reach up to 100% (Shabi, 1990; Sugar and Hilton, 2011). The pear scab infects and causes disease also on ornamental pears planted in urban landscapes. *V. pyrina* causes also severe twig infections and repeated plant infections can lead to tree mortality in urban landscapes (Shabi, 1990; Percival and Noviss, 2010).

Characterization of the pathogen

The scab on European pear is caused by ascomycetous fungus *Venturia pyrina* Aderh. (Sivanesan and Waller, 1974). *V. pyrina* has been classified to *Pezizomycotina* subdivison, *Dothideomycetes* class, *Pleosporomycetidae* subclass, *Pleosporales* order, *Venturiaceae* family (Kirk et al., 2004; Lumbsch and Huhndorf, 2010). Recently, based on molecular phylogeny and morphological and ecological grounds, *V. pyrina* was re-classified and placed in the newly described order *Venturiales*, C.L. Scoch and K.D. Hyde within *Dothideomycetes* (Zhang et al., 2011). The fungus is heterothallic and similarly as other *Venturia* species is hemibiotrophic (Langford and Keitt, 1942, cit. in. Spots and Covey, 1990; Stehmann et al., 2001). *V. pyrina* develops sexual stage in leaf litter, where it overwinters as a saprotroph during the dormant season, and asexual (conidial spores) state is formed on infected plants during the season (Stehman et al., 2001). Species of *Venturia* are mostly identified based on morphology and host (Stehman et al., 2001). The detailed morphology of the fungus is summarized and described by A. Sivanesan and J.M. Waller (1974).

European pear (*Pyrus communis* L.), Syrian pear (*Pyrus syriaca* Boiss.), other *Pyrus* species and also loquat (*Eriobotrya japonica* Lindl.) are mentioned as the hosts for *V. pyrina* among which European pear is considered to be the principal host (Sivanesan and Waller, 1974). The loquat *E. japonica* as a host for *V. pyrina* is doubtful and most likely the scab reported on this plant is caused by another *Venturia* species phylogenetically closely related to *V. inaequalis* as revealed by P. Sánchez-Torres et al. (2009). As summarized by P. Zhao et al. (2012) Asian pear (*Pyrus pyrifolia* (Burn) Nak.) was considered a host for *V. pyrina* until another species *V. nashicola* was described and proved to be the causal agent for scab on this plant.

Studies on *V. pyrina* diversity in general and among different geographical regions are scars at an early stage (Shabi et al., 1972; Chevalier et al., 2008). The variation of the pathogen was shown in cultivar resistance studies, where cultivar resistance or susceptibility correlated to the origin of inoculum (Shabi et al., 1972; Zhao et al., 2011) and in multigene phylogenetic studies (Zhao et al., 2011). The first indications of diversity among *V. pyrina* populations were based on inconsistency in pear cultivar resistance in different geographical regions. Possible presence of variable *V. pyrina* biotypes in each location was concluded as the main reason for this inconsistency (Brown, 1960). In the limited number of studies reported so far some attempts have been made to characterize and define races or biotypes of *V. pyrina*. In Israel, four races were defined on European pear and one race on Syrian pear (Shabi, 1972). Among defined races in Israel only race 2 was considered as important in pear growing (Shabi, 1989). During more recent studies in France, it was found out that *V. pyrina* population is highly divergent in terms of specificity and aggressiveness on different pear cultivars and strong co-adaptation of new strains to the so far resistant cultivar 'Conference' was detected (Chevalier et al., 2004; Chevalier et al., 2008). Due to *V. pyrina* life cycle, each spring plant is infected by the newly released ascospores representing new genotypes, and therefore ensuring high potential for genetic diversity and adaptation ability of the pathogen. This phenomenon has been reported for apple pathogen *V. inaequalis* as one of the driving forces for diversity and formation of more aggressive races (MacHardy et al., 2001).

In those few phylogenetic studies on *Venturia* species conducted so far (Schnabel et al., 1998; Beck et al., 2005), only isolates of *V. pyrina* from New Zealand, Japan and Israel were included and phylogenetic relationships of isolates with other origin have not been studied. In the recent phylogenetic study based on rDNA-ITS, partial ß-tubulin and elongation factor 1a gene sequences using *V. pyrina* isolates originated from Japan and Israel revealed two distinct evolutionary lineages (Zhao et al., 2012). Isolates from Israel belonging to the race 2 and Japanese *V. pyrina* isolates were closely related and formed separate clade, while other isolates grouped together in another lineage (Zhao et al., 2012).

Pathogen populations differ among the regions due to the factors of host genotypes, climate conditions and management strategies. Continuous use of fungicides leads to adaptation of the pathogen populations and formation of the resistance (Koenraadt et al., 1992). Closely related to *V. pyrina*, the apple scab pathogen *V. inaequalis*, has a high ability for adaptation to the environment of continuous fungicide pressure and as a result fungicide resistance in the populations is formed (Chapman et al., 2011). Fungicide resistance and genetic bases for it in *V. pyrina* populations, particulary resistance to benomyl, was studied during 1970^{ies} to 1980^{ies} in pear orchards in Israel (Shabi and Katan, 1979; Shabi et al., 1986). During these studies it was found out that benomyl resistance of *V. pyrina* is regulated by a single gene (Shabi and Katan, 1979). Resistance remained consistant in the pathogen populations for 10 years without benomyle applications (Shabi, 1989).

Symptoms, life cycle of the pathogen and disease epidemiology

V. pyrina attacks buds, leaves, fruits and young shoots and the first symptoms appear usually within two weeks after infection (Eglītis et al., 1943; Sivanesan and Waller, 1974; Bearden et al., 1976; Shabi, 1990; Liu et al., 2009). Symptoms on leaves and fruits appear as olive green to dark brown, usually circular spots that become velvety because of pathogen conidial sporulation, and growth distortion on scabbed organs is often observed (Eglītis et al., 1943; Sivanesan and Waller, 1974; Jones and Aldwinkle, 1997). With age lesions on fruit become cracked and corky, and the velvety look disappears on infected areas (Jones and Aldwinkle, 1997). Unlike apple pathogen *V. ineaqualis*, *V. pyrina* attacks also young wood and infection appears as pale brown blister-like lesions (Kienholz and Childs, 1937; Sivanesan and Waller, 1974).

V. pyrina overwinters as a saprotroph in the litter on infected leaves and as mycelium in infected twigs (Kienholz and Childs, 1937; Eglītis et al., 1943; Bearden et al., 1976; Spotts and Covey, 1990; Rossi et al., 2009). Pseudothecia are formed on the old leaf tissue during the winter. Psedothecia are formed only from heterothallic mating, requiring two different mating types (Keitt and Palmiter, 1938; Langford and Keitt, 1942). The ascospore maturity and release usually occurs about the time when pear buds are unfolding and this is the most important source for primary infections in the spring (Shabi, 1990; Liu et al., 2009). When moisture and temperature conditions are favourable, airborne ascospores are discharged, and they are carried by air to the surrounding trees, where they germinate and cause primary infections (Latorre et al., 1985). The ascospore discharge from overwintered pseudothecia usually occurs after spring rainfals and dews in a wide range of air temperature and may last up to four months in several events when faivaroble conditions are present (Lattore et al., 1985; Spotts and Cervantes, 1994; Liu et al., 2009; Rossi et al., 2009; Rancāne et al., 2013). In the studies on *V. pyrina* epidemiology has been shown that light stimulates ascospore discharge, but some amount of ascospores was also trapped during the darkness, especially during dew periods (Spotts and Cervantes, 1994). In the spring, conidia are also formed on overwintering lessions on young wood and in some years they are important source of primary infections (Sivanesan and Waller, 1974; Timmermans et al., 2010). Because of overwintering conidia the disease was more difficult to control when twig infection has occurred (Marsh, 1933; Smith, 1905). However, the occurrence and importance of twig infections differ among geografical regions (Spotts and Covey, 1990; Rossi and Pattori, 2009).

Soon after the first infection conidial sporulation appears on the lesions, and secondary infections occur when conditions are favorable for the fungus, and in warm and humid conditions conidia are formed in great numbers (Rossi and Pattori, 2009; Liu et al., 2009). The conidia are dispersed by rain and wind (Shabi, 1990). Conidia of *V. pyrina* need free water to germinate and infect pear leaves similarly as for other closely related pathogens *V. nashicola* and *V. inaequalis* (Shabi, 1990; Li et al., 2003). During the growing season the fungus lives as a true parasite within the pear tissue (Isshiki and Yanase, 2000). The new infections from sporulating lesions may occur several times per season depending on environmental conditions (Shabi, 1990). For long distance spread picking bins with scabbed leaves have been suspected as a possible carrier of spores and suggested as possible means of spreading *V. pyrina* among orchards (Spotts and Covey, 1990).

Resistance of varieties and control possibilities of the disease

Due to the public demand and new legislation annualy banning several fungicides important for agriculture, a great demand for elaboration of alternative control methods or compounds has been raised for scientific community and growers. Studies on resistance to the pear scab pathogen *V. pyrina* in European pear are at an early stage (Postman et al.,

2005; Faize et al., 2007; Pierantonie et al., 2007; Liu et al., 2009). The knowledge on pear resistance is mostly as a general description of cultivar performance in the field (Kemp et al., 2000; Fisher and Mildenberger, 2004). Only in few cases systematic field evaluation results based on wide screening of collections are published (Brown, 1960; Chevalier et al., 2011; Lācis et al., 2012). Currently grown pear cultivars have different susceptibility to pear scab, and only cultivars 'Abbé Fètel' and 'Navara' (Zhao et al., 2011; Bouvier et al., 2011) have been described as resistant. For a long time the cultivar 'Conference' remained resistant to pear scab (Bell, 1991). However, since the 1990's 'Conference' has become very susceptible in some French orchards (Chevalier et al., 2008). The pear cultivar 'Navara' was shown to be a resistant cultivar in Angers environmental conditions and several experiments have shown a pinpoint reaction and stellate necrosis indicating presence of resistance genes (Chevalier et al., 2004; Lespinasse et al., 2008).

Mechanisms of European pear resistance to scab remains uncertain and currently only one major resistance gene *Rvp1* has been identified in the cultivar 'Navara' (Bouvier et al., 2011). Development of QRL markers for *Rvp1* gene showed the synteny between apple and pear linkage groups (Pierantoni et al., 2007; Bouvier et al., 2011). Evidence of polygenic resistance of pears to *V. pyrina* has also been shown (Chevalier et al., 2004; Pierantoni et al., 2007). The importance of resistance to pear scab is highlited by several authors and some breeding programmes aimed to develop scab resistant pear cultivars are in progress (Faize et al., 2007; Chevalier et al., 2004; Lespinasse et al., 2008; Liu et al., 2009; Chevalier et al., 2011).

Despite the long history, worldwide distribution and increasing economic significance of the European pear scab, the research on control methods, and pathogen biology and disease epidemiology that could aid to develop more effective and also alternative to fungicide application control methods are still limited. Previously benzimidazole fungicides have been used extensively in some areas to control pear scab and lead to fast and stable resistance formation in the pathogen populations, which forced to switch to other type of fungicides and to find alternatives (Shabi and Katan, 1979; Shabi et al., 1981; Bakker, 1999; Washington et al., 1998; Sugar and Hilton, 2011). The salts like bicarbonates and silicon against various fungal diseases have been tested on a wide range of diseases including apple scab (Laffranque and Shires, 2005; Conway et al., 2007; Creemers et al., 2007). In case of pear scab, potassium phosphite in combination of reduced amount of fungicide myclobutanil was tested as alternative to existing control system and was found that potassium phosphate alone significantly reduced the pear scab (Percival and Noviss, 2010). Some limited efforts have also been made to control pear scab by control of the tree vigour and by applications of systemic induced resistance agents (Jansonius, 2008; Perciaval et al., 2009).

Nowadays the pear scab control basically relies on protective fungicide applications on the regular bases during the primary infection period, which is the most essential step for successful scab control (Bearden et al., 1976; Liu et al., 2009; Rossi and Pattori, 2009; Teng, 2011; Villalta et al., 2013). The number of sprays needed during the primary infection period and later in the season to control secondary infections greatly depends on infection background in the previous year, wetness periods and temperature (Bearden et al., 1976; Shabi, 1989; Rancāne et al., 2013). Combination of protective fungicide sprays with potharwest sanitation to reduce amount of pseudothecia in overwintered leaf litter have been successfully used (Eglītis et al., 1943; Spotts et al., 1997; Rancāne et al., 2013). Several sanitation methods and compounds have been evaluated for their efficency to reduce primary inoculum from leaf litter including application of dolomitic lime in autumn, beetroot Vinasse, and leaf collection with different level of succsess (Spotts et al., 1997; Timmermans and Jansonius, 2012; Timmermans et al., 2010). Among compounds used to reduce ascospore inoculum in the spring, urea applications on the leaf litter and on the tree canopy have proved as the most efficient not only for pear scab, but also for reduction of apple scab (Burchell et al., 1965; Sutton et al., 2000; Holb, 2006; Mac and tSaoir, 2010; Rancāne et al., 2013).

In order to decrease the use of pesticides due to the environmental and food safety concerns, the early warning and disease-modeling systems are developed. They are based on careful monitoring of meteorological parameters and pathogen development. Despite similarities in several aspects, including a life cycle, it was concluded that epidemiology of pear scab differs from apple scab and knowledge on pear scab is missing for development of effective warning system for pear scab control (Jansonius, 2008). Limited number of studies has been carried out to develop models for prediciting risk of primary infection (Rossi and Pattori, 2009). Need for adaptation to different growing regions and validation of existing warning models, which currently are based on limited factors (e.g. temperature and time) influencing ascospore matuaration and release have been highlited by some authors (Villalta et al., 2013). Necessity for more accurate prediction of the first ascospore release at the beginning of the season as well as integration of other factors influencing pear scab development, such as weather, tree growth and time since the last spray, has also been pointed out (Villalta et al., 2013).

Further investigations are necessary to develop the system of pear scab integrated control, which include knowledge about pathogen biology, diversity, resistance of cultivars and forecast and warning system of spores realising.

Conclusions

1. Nowadays, the European pear scab is common and considered as an economically important disease in commercial orchards in most of the pear growing areas worldwide. Pear scab is an especially serious and increasing problem in organic orchards.
2. Studies on pear scab pathogen *V. pyrina* diversity are scars at an early stage. The variation of the pathogen was shown in cultivar resistance studies and in multigene phylogenetic studies. Some attempts have been made to characterize and define races of *V. pyrina* and new biotypes co-adaptated to specific cultivars have been recorded recently indicating adaptation of the pathogen.
3. Due to environmental and food safety concerns and high adaptation capability of the pathogen to overcome resistance, need for changes in control strategies has been highlighted by pear scab research community. Deeper knowledge on the resistance mechanisms, pathogen diversity worldwide, and identification of pathotypes/races and evaluation of population diversity can provide the basic knowledge required for development of an effectively integrated pest management (IPM) system and breeding strategies of durable resistance in pear.
4. Despite the long history, worldwide distribution and increasing economic significance of the European pear scab caused by *V. pyrina*, the research on control methods, and pathogen biology and disease epidemiology that could aid to develop more effective control methods are still limited.

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STINGING NETTLE – THE SOURCE OF BIOLOGICALLY ACTIVE COMPOUNDS AS SUSTAINABLE DAILY DIET SUPPLEMENT

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Abstract
Environmental conditions and climate change on a global scale affects the overall agriculture and food supply. Consumers demand for vegetables with high nutritional value is increasing. Consumers more and more are thinking about a healthy and balanced diet, but it is not easy to provide year-round fresh vegetables. Nettle (*Urtica)* leaves traditionally are used in early spring as a leafy vegetable in salads and soups. Young leaves before flowering are used for human consumption. Nettle contains a lot of vitamins and biologically active compounds. The research aim was to evaluate different stinging nettle clones, which grow in Pūre village (Tukuma district, Latvia). Samples were collected, when shoots were 10 – 15 cm long. Content of chlorophyll, carotenoids and anthocyanins in five nettle clones leaves were analysed. Biochemical analysis was done in Latvia University of Agriculture, Institute of Soil and Plant Science laboratory. Differences were observed between all clones. Significant difference between genotypes was observed in anthocyanins content, but not in chlorophylls and carotenoids content. Higher anthocyanins content was observed in samples, which grow in places with low nitrogen and phosphorus content. Content of biochemical compounds can influence some metal ions, environmental and other factors.
Key words: nettle, carotenoids, chlorophylls.

Introduction

Nettle (*Urtica)* has a long history as herbal medicine and nourishing food. Nettle species, also known as weed, are widely used as food in early spring. Stinging nettle (*Urtica dioica* L.) young leaves are added to soups or salads as well as dried for winter use (Guil-Guerrero et al., 2003). Lately producers more and more are focusing on nettle as an industrial crop. Also, it is used for textile production.

Nettle production in Europe began in the 19th century. About 500 ha of nettles were cultivated in Germany and Austria and used for textile production. Nettle crop can produce economically significant yields for four years. If grown longer, weed infestations tend to increase and yields reduce. Extensively nettle can be grown for 10 – 15 years, or even without time limit (Vogl and Harti, 2003).

More than 1000 plant species of nettle family (*Urticaceae*) are known across the world. In Latvia, only two nettle species are found:

- *Urtica dioica* L. – often called common nettle or stinging nettle, fibber nettle. It is a perennial herb, 30 – 150 cm in height, lightly green in colour, usually dioecious. Leaves are ovate, rarely lanceolate acuminate. The male and female flower heads are similar in form, branched. The female flowers have purplish stigma.
- *Urtica urens* L. – known as annual nettle, dwarf nettle, small nettle, dog nettle or burning nettle. It is an annual herb, monoecious, 10 – 60 cm in height, clear green in colour. Leaves are ovate and deeply serrate. The male and female flowers are numerous, centrally glabrous or sparsely hispid on the back. It reminds the common nettle in habit but has smaller leaves and short flowers (Kavalali, 2003a).

Common nettle is the only nettle, which can be used for consumption and for medical purposes. Its nutritional value is very high, much higher than other vegetables and herbs usually grown in the gardens. *Urtica* leaves have relatively high level of protein (66%), which is of better quality if compared with the proteins of other leafy vegetables (Hughes et al., 1980). The leaves of nettle are good sources of different significant minerals and vitamins (Adamski and Bieganska, 1980; Kukric et al., 2012). Nettles contain flavonoids, fatty acids, terpenes, protein, vitamins, and minerals. Stinging nettle leaves are rich in vitamin C (20 – 60 mg 100 g^{-1}), B groups vitamins, vitamin K (0.16 – 0.64 mg 100 g^{-1}) and some minerals, such as calcium, iron, magnesium, phosphorus, potassium and sodium (Upton, 2013). Nettle leaves contain nine carotenoids. Lutein and lutein isomers, β-carotene are the basic carotenoids in nettle leaves (Guil-Guerrero et al., 2003). Also important substances are amino acids, glucokinins and chlorophylls (Upton, 2013). Nettle leaves contain significant amount of chlorophylls, 2.5 mg g^{-1} fresh weight (Hojnik et al., 2007). Younger leaves contain more chlorophylls and carotenoids than older ones. Chlorophyll content is very different in leaf vegetables. Lettuce (*Lactuca sativa* L.) total chlorophylls content on average is 0.2 mg g^{-1} (Cruz et al., 2012), dandelion (*Taraxacum officinale* L.) and chicory (*Cichorium intybus* L.) 2.5 mg g^{-1}, rocket (*Eruca sativa* L.) 3 mg g^{-1} (Žnidarčič et al., 2011).

Nettle for medicine is used as a nourishing blood tonic, diuretic, haemostatic, purgative vermifuge, blood purifier, antiarthritic, for seasonal allergiesand for the treatment of eczema, rheumatism, haemorrhoids, hyperthyroidism, bronchitis, cancer and also used for sprains and swellings (Kavalali, 2003b; Upton et al., 2011). *Urtica* species are also used in many phytotherapeutic preparations (Kavalali, 2003b). Different parts of the nettle plant can be used as food, fodder and as material for cosmetics and medicine industry (Vogl and Harti, 2003). Since ancient times, people have used nettle for flailing arthritic or paralytic limbs with fresh stinging nettle to stimulate circulation of blood. Dried herbs used for teas, tablets, capsules and other preparations. Nettle tea is recommended if you have problems with breast, lung, stomach and urinary tract. Preparations from fresh plant material include juice, homeopathic products, liquid extracts (Randall, 2003).

Clinical studies did not show negative effect with nettle product properties. Experiments with animals showed analgesic effect. Nettles have antioxidant and antiviral properties. Higher doses of nettle can cause blood – vessel narrowing (Upton, 2013).

In Latvia, nettles are not cultivated yet. With each year consumers interest on nettle as leafy vegetable and food additive is gradually rising. Therefore, the aim of the research was set: to collect local wild nettle genotypes in the surroundings of Pūre village (Tukuma district) and evaluate the accumulation of biologically active compounds in the clones.

Materials and Methods

Five local nettle (*Urtica dioica* L.) clones were collected in the Pūre village (Tukums district, Latvia 57°2′9″N 22°54′25″E) in the spring of 2013. All clones were grown in places, with soil acidy pH KCl 7±0.2. Growing conditions were different. Content of nitrogen and phosphorus in soil was various. Two clones (clone 1 and clone 5) were collected in places where low phosphorus and nitrogen content was stated. Other two clones (clone 2 and clone 4) were collected in places with medium level content of phosphorus and nitrogen. One clone (clone 3) was growing in the place with relatively higher N, P content in comparison with others. Nitrogen and phosphorus content in soil was determined using express methods. Content of nitrogen and phosphorus was gradation from 1 to 4 (1 – lower lever, 4 – highest level) (Tab.1).

Nitrogen content was determined by using diphenylamine $(C_6H_5)_2NH$) express method, based on the formation of intense blue tint (N,N'-difenil benzidin violet) that results from the reaction between nitrate and diphenylamine ions.

Phosphorus content was determined with express method by using solutions of ammonium molybdate, benzidine and sodium acetate. Soluble silicates and phosphates react with ammonium molybdate in nitric acid solution to form yellow complex molybdates. These complex molybdates bring about the oxidation of benzidine in alkaline solution with the formation of a blue quinoid compound and molvbdenum blue (Магницкий, 1972).

All samples were harvested in one day, when shoots were 10 – 15 cm long. In one sample, there were five shoots. Samples were cooled, packed in a thermo bag and transported ensuring low temperature to the laboratory. On the same day the samples for biochemical analysis were prepared. Analyses were performed in Latvia University of Agriculture, Institute of Soil and Plant Sciences.

The content of chlorophyll, carotenoids and anthocyanins in nettle leaves was analysed spectrophotometrically (Shimadzu Spectrophotometer UV-18000) in two replicates.

Leaf average sample was weighed and placed in a pestle and added a bit of quartz sand, some crystals of $CaCO_3$, and added 1 – 2 mL of 960 g kg^{-1} ethyl alcohol and ground. Samples were placed in a graduated tube, filled up to 10 mL of ET-OH and then centrifuged HermleZ383. The solution optical density at different wavelength (nm) was determined and content of chlorophyll a, chlorophyll b, total chlorophylls and carotenoids were calculated by equations (1) – (4)

$$C_{hla} = 13.7 \times A_{665} - 5.76 \times A_{649} \quad (1)$$

$$C_{hlb} = 25.8 \times A_{649} - 7.60 \times A_{665} \quad (2)$$

$$C_{hl(a+b)} = 6.1 \times A_{665} + 20.04 \times A_{649} \quad (3)$$

Table 1

Nitrogen and phosphorus content in soil, mg kg^{-1}

	1 (no color)	2 (pale blue)	3 (blue)	4 (dark blue)
Nitrogen	<50	50-100	100-150	>150
Phosphorus	<100	100-300	300-600	>600

$$C_c = 4.695 \times A_{440.5} - 0.263 \times C_{(a+b)} \quad (4)$$

where

$C_{hla.} C_{hlb.} C_{hl(a+b)}$ and C_c – concentration of pigments mg dm^{-3};

A – light absorption, at appropriate wavelength;

Calculated content of pigments (mg g^{-1} fresh weight):

$$m = \frac{c \times m_{wei}}{P \times 1000} \quad (5)$$

where

m – content of pigments in plant material, mg g^{-1};

c – concentration of pigments, mg dm^{-3};

m_{vei} – weighed amount of plant material, g;

1000 – Coefficient to recalculate from dm^3 to cm^3 (Гавриленко и Жигалова, 2003).

Leaf average sample was weighed and placed in a pestle and ground. Samples were placed in a graduated tube, 10 mL of 10 g kg^{-1} hydrochloric acid was added, and then centrifuged. The optical density of solution was fixed at 535 nm wavelength and the content of anthocyanins in plant material was calculated (Moor et al., 2005).

The results were analyzed using ANOVA at significance level of $\alpha = 0.05$.

Results and Discussion

Morphological differences of clones were stated. Leaf samples were of different intensity green colour. For some samples purple – violet hue was observed (clone 1 and clone 4). Differences in green pigment - chlorophyll content in analysed clones were also stated. Chlorophyll content in the samples ranged between 1.87 – 2.51 mg g^{-1} fresh weight (Fig. 1).

A result confirms findings of others scientists. Our results were very similar to results obtained in the research performed in Slovenia, where chlorophyll content was on average 2.50 mg g^{-1} fresh weight (Hojnik et al., 2007). A.M. Humphrey (1980) mentions that nettle leaves contain approximately 2.5 mg g^{-1} total chlorophylls. Y.F. Kopytko (2012) refers that nettle leaves contain chlorophyll 10 – 50 mg g^{-1} dry mass, of which 75% are chlorophyll a and 25% chlorophyll b. Also, in our research relation 3:1 to chlorophyll a chlorophyll b was observed. Chlorophyll content in nettle samples was different, although significant differences between clones in chlorophyll content were not observed in the nettle leaves ($p = 0.33$).

The content of carotenoids in nettle leaves was four times lower than chlorophylls content (Fig. 2).

Results show that carotenoids content ranged between 0.52 – 0.63 mg g^{-1} fresh weight. Significant differences between clones are not observed also in carotenoids content in the analysed nettle leaves ($p = 0.28$). In research performed in Serbia leaf samples were collected at different times during vegetation period. It showed differences during carotenoids content, it ranged between 0.22 mg g^{-1} – 0.22 mg g^{-1} fresh weight (Kukric et al., 2012).

Significant differences between clones are observed in anthocyanins content ($p = 0.03$) (Fig. 3).

Anthocyanins content in the analysed samples ranged between 0.22 – 1.99 mg g^{-1} fresh weight. Higher anthocyanins content was observed in clones 1 and 5, which grew in place with phosphorus deficit. It is reported that deficit of nutrients, especially phosphorus and nitrogen, promote the accumulation of anthocyanins was found (Biesiada and Tomczak, 2012). It was stated that in the case when plants have phosphorus deficit, their leaves are dark green-violet colour tone. In this case plants accumulate much more athocyanins as normally. Also, R. Piccaglia with

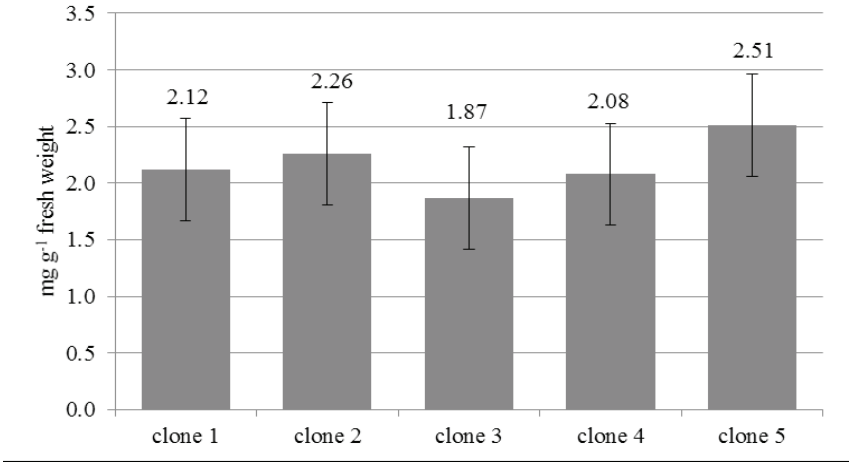

Figure 1. Chlorophylls content in the analysed nettle leaves.

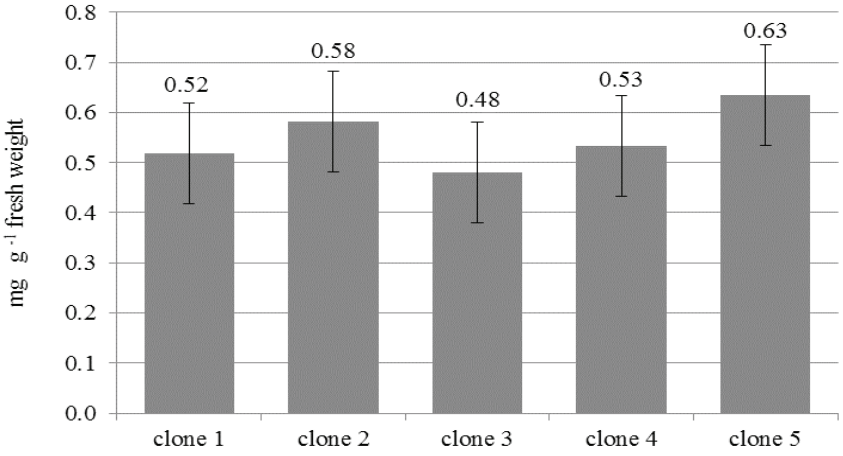

Figure 2. Carotenoids content in nettle leaves.

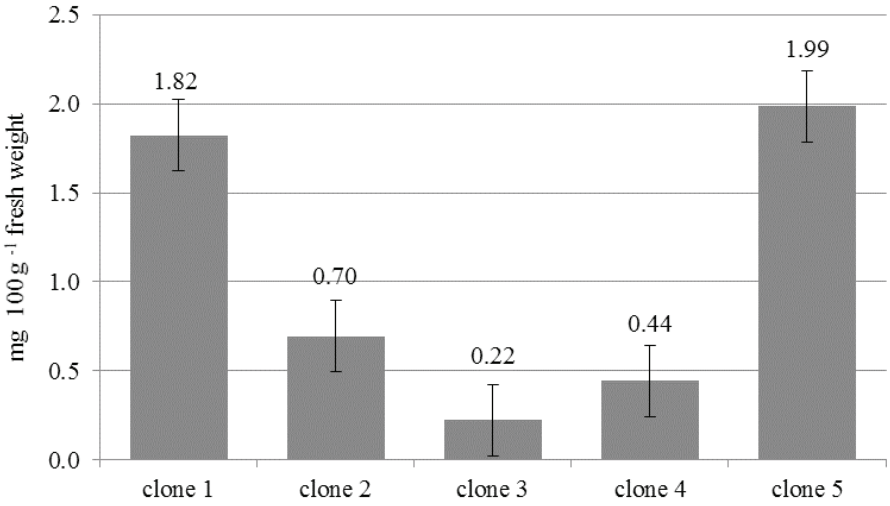

Figure 3. Anthocyanins content in the nettle leaves.

colleagues (Piccaglia et al., 2002) reports that the lower content of anthocyanins was detected if higher doses of phosphorus were added. Our results show that correlation coefficient between nitrogen content in soil and anthocyanins content in leaf sample was $r = -0.95$ ($r > r_{0.05} = 0.878$) and between phosphorus content in soil and anthocyanins content in leaf sample was $r = -0.10$ ($r < r_{0.05} = 0.878$). Higher phosphorus and nitrogen content (clone 3) had negative influence on the anthocyanins accumulation. Clone 3 grew in the place where there was a greenhouse some years ago.

The amount of anthocyanins in leaves is influenced by many factors. One of these factors is presence of some metal ions: aluminum, iron and magnesium in soil. Environmental factors also change leaf tone (Mlodzinska, 2009). Therefore, it is considered essential to plant all the clones in one place where similar agro-ecological conditions are ensured in order to evaluate influence of genotype on the content of biochemically active compounds and exclude the genotype-environment interaction.

Conclusions

High amounts of chlorophyll were detected in the analysed samples. It should be stressed as an important factor that the use of nettle is an important component for biologically functional food. Significant difference between genotypes was observed in anthocyanins content, but insignificant one in chlorophylls and carotenoids content. Stinging nettle leaves have high level of pigments, especially chlorophylls, which provide our daily diet with biologically active compounds.

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AGROBIOLOGICAL ESTIMATION OF INTRODUCED GRAPE VARIETIES IN THE CONDITIONS OF THE SOUTH - EAST OF KAZAKHSTAN

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Abstract
Assortment improvement is a natural process of updating varieties and improving the quality of variety assortment. The article presents the results of studying the introduced grape (*Vitis vinifera*) varieties in the conditions of the south-east of Kazakhstan, in particular in the bottom-mountain area of Almaty region. The beginning and the end of the main phenological phases passing for the vegetative period in a grape plant directly depended on climatic and weather conditions of this district. It is established that varieties 'Kuibyshevsk early-maturing', 'Iyulsky' and 'Priusadebny' have a good degree of eyes wintering buds and are able to give a yield of high quality in this area in comparison with recognized variety 'Almaty early-maturing'.
Key words: grape, variety, phenological phases, harvest, quality, wintering degree.

Introduction

It is known that a major factor determining the increased efficiency of grape (*Vitis vinifera*) plants is an assortment which is improved both by introduction of varieties based on soil and climatic analogs, and removal and introduction of the genotypes created by methods of combinative selection on a genetic basis.

Assortment improvement is a natural process of updating varieties and improving the quality of variety assortment. Varieties with high adaptation level reduce the degree of risk of harvest failure due to the adverse weather factors. All this significantly raises the profitability level of grape growth, improves economic indicators of economic entities and an ecological situation in regions of grape cultivation (Troshin and Radchevsky, 2001).

The Republic of Kazakhstan has developed the 'Concept' on the basis of which RK (Republic of Kazakhstan) Cabinet approved 'The program of grape and wind growing restoration and development in Kazakhstan in 2001'. According to this Program the vineyard area in Kazakhstan shall be brought to the level of the 70 – 80^{ies} of the last century (26-27 thousand hectares) in the long term. In addition, the efficiency of vineyards as a whole by Republic of Kazakhstan shall significantly increase even in comparison with the 1970 – 80^{ies} and gross production of grapes may really reach 200-220 thousand tons in favorable years. Improvement of variety assortment of grapes will promote the performance of these tasks.

At cultivation of table grapes, the absolute productivity value is not the main criterion by which the best of compared grades shall be determined. For such conclusion it is necessary to determine the commodity grapes yield: the sale price and productivity in harvesting and sorting depend on this factor. Much attention shall be given to the size of grapes and berries at introduction of new highly productive table grapes possessing a complex of economic and valuable characteristics. Only large berries with beautiful coloring provide elegance and attraction of table grapes (Aliyev, 2002).

Formerly the most widespread way of assortment improving was the direct introduction of varieties, but in recent years it has become almost impossible. According to UPOV Convention, it is prohibited to import not patented or counterfeit varieties for economic use, also because of the risk of delivering the quarantine pest – phylloxera (*Phylloxera vitifoliae*) (Izbasarov and Madenov, 1997).

Kazakhstan is in the zone where the scion-rooted grape culture still remains. In case of phylloxera delivery, the Republic shall pass to the imparted grapes culture. Kazakhstan unlike the main wine growing regions of the world uses covering labor-intensive culture of grapes, and it may result in the increased cost of a landing material and product cultivation. Therefore, the delivery of new varieties is made through *in vitro* culture.

Kazakh scientific research institute of fruit and grape growing has been introducing grapes from the near and far abroad countries for many years. The Ampelografic collection is constantly replenishing with new varieties.

In the Kazakh scientific research institute of fruit and grape growing for the purpose of replenishment of assortment of table varieties of grapes long-term selection work on creation of new large clusters of table varieties of different term of maturing which could compete in a domestic and foreign market of the republic is carried out. As a result of selection work of a number of scientists, the extensive hybrid fund of grapes of the table direction from which by a "step" technique the best hybrids – candidates for varieties are selected has been created (Kazybaeva, 2006).

Table 1

Average monthly air temperature in south-east zone of Almaty area (2003 – 2013) on the meteorological station Chilik

Months	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Average annual
Average temperature	-9.2	-6.0	3.0	12	17	22	24	23	17	10	1	-6	9
Absolute minimum	-37	-39	-29	-8	-7	2	6	5	-3	-15	-38	-32	-3.9
Absolute maximum	13	19	28	34	40	41	41	42	38	32	23	14	42

The purpose of work is to study phenological features of the introduced grape varieties and a comparative assessment of performance and quality of indicators of the harvest.

Materials and Methods

Researches were conducted in 2012 – 2013 in a bottom mountain zone of Zailiysky Alatau at the height of 1070 meters above the sea level, 43°18' north latitude and 77°14' east longitude, in Almaty region on the pilot farm base 'Pomology garden' by Kazakh scientific - research institute of fruit and grape growing. The introduced table varieties of early and middle term of maturation served as objects of researches: Iyulsky, Kuibyshevsk early-maturing, Muscat Yubileyny, Priusadebny, Almaty early-maturing (standard of Kazakhstan selection). The year of landing is 2001, the planting scheme of bushes of 2.75 × 1.5 m. Culture of grapes is covering.

The climate of the zone is sharply continental. According to mean annual data, the average daily temperature is +8.9 °C, an absolute maximum +39 °C, minimum -34.5 °C. The annual amount of precipitation is 650 mm with uneven distribution within a year (table 1).

Phenological phases were noted: blooming of kidneys, beginning and end blossoming, maturing of berries. The average mass of clusters was established by a blossoming weighing way on 100 clusters. The crop from a bush was defined by multiplication – quantities of clusters on their average weight. Productivity from a hectare was defined by crop multiplication from a bush on quantity of plants/hectare at this scheme of landing.

All these calculations were performed using the standard technique of primary cultivar. Yield data was processed by the analysis of variance (Dospekhov, 1979).

Observations were made by the standard procedure 'Methodical instructions on grape selection' (Yerevan, 1973), 'Studying the grape varieties' (Lazarevsky, 1963).

Results and Discussion

The beginning and the end of the main phenological phases passing for the vegetative period in a grape plant directly depended on climatic and weather conditions of this district.

Weather conditions in 2012 differed from mean annual data and were characterized by steady and continuous low temperatures during the winter period. An average daily temperature of December and January was 4 – 5 °C, and in February about 2 – 3 °C lower than mean annual values. In the third decade of January the frosts reached -25 °C. Precipitation dropped out for three times more than the norm. Considerable thaw had not been noted.

Spring was amicable and warm that accelerated passing of phenological phases (with-sap flow and bud pushing). Especially the varieties Priusadebny (12.04) and Iyulsky (13.04) had early bud pushing. The bud pushing of varieties Kuibyshevsk early-maturing and the Muscat Yubileyny had been observed for 3 – 4 days later. The bud pushing of a control variety Almaty had been observed on April 14.

Spring frosts to -2 °C (on April 23) damaged part of already dismissed buds of grapes that had an adverse effect on productivity of all varieties.

The beginning of blossoming passed in earlier terms from 26 May to 31 May, mass blossoming from June 2 to June 7, the end of blossoming was from June 10 to June 13. The summer was hot and dry. In June temperature reached an absolute maximum (+39 °C), a precipitation dropped out for 30% less than norm. All this determined the intensive growth and maturing of berries. The removable maturity of berries was noted for 8 – 10 days before the usual term. The earliest maturing of berries was noted at varieties 'Iyulsky' (24.07), Almaty early-maturing (1.08) and at 'Priusadebnyi' (8.08). Maturing of berries at varieties the Muscat Yubileyny and Kuibyshevsk early-maturing is - 14.08.

Weather conditions of the winter period 2013 were close to mean annual values, thaw was not observed during the winter period. The spring was early with

intensive increase of temperature. The bud pushing in this year was for 2 – 3 days earlier than in 2012. However, the damp spring decreased a temperature mode at the end of April and May. Therefore, blossoming of varieties took place in usual terms. Blossoming began on June 5 – 7, mass blossoming was observed on June 14 – 16 and the end of blossoming was noted on 20 – 23 June. The summer was hot, but damper than in previous year. The harvest formation took place in favorable conditions. The removable maturity of berries was noted at varieties 'Iyulsky' (8.08), 'Almaty early-maturing' (10.08) and at 'Priusadebny' (14.08). At varieties 'Muscat Yubileyny' and 'Kuibyshevsk early-maturing' - 19.08.

Weather conditions in these years determined the degree of grapes eyes safety, which was different in varieties. The best variety of eyes wintering had been noted at grapes 'Priusadebny' (64.7%) and 'Iyulsky' (63.3%) for 2 years on average. The variety 'Muscat Yubileyny' endures winter conditions of this district less well; therefore, the wintering of buds was lower than at standard and amounted to 57.6% only. The degree of eyes safety at a variety the 'Kuibyshevsk early-maturing' was slightly higher than control one (figure 1).

Determining the levels of varieties productivity enables to reveal the maximum ability of a variety to yield harvest in specific conditions of growth, in comparison with standards. According to Table 1, it is obvious that the studied varieties of grapes are capable to yield the harvest of high quality in the conditions of a bottom mountain zone of Almaty area. Despite the spring frost in 2012, which sharply reduced the number of scions and consequently the grapes, the productivity of almost all varieties was higher than at the control variety 'Almaty early' – maturing (except for 'Muscat Yubileyny'). The comparison of varieties shows that the greatest number of grapes is noted at the variety 'Iyulsky' (22 pieces/bush) for an average of 2

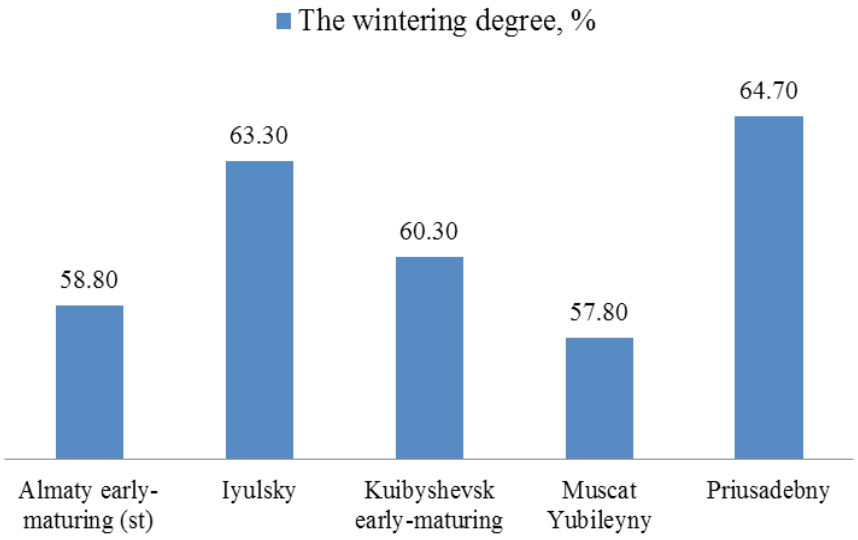

Figure 1. The wintering degree of the introduced grape varieties (2012 – 2013).

Table 2

Agrobiological indicator of the introduced grape varieties (2012 – 2013)

Name of the variety	Quantity of grapes, pc	Average mass of grapes, g	Crop of a bush, kg	Content of sugar in berries, g kg^{-1}	Titrable acidity, g L^{-1}	Productivity, t ha^{-1}
Almaty early-maturing (st)	9	270	2.5	19	5.2	6.06
Iyulsky	22	188	4.1	18	5.6	9.94
Kuibyshevsk early-maturing	12	230	2.8	18	5.4	6.79
Muscat Yubileyny	11	218	2.4	18	4.9	5.82
Priusadebny	9	360	3.2	19	5.0	7.76
$LSD_{0.05}$	-	56.7	0.8	-	-	1.60

Table 3

Biometric indicators based on the growth and ripening of scions of grape varieties (2012 – 2013)

Name of the variety	Total amount of growth, m			Average length of a scion, cm			Extent of ripening of escapes, %		
	2012	2013	average	2012	2013	average	2012	2013	average
Almaty early-maturing (st)	23.88	26.80	25.34	177.0	127.6	152.3	65.3	65.9	65.6
Iyulsky	22.22	28.10	25.16	177.4	144.8	161.1	54.6	51.5	53.0
Kuibyshevsk early-maturing	27.14	37.00	32.07	181.0	161.2	171.1	82.9	73.8	78.3
Muscat Yubileyny	30.61	32.20	31.40	175.3	168.1	171.7	53.0	43.4	48.2
Priusadebny	19.35	30.45	24.90	122.7	148.8	135.7	57.2	60.4	58.8
$LSD_{0.05}$	7.00	6.30	-	36.2	25.1	-	-	-	-

years. In spite of the fact that among studied varieties the Iyulsky has the least average mass of grapes (188 g) due to the good set of grapes, the crop from the bush is higher (4.1 kg) in comparison with other varieties that in terms of hectare amounted to 9.94 t. The good harvest is received at the variety 'Priusadebnyi' (7.76 t ha^{-1}). The critical factor of this variety is an average mass of grapes (360 g). Despite the good wintering of eyes this variety had a smaller quantity of grapes per bush than other varieties (Table 2). The small crop is received at the variety 'Muscat Yubileyny', the yield per hectare amounted to 5.82 t ha^{-1} that is 2.4 c less than at a standard one. The variety 'Kuibyshevsk early-maturing' had a productivity exceeded the limit for 0.73 t ha^{-1}.

The content of sugar in berries at the varieties 'Iyulsky', 'Kuibyshevsk early-maturing'. 'Muscat Yubileyny' equaled to 18 g kg^{-1}. For the varieties 'Priusadebnyi' and 'Almaty early-maturing' it has been 19 g kg^{-1}. According to the content of titrable acid, the first place takes 'Iyulsky' (5.6 g L^{-1}), then 'Kuibyshevsk early-maturing' (5.4 g L^{-1}), 'Almaty early-maturing' (st) (5.2 g L^{-1}), 'Priusadebny' (5.0 g L^{-1}). The content of titrable acid at the 'Muscat Yubileyny' did not exceed 4.9 g L^{-1}.

Total amount of the growth and ripening grade of one-year scions characterize the plant readiness for wintering and ability to fructify well the next year. The assessment of biometric indicators of one-year scion of studied grape varieties makes it possible to conclude that on average within 2 years the growth amount, average length of scions and the ripening grape allow to cultivate these varieties in this zone. The analysis showed that in 2012 the growth amount was higher at the varieties 'Muscat Yubileyny' – 30.61 m $bush^{-1}$ and 'Kuibyshevsk early-maturing' – 27.14 m $bush^{-1}$, but the smallest - at the variety 'Priusadebny' – 19.35 m $bush^{-1}$ and 'Iyulsky' – 22.22 m $bush^{-1}$.

In 2013, these indicators were better than in the previous year. The growth amount of all studied varieties exceeded a control variety (Table 3). A good performance has been observed at the varieties 'Kuibyshevsk early-maturing' (37.00 m $bush^{-1}$) and 'Muscat Yubileyny' (32.20 m $bush^{-1}$).

According to the factor average length of a scion, the best result is received at the variety 'Kuibyshevsk early-maturing' (171.1 cm) on average for two years, though this factor for this variety was much lower in 2013 than in 2012. The smallest average length of a scion has been noted at the variety 'Priusadebny' (135.7 cm). The extent of ripening of studied varieties for this zone is satisfactory on average for 2 years. The variety 'Kuibyshevsk early-maturing' (78.3%) stands out by this factor. The ripening of varieties 'Priusadebny' and 'Iyulsky' has been slightly lower than at the standard variety 'Almaty early-maturing' (table 2). The lowest extent of ripening is noted at the variety 'Muscat Yubileyny' (48.2%). Bad ripening of scions during the autumn period probably affects the wintering degree of this variety (Fig. 1).

Conclusions

According to the results of researches, it is established that the varieties 'Kuibyshevsk early-maturing', 'Iyulsky', 'Priusadebny' are possible to be cultivated in the bottom mountain zone of Almaty region based on eyes wintering degree, productivity, the growth amount and extent of ripening. The variety 'Muscat Yubileyny' had a low degree of eyes safety during the winter period due to the bad ripening of rods, and, as a consequence, a low productivity. This variety is not recommended for cultivation in this zone.

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YIELD MATURITY PARAMETERS OF HYBRID GRAPEVINE (*VITIS SP.*) CULTIVAR 'ZILGA'

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Abstract
The number of grape (*Vitis* sp.) cultivars is increasing every year, the focus point of recent grapevine studies are heading to producing high quality fruits for producing quality wine. A field trial with cultivar 'Zilga' was established in 2007 at the Estonian University of Life Sciences and in 2010 in a greenhouse. 'Zilga' is one of the well adopted cultivars in Estonia, which has been used mostly for producing wine and grown in open field conditions. The aim of the present experiment was to find out the yield maturity parameters of cultivar 'Zilga' for red wine in Estonian conditions. Data was collected from the year 2011 to 2013. The bunch weight and °Brix were determined from fresh materials, but all the other biochemical parameters were determined from frozen (-20 °C) grapes. The recommended content (20 °Brix) of soluble solids was not obtained in open field conditions, but reached to 24.1 °Brix in greenhouse conditions in 2013. Titratable acids content was higher than recommended values, ranging from 1.4 to 1.6 mg 100 g^{-1} in open field and showing the lowest content (1.2 mg 100 g^{-1}) in greenhouse conditions. Maturity index values ranged from 117 to 224, and the optimum was reached in two years from three. The highest total phenolics content, 293 mg 100 g^{-1} was obtained in 2011, while anthocyanin content was significantly increased in 2013.
Key words: berry ripening, soluble solids, titratable acids, total phenolics, anthocyanins, maturity index.

Introduction

The increasing interest of viticulture in cool climate countries leads to the demand for winter hardy cultivars. Good results have been achieved in grape (*Vitis* sp.) breeding by P. Sukatnieks and G. Vēsmiņš in Latvia (Kaufmane et al., 2013). In cool climatic conditions, cultivars of hybrids of *V. labrusca*, *V. riparia* and *V. amurensis* are the ones with good winter hardiness and disease resistance. The day and night temperature fluctuation and direct sunlight interception are the main factors that affect the accumulation of biochemical compounds and grape quality as well (Gustafsson and Mårtensson, 2005; Nicolosi et al., 2012).

A common problem in cool climate conditions for wine producers is insufficient grape maturation – titratable acids (TA) being high but soluble solids content (SSC) too low (Gustafsson and Mårtensson, 2005). H. Schalkwyk and E. Archer (2000) recommend SSC for red wine grapes from 20 to 23 °Brix and TA from 0.6 to 0.7 g 100 g^{-1} FW. Thereby the measurement of SSC alone is not enough to ensure the maturity of grapes (Ferrer-Gallego et al., 2012; Iyer et al., 2012). Additionally, a combination of SSC and pH values is generally used to determine the optimum ripeness of red wine grapes, maturity index (MI = °Brix × pH^2) optimally ranging from 200 to 270 (Coombe et al., 1980; Hunter et al., 1991; Schalkwyk and Archer, 2000; Iyer et al., 2012). Too high values are also not preferred – optimal ripening conditions and sufficient maturity could be obtained in greenhouse, but it is not common practise to grow wine grape cultivars in indoor conditions.

In addition to sugars and acids, the content of phenolic compounds (including anthocyanins) is important. Total phenolics content (TPC) varies depending on multiple factors such as temperature, cultural practices and grape developmental stage (Haselgrove et al., 2000; Peña-Neira et al., 2004; Ferrer-Gallego et al., 2012; Palliotti et al., 2012). For red grapes, anthocyanins content (ANC) varies among cultivar and grape maturity, production area, seasonal conditions and yield (Haselgrove et al., 2000; Ryan and Revilla, 2003; Fournand et al., 2006; Hülya Orak, 2007; Falcão et al., 2008; Topalovic, Mikulic-Petkovsek, 2010; Ferrer-Gallego et al., 2012; Pedneault et al., 2013). Fruit biochemical composition depends on the grapevine cultivation area and specific conditions of the growing year (Martin and Dunn, 2000). In Nordic climate, differences between the years can significantly affect ripening; therefore, the expected concentrations may not be achieved (Pedneault et al., 2013), as cool and wet weather conditions may influence ripening (Nicholas et al., 2011).

Latvian origin 'Zilga' is one of the well adopted cultivars, which has been on the list of recommended cultivars suitable for growing in Estonia from the year 2004 and used mostly for producing wine. Open field trials with this cultivar was conducted in 2007 at the Estonian University of Life Sciences, and it has been under investigation since. Through the years the research objectives have been changing in relation to the development of viticulture. At the beginning of vine research, the main aim was selecting suitable species and their cultivars for growing in Nordic climatic conditions. For today, as the number of suitable grape cultivars is increasing constantly, the focus point of recent studies is heading for producing high quality fruits for producing quality wine. The

aim of the present experiment was to find out the yield maturity parameters of cultivar 'Zilga' for red wine in Estonian conditions.

Materials and Methods

Experimental site

The vineyard was established at the Rõhu experimental station of the Estonian University of Life Sciences (58° 23′ 17″ N, 26° 41′ 50″ E) in June 2007 and at the Saare-Tõrvaaugu (58° 37′ 42″ N, 25° 8′ 17″ E) greenhouse in May 2010. The data was collected and samples were taken from 2011 to 2013 in open field and in 2013 in greenhouse conditions. Plantation soil was sandy loam with pH (KCl) 5.8, with 4.4% humus content and 50 cm thick humus layer. The content of P, K, Ca and Mg was sufficient in the soil of both experimental areas and hence, no fertilizers were used. The grapevines were propagated *in vitro* and grown as own-rooted. Vines were spaced 2 × 2 m apart and planted in single rows with 0.04 mm thick and 1 m wide black polyethylene mulch with turf between the mulched beds. In the greenhouse, the mulch is all over the floor area. The experiment was conducted using randomized block design in 4 replications and 8 vines in each. Vines were neither irrigated nor covered for winter. For greenhouse cultivation, plastic tunnels 45 m in length, 8 m in width and 4 m in height were used. Vines were planted in 2010, in 1.65 × 3.5 m spaces and trained in high double trunk trellis. The greenhouse area was covered with 0.18 mm thick UV-stable low-density polyethylene at the end of April. White polypropylene fabric and spruce (*Picea*) branches were used as a winter cover; no additional heating system was used in plastic tunnels. Rows were oriented from north to south. Vines were neither irrigated nor covered for winter. The training system was low in open field and a high double trunk in the greenhouse. Leaves adjacent to berry clusters were removed at the beginning of veraison from the east side of the canopy to allow the morning sun exposure due to the occurrence of dew.

Cultivar

Grape cultivar 'Zilga' is a hybrid (*V. amurensis* × *V. labrusca* × *V. vinifera*) bred in Latvia by Pauls Sukatnieks (Vēsmiņš, 2012). Berries are medium sized and able to obtain 16…19 °Brix. They ripen in the middle of September in Latvian agroclimatic conditions with clusters from 100 to 400 g weight. 'Zilga' is one of the cultivars most often used for producing wines in Latvia (Dishlers, 2003). In Estonian conditions, 'Zilga' has been characterised as a cultivar with roundish and uniform sized berries covered with greyish wax layer (Kivistik, 2012). Cane maturation occurs early, and in autumn the foliage is nicely red-coloured. The cultivar is winter hardy (-30…-35 °C). 'Zilga' is also on the list of recommended cultivars for growing in commercial or home gardens in Estonia from 2004 onwards.

Weather conditions

On 15 April 2011, temperatures were above 5 °C and rose to 20 °C by the end of the month. The period of active plant growth temperatures (>10 °C) in 2011 was from 7 May to 8 October. The summer of 2011 was warm – mean temperature in July was 3.3 °C higher than the long term mean (Table1). First night frosts occurred on 20 October. In 2011, the sum of active temperatures was 2498 °C, and the length of active plant growth period was 155 days. In 2011, there was almost no rain in April and 27 mm more rain than the long term mean in September (Table1).

In 2012, temperatures were above 10 °C from the beginning of May until 6 October. The active plant growth period was 150 days. Late spring frosts stopped in the mid-May and temperature remained >0 °C until the 20 October. Mean temperatures in June and July were cooler than in 2011; respectively 3.9 °C and 2.3 °C lower (Table1). The sum of active temperatures from 1 April until 31 October 2012 was 1967 °C. In 2012, the precipitation level was significantly higher in April, May and June but almost at the same level in August and September in comparison with many years.

In 2013, the active plant growth period started from the 7 May and ended on 23 September. Frost-free period was 145 days long, which is 22 days more than long-term mean. The last spring frost occurred on 3 May, but the first autumn frost on 26 September 2013. The sum of active temperatures (>10 °C) from 1 April to 31 October was 2263 °C. This is 332 °C more than many years mean (1936 °C) but less compared to the year 2011. The precipitation sum from 1 April to 31 October was 352 mm which is 152 mm less than in 2012, 43 mm more than 2011 and 86 mm less than the mean (438) of 1971 – 2000.

Biochemical analysis

Grapes were picked every year in September in open field conditions from 2011 to 2013 and in the greenhouse in 2013. The weight of ten randomly selected bunches of vine was determined in each replication. SSC was measured from fresh berries by refractometer (Atago Pocket Refractometer Pal-1). For °Brix measurement, 30 grapes in 3 replications from the different parts of a cluster were picked and analysed.

All the other biochemical parameters were determined from frozen (-20 °C) grapes. TA content was determined by the titration method (Mettler Toledo EasyPlus Pro Titrator) with aqueous 0.1 M NaOH solution, using pH 8.2 endpoint titration type.

Table1

Weather conditions in 2011 – 2013: monthly mean air temperature and total monthly precipitation compared to many years mean (1971 – 2000)

Month	Air temperature (°C)					Precipitation (mm)			
	2011[a]	2012[a]	2013[a]	2013[c]	Mean [b]	2011[a]	2012[a]	2013[a,b]	Mean [b]
April	5.7	4.6	4.0	-	4.7	1	45	36	33
May	11.0	11.4	15.5	-	11.1	58	78	65	53
June	17.2	13.3	17.8	21.6	15.1	35	98	29	69
July	20.0	17.7	17.5	21.1	16.9	48	80	67	76
August	15.9	14.8	16.6	18.9	15.6	55	80	73	80
September	12.3	11.9	10.8	12.8	10.4	80	61	38	67

[a] Data was collected from automatic weather station of open field experimental station
[b] Data according to the Estonian Hydrological and Meteorological Institute (www.emhi.ee) database
[c] Temperatures were measured with specially installed thermometers in the greenhouse

The TA was expressed as g of tartaric acid per 100 g of fresh weight (FW). pH was measured from grape juice with a pH electrode of Mettler Toledo EasyPlus Pro Titrator. MI was calculated according to the formula determined by B.G. Coombe et al. (1980): MI = °Brix × pH^2. ANC was estimated by a pH differential method from grape skin (Cheng and Breen, 1991). Absorbance was measured with a UVmini-1240 Shimadzu spectrophotometer at 510 and at 700 nm in buffers at pH 1.0 (HCl 0.1N) and pH 4.5 (citrate buffer). The results were expressed as mg of malvidin-3-glucoside equivalent per 100 g of FW. TPC was determined from grape skin with the Folin-Ciocalteau phenol reagent method, using a spectrophotometer (UVmini-1240 Shimadzu) at 765 nm. The TPC was expressed as mg of gallic acid equivalents per 100 g of FW.

Statistical analysis

The results of grape chemical composition were tested by one-way analysis of variance. To evaluate the effect of year, the least significant difference ($LSD_{0.05}$) was calculated. Different letters on figures and tables mark significant differences at $p \leq 0.05$.

Results and Discussion

Soluble solids content

The SSC ranged from 13.5 to 24.1 °Brix (Figure 1). In open field conditions, the SSC did not reach the optimum, but the recommended °Brix values (20 °Brix) were obtained reaching to 24.1 °Brix in greenhouse conditions in 2013. SSC was the lowest (13.5 °Brix) in 2012, when summer temperatures in June, July and August were respectively 3.9, 2.3 and 1.1 °C lower than in 2011.

According to literature, 'Zilga' is able to obtain 16…19.5 °Brix depending on the growing site (Dishlers, 2003; Vēsmiņš, 2012). Similar results were also achieved in our experiment in 2011 and 2013. The weather conditions in 2011 and 2013 were considered to be warmer according to the sum of active temperatures (>10 °C) (respectively 2498 °C and 2263 °C) than in 2012 (1967 °C) and long-term mean (1936 °C). Therefore, it can be suggested that sugar accumulation was advanced, grape berries had sufficient time and temperatures for ripening. In 2012, there was also higher precipitation level through the vegetation period, which could affect the biochemical composition and also sugar accumulation. K.A. Nicholas et al., (2011) indicate cool and wet weather as inhibiting factors for grape ripening. The recommended SSC in 'Zilga' grapes, up to 24.1 °Brix, was obtained in comparison to the variant in a greenhouse, which indicates a high potential of the cultivar.

Titratable acids

TA content ranged from 1.4 to 1.6 mg 100 g^{-1} in open field and showed the lowest content, 1.2 mg 100g^{-1}, in greenhouse conditions (Figure 2). These concentrations are extremely high compared to H. Schalkwyk and E. Archer (2000) who suggest optimally for red table wine grapes TA content from 0.6 to 0.7 mg 100 g^{-1}. In Latvian climatic conditions, 'Zilga' TA has been close to 1.0 mg 100 g^{-1} (Dishlers, 2003). In our experiment, only the greenhouse-grown berries TA content (respectively, 1.2 mg 100 g^{-1}) remained close to the previously indicated value. Organic acids accumulate in the berry during berry formation, yet due to the increasing fruit size, their concentration is reduced (Kennedy, 2002; Topalovic and Mikulic-Petkovsek, 2010). The content of TA can roughly be climate dependent. As a result, the acids tend to show higher concentrations in cooler regions (Kennedy, 2002). This suggests that the temperatures in Estonia are too low in the first period of berry development, which influences the berry composition significantly.

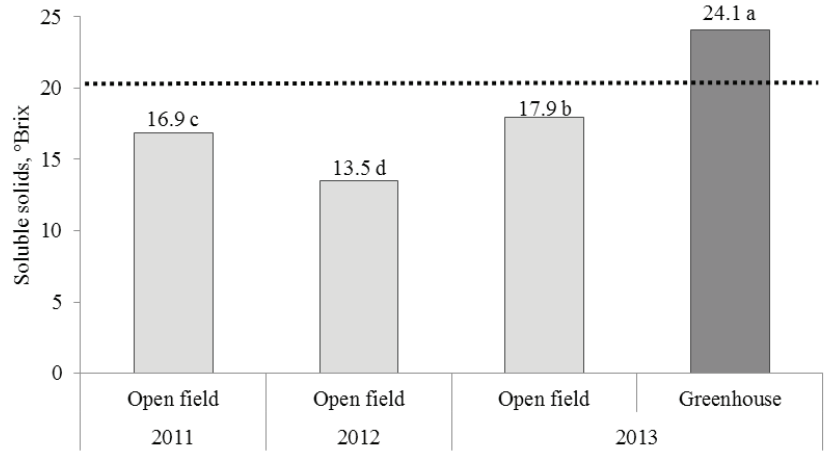

Figure 1. Soluble solid content of open-field (2011 – 2013) and greenhouse-grown 'Zilga' (2013). Horizontal line on the figure indicates the recommended 20 °Brix for wine berries (Schalkwyk and Archer, 2000).

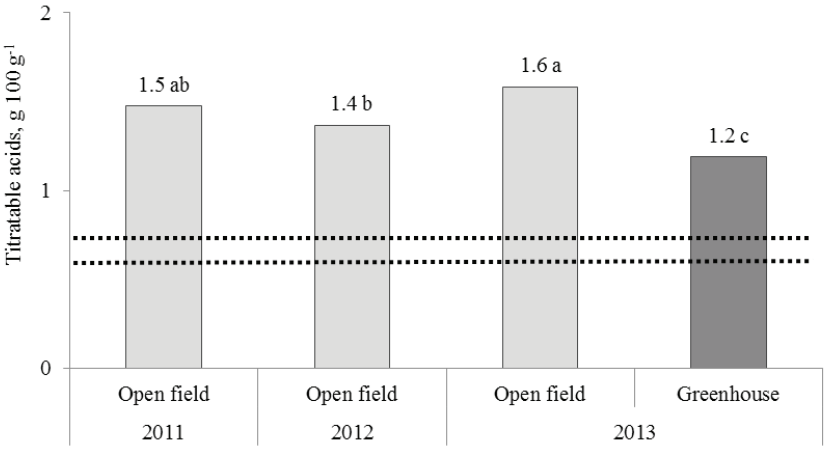

Figure 2. Titratable acids content of open-field (2011 – 2013) and greenhouse-grown 'Zilga' (2013). Horizontal lines on the figure indicate the recommended interval of TA for red table wine (Schalkwyk and Archer, 2000).

Maturity index

MI values ranged from 117 to 312, and did not reach the optimum only in 2012 (Figure 3). In a greenhouse, the MI increased above the optimum. MI is a combination of SSC and pH values that is generally used to determine the optimum ripeness of red wine grapes (Coombe et al., 1980). Our results indicate that there is a problem with obtaining MI because of SSC being low (Figure 1) and pH values high. Agroclimatic conditions and differences between the years lead to the fact that the weather has essential importance when growing high quality grapes (Martin and Dunn, 2000; Pedneault et al., 2013). This refers especially to cool climate conditions like in Estonia.

Total phenolic content

TPC varied from 214 to 540 mg 100 g^{-1} (Figure 4). In open field trials, the highest contents (293 mg 100 g^{-1}) were obtained in 2011, while the TPC showed equally lower concentrations in 2012 and 2013. Extremely high TPC (540 mg 100 g^{-1}) in greenhouse-grown berries could be related to day and night temperature fluctuations. TPC depend on several factors such as temperature (Haselgrove et al., 2000; Ferrer-Gallego et al., 2012); cultural practices (Peña-Neira et al., 2004; Palliotti et al., 2012) and grape developmental stage (Haselgrove et al., 2000). Phenolic compounds accumulate rapidly during the few weeks after veraison and may be variable according to the vine growing conditions (Topalovic and Mikulic-Petkovsek, 2010).

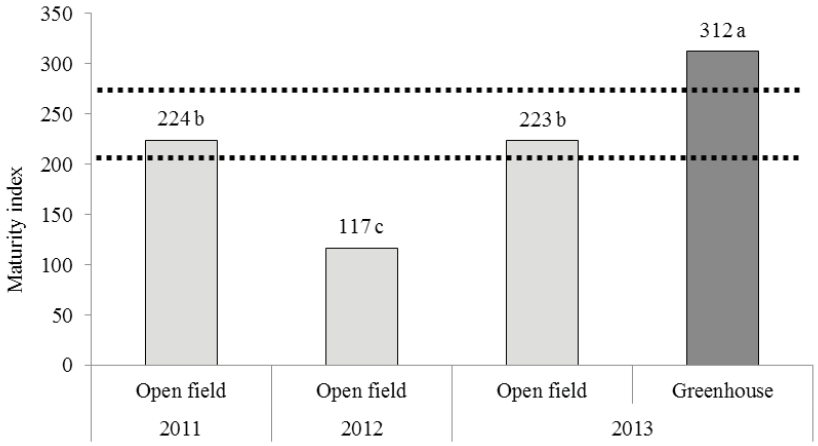

Figure 3. Maturity index of open-field (2011 – 2013) and greenhouse-grown 'Zilga' (2013). Horizontal lines on the figure indicate the recommended interval for wine grapes (Coombe et al., 1980).

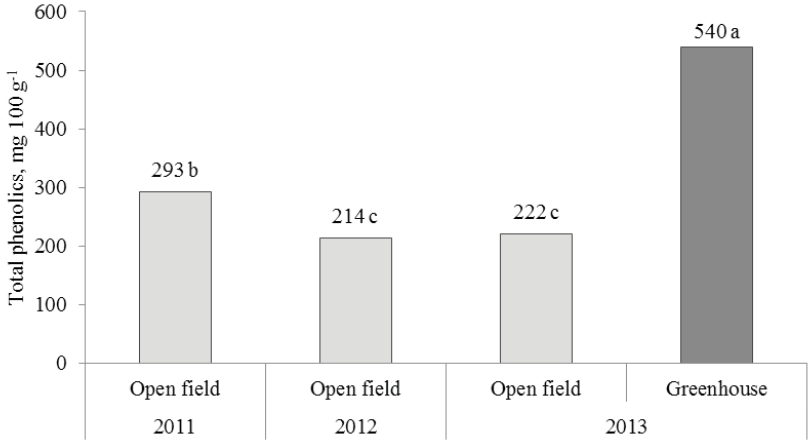

Figure 4. Total phenolic content of open-field (2011 – 2013) and greenhouse-grown 'Zilga' (2013).

High variability on temperature scale decreases water status and is more stressful to vines when compared to open field conditions. More phenolic compounds (including anthocyanins) accumulate under dryer and warmer conditions (Rio Segade et al., 2008). The temperature fluctuations and precipitation rate play the major role in grape ripening; even more when the minimum and maximum rates vary differently under the greenhouse conditions.

Anthocyanin content

ANC varied greatly ranging from 50 to 112 mg 100 g^{-1} (Figure 5). In open field conditions, ANC was significantly increased in 2013 (64 mg 100 g^{-1}), probably due to dryer and sunnier weather conditions in August and September compared to other experimental years (Table 1). Similar levels of ANC were determined in 2011 and 2012, 54 and 50 mg 100 g^{-1} respectively. The ANC varies greatly depending on cultivar and grape maturity (Ryan and Revilla, 2003; Fournand et al., 2006; Topalovic and Mikulic-Petkovsek, 2010). Secondly, the production area and seasonal conditions play the major role in red pigment accumulation (Ferrer-Gallego et al., 2012). Light interception and temperatures influence anthocyanin metabolism significantly, but too high temperatures (above +35 °C) inhibit their accumulation (Haselgrove et al., 2000). Though in the greenhouse the temperatures rose in some days extremely above +40 °C maximum, but the day and night temperature variations could have been affecting the ANC.

Conclusions

The present experiments with grape cultivar 'Zilga' can be concluded as follow:

1. Yield maturity parameters were significantly influenced by the weather conditions occurring in the specific growth year.

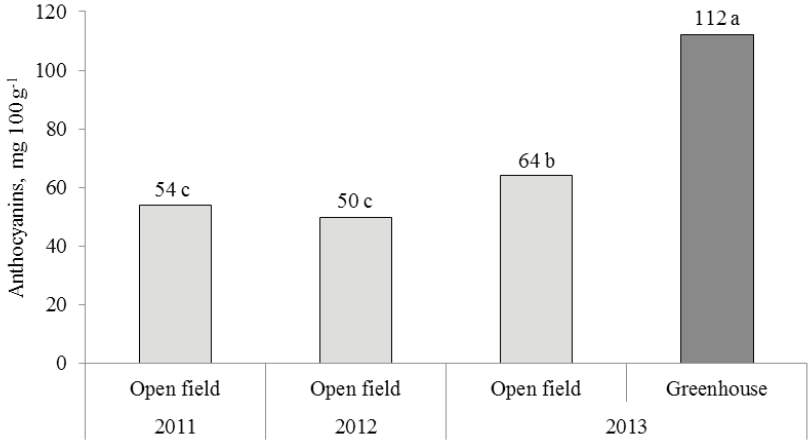

Figure 5. Anthocyanin content of open-field (2011 – 2013) and greenhouse-grown 'Zilga' (2013).

2. The recommended SSC was not obtained in an open field, but was reached in greenhouse conditions. TA remained too high compared to recommended values despite the conditions. MI (°Brix × pH^2) optimum was reached in two years from three, and the values were above the recommended level in greenhouse-grown grapes. Phenolic maturity was different according to the year – the highest TPC was obtained in 2011, but ANC in 2013.
3. The results of the present experiment suggest that Latvian origin hybrid grape cultivar 'Zilga' is with a good potential of quality red table wine production, but maturity parameters differ among the years. Therefore, further experiments with suitable growing technologies (like pruning, removing the leaves etc.) are in need to find out possibilities for achieving optimal maturity parameters in open field conditions.

Acknowledgements

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ANALYSIS OF CLIMATIC FACTORS IN CONNECTION WITH STRAWBERRY GENERATIVE BUD DEVELOPMENT

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Abstract
Strawberries (*Fragaria× Ananassa* Duch.) are widely grown in Latvia, and it is the first berry crop that ripens in summer. In Latvia, climate conditions are very suitable for strawberry growing. Unfortunately, meteorological conditions have become very changeable in recent years. The short day (SD) strawberry cultivars are the most important ones for Latvia, as they initiate flower buds in autumn. For these cultivars it is important to initiate flower buds at the end of September till the end of October. The day length has to decrease less than 14 hours per day to begin this process. In Latvia, the day length below 14 hours per day is at the end of August. The second important limiting factor for initiation of flower buds is the average day temperature, which must be between 10 and 15 °C. The studies on strawberry flower bud initiation in Latvia were done long time ago, on a very small scale and fragmentary. The current paper analyzes the temperature regime during the period of previous ten years (2004 – 2013) in autumn in Dobele in relation to suitability of this climatic factor to the flower bud initiation and strawberry yield next year. The soil surface temperatures during the last ten years in August were about +20 °C, which means that the flower bud initiation could not occur as early as it was described in the previous study.
Key words: temperature, day length, bud initiation.

Introduction

June-bearing strawberries (*Fragaria × Ananassa* Duch.) are short-day plants and initiate their flowers in the fall of previous year as day length shortens until 12 hours (Temperate zone…., 1993).

The strong interaction between photoperiod and temperature on flower induction shows that a lower temperature is required to achieve successful induction at longer photoperiod. Moreover, each cultivar has its own specific photoperiod × temperature interaction response curve for flower induction (Taylor, 2002). Only one study was found about strawberry plants flower bud initiation, which was carried out in Latvia 40 years ago by E. Muižarāja (Dūks, 1976). The research was carried out with cultivars bred in Russia. In this study it was concluded that the strawberry bud initiation for short day (SD) cultivars in weather conditions typical in Latvia for early cultivars begins in mid – August but for late cultivars in mid – September (Dūks, 1976).

According to research about the Latvian climate change carried out during the period from 1950 to 2010, an increase in the number of warm nights and a decrease in the number of cold days and nights in the second half of the 20th century has been observed as well as a slightly increased number of summer days with daily maximum temperatures above 25 °C (Avotniece et al., 2012), which could possibly affect the beginning of buds initiation since the year 2000 compared to the 70-ies when the only study was conducted.

Mostly the research on strawberry flower buds initiation had been done under controlled climatic and day conditions (Heide, 1977; Opstad et al., 2011), but only few studies in open field conditions (Opstad et al., 2011).were found.

The aim of the study was to evaluate the temperature regime from August to October during last ten years for strawberries in the open field to determine the potential of strawberry bud initiation time for short day's varieties.

Materials and Methods

Materials

Soil surface temperature data from 2004 till 2013 was collected by the meteorological station in Dobele (www.meteo.lv). Soil surface temperature was recorded every hour, and average soil surface temperature was assessed, as well as determined maximum and minimum soil surface temperatures per decade (October I,II,III) and per month (August, September and October). Soil surface temperature was reviewed for 10-year period, but closer studied data from 2008, when the first strawberry trial in the Latvia State Institute of Fruit-Growing in Dobele was planted. Day length was calculated from data http://www.suncalc.net. Flower bud initiation was determined from literature studies. Yield per plant (g) was calculated for the cultivar 'Polka' per year at the end of the harvest period. Data were collected for two planting periods 2008 and 2010. Yield was harvested per two cycles 2009-2010 and 2011-2012.

Statistical analysis

Data are expressed as mean ± standard deviation. For the statistical data analysis 95% probability was used to determine the significance of differences.

Results and Discussion

Studies on the bud initiation for strawberries have not been conducted in Latvia during past 40 years. However, foreign studies have shown that the flower buds can do initiation only when the day length reduces to 14 hours. The day length observations show that the day length in Latvia above 14 hours falls after August 25, and 12 hours achieves on September 25. If the day length achieves 14 hours at the end of August, cultivars flower bud initiation for SD strawberry can start at this time, not as it was mentioned in literature – at the beginning of August (Dūks, 1976).

Not only the day length is important to bud initiation, but also the adequate temperature is needed at this time. In contrast to research done by E. Muižarāja (Dūks, 1976), the flower bud initiation in Latvia can occur in August. The analyses of temperature and day length data shows that it could be impossible, because the day length was longer than 14 hours and average temperature in August was 19 – 22 °C (Fig. 1.). According to A. Sønsteby and O.M. Heide (2006) and M. J. Verheul et al. (2007), it is not suitable for the flower bud initiation.

Photoperiod and temperature are considered as two the most important environmental factors controlling the transition from vegetative to floral growth (Guttridge, 1985; Durner and Poling, 1988). Greatly varying threshold photoperiods (11 – 16 h) and temperature (9 – 25 °C) have been reported in various studies (Larson, 1994; Taylor, 2002; Hytönen et al., 2004). Temperature is also important for floral initiation under SD conditions. The optimum temperature for SD floral initiation is 15 – 18 °C, while below 10 °C and above 25 °C SD induction is rather ineffective (Sønsteby and Heide, 2006). G.M. Darrow and G.F. Waldo (1934) described the influence of temperature on flower initiation in strawberry, indicating the potential difficulty in photoperiodic classification of cultivars. Flower induction of SD types can occur under any photoperiod if the temperature is cool enough, generally <15 °C (Guttridge, 1985).

The effects of temperature and photoperiod on flower and inflorescence initiation during the autumn for the strawberry cultivar 'Elsanta' in the research carried out by P. Le Miere et al (1996) were investigated. This study shows that photoperiod had no effect on the rate of flower initiation or final flower number in the primary, secondary or tertiary inflorescences. The temperature had a little effect on the final flower number in the primary inflorescence. However, the rate of flower initiation increased linearly with increasing temperature in the secondary and tertiary inflorescences, namely to an optimum of 18.6 °C for the secondary and 19.9 °C for the tertiary inflorescence, and declined at temperatures above these.

The data analyses shows that in the years 2008, 2011 when autumn periods were relatively very warm, the next year berry yields, namely in 2009 and 2012, were much higher (for about 30 – 50%).

The average soil surface temperature at the 1st decade of October in 2008 was 9 °C, in 2011 it was 12 °C (Table). It can be concluded that enough high average air temperatures in October promoted better flower bud initiation in comparison to years when temperatures were lower (Fig. 2., Table).

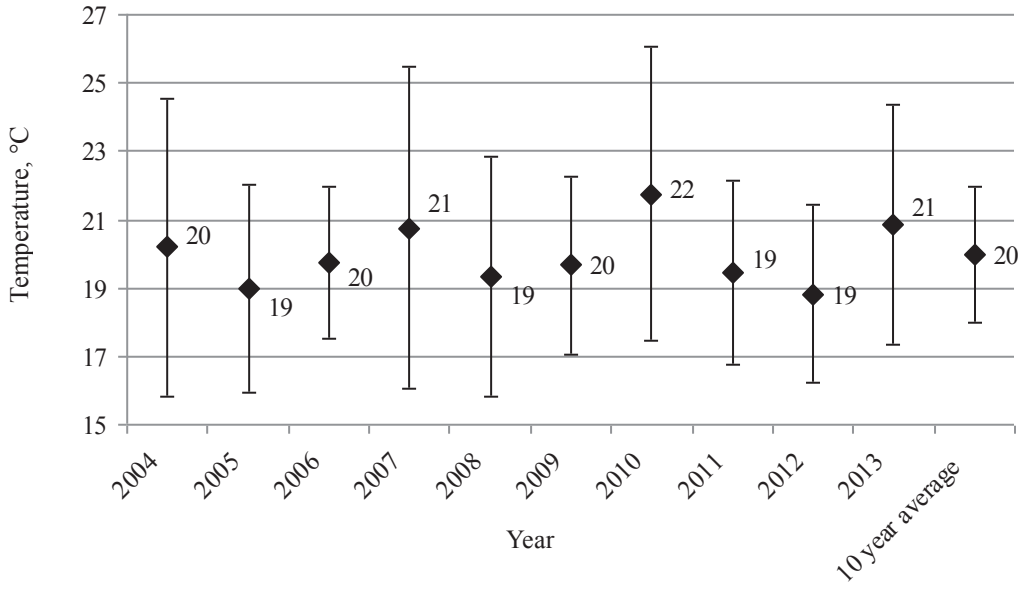

Figure 1. Average soil surface temerature in August years 2004 – 2013.

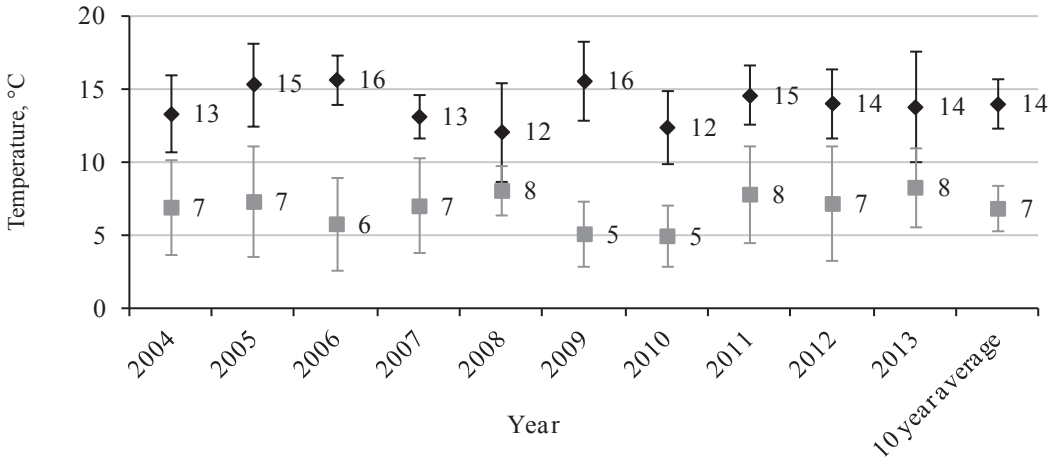

Figure 2. Average soil surface temerature in September and October years 2004 – 2013:
◆ September ■ October

Table

Temperature influence on the next year's strawberry yield

Planting year	Years of harvesting	Yield per plant, g	Soil surface temperature in the I decade of October:
2008	-	-	**8.6**
-	2009	**229**	6.9
-	2010	187	6.8
2010	2011	165	**11.8**
-	2012	**463**	9.8

In 2008, 2011 and 2013 there were no significant ($p>0.05$) differences in soil surface temperature among years. Data showed that only in October of 2008 both I and II decades' average soil surface temperatures were similar, 9 °C (Table, Fig. 3.). This taken together with a shorter day length may have prolonged bud initiation in the autumn as shown by higher yield next year.

In 2008, 2011 and 2013 were no significant ($p>0.05$) difference between soil surface temperature among years. Description of temperatures in October showed that in 2008 the I and II decades' average temperatures were similar (9 °C) (Table, Fig. 3.). It can prolong bud initiation in the autumn.

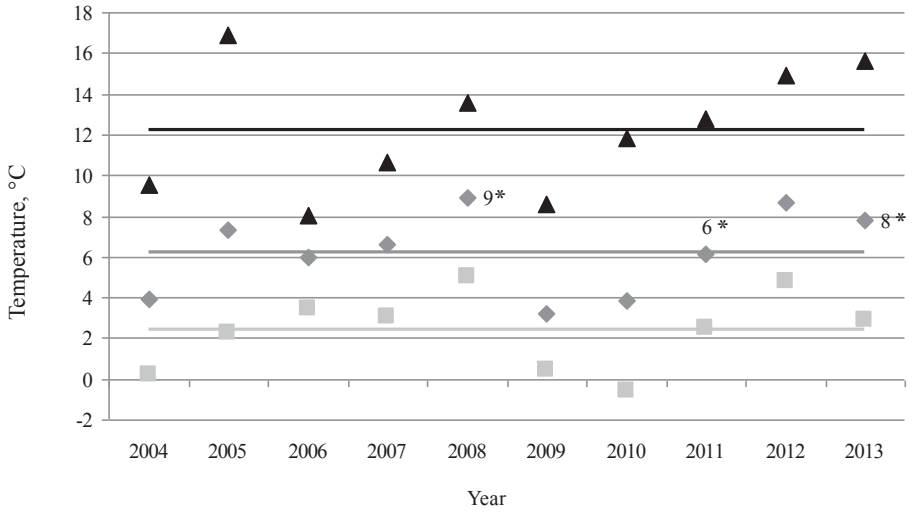

Figure 3. Soil surface temperature decade II of October years 2004 – 2013:
◆ Average ■ Minimum ▲ Maximum
10 Year Average 10 Year Minimum 10 Year Maximum

*– author observation

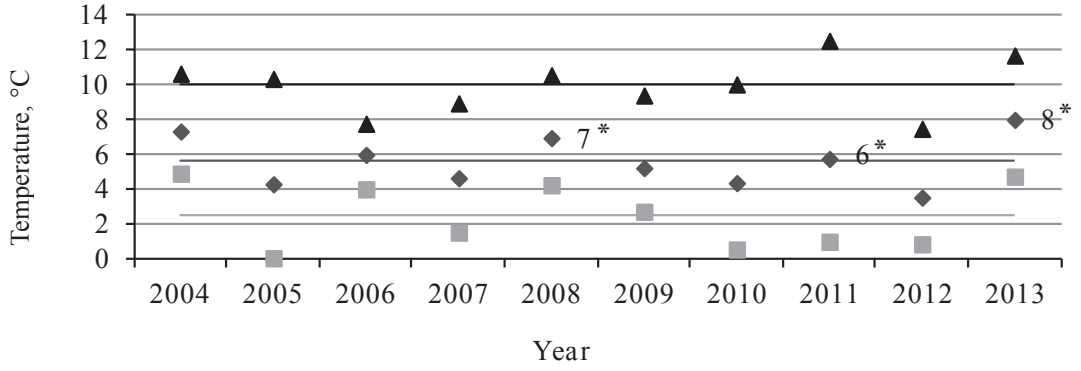

Figure 4. Soil surface temperature decade III of October years 2004 – 2013:

◆ Average ■ Minimum ▲ Maximum

—— 10 Year Average —— 10 Year Minimum —— 10 Year Maximum

*– author observation

Temperature observations in the third October decade in the period of ten years show that in 2004, 2008 and 2013 temperatures did not decrease below 7.0 °C. In other years temperature was lower and plant vegetative growth stopped (Fig. 4.).

Conclusions

1. Strawberry flower bud initiation during the period of last ten years could not occur in August because of too high temperatures. It could be related to long-term trends in climate changes in Latvia.
2. According to meteorological observations in Latvia, strawberry bud initiation time could be in September when the day length becomes less than 14 hours, and temperatures are suitable, 10…15 °C.
3. In 2008, 2011 and 2013 average soil surface temperature was more suitable for strawberry bud initiation, because average temperature was above10 °C till the beginning of October.

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LODGING CAUSE HEIGHT AT THE CENTRE OF GRAVITY CHANGES DURING VEGETATION PERIOD FOR OAT

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Abstract
Height at the center of gravity is a part of mathematical model to interpret risk of lodging used for cereal crops. Because of the anatomical changes during vegetation period, several measured parameters in early milk stage (stem and panicle weight) will differ from over-ripe stage results in their values. The aim of this study was to define approximate values of calculated parameters describing oat (*Avena sativa* L.) height at the center of gravity in early milk and over-ripe stages as well as determine connection with lodging risk. The trial was carried out at the State Stende Cereals Breeding Institute in 2013. There were 5 oat cultivars studied. At the investigated growing stages plant samples were taken from a field and in laboratory conditions stem/panicle weight and length measured. The height at the center of gravity and gravity ratio were calculated using mathematical model by Berry (1998). Results showed that the value of the height at the center of gravity in over-ripe stage was significantly higher ($p<0.01$) than in early milk stage, but correlation with risk of lodging remained. Such lodging resistance describing mathematical model can be useful for oat as well as wheat cultivars.
Key words: oat, stem lodging, centre of gravity, vegetation period.

Introduction

Lodging is a complicated process observed in field conditions, usually after the ear or panicle has emerged. It is the process by which the cereals are displaced from their upright position. Lodging in cereals generally occurs only after the ear or panicle has emerged and can seriously reduce profitability through reduced yield, delayed harvest, increased grain drying costs and reduced grain quality. It is especially unwelcome and dangerous in early milk stage, when the grains are in forming process (Tripathy et al., 2003; Berry et al., 2004).

There are two possible points of failure in the plant structure, the stem and the root. The elongated stem consists of a series of jointed hollow internodes connected by solid swollen meristematic nodes. The stem is strengthened by lignin, but may fail due to bending or buckling of the lower stem internodes. Root lodging results from a failure in root-soil integrity so that straight, unbroken culms lean or fall from the crown. Commonly lodging in oat occurs as a result of culm structural failure rather than loss of root anchorage.

Lodging risk is strongly influenced by wind, rain, cultivar and agronomical factors (variety choice, sowing date, seed rate, drilling depth, rate of nitrogen application, as well as the application of growth regulators). It is clear that weather plays an important role in determining lodging risk, but there is little published information quantifying the weather conditions needed to cause lodging event. Lodging was also found to be more closely associated with the occurrence of rainfall than the amount of rainfall (Baker et al., 1998). Dwarfing genes and growing regulators have been very effective tools for reducing lodging risk and maintaining steady improvements in yield. But on the other hand, in years of severe lodging, the applications of growth regulators have not prevented lodging completely and an analysis of the effects of dwarfing genes on wheat yield showed that the minimum crop height for optimum yield can be 0.7 m (Sterling et al., 2003, Berry et al., 2004).

A model of wheat canopy/root/soil system has been developed, which calculates the risk of stem and root lodging from crop parameters and soil characteristics. The two of the most important components of bending moment model, which describes lodging: height at the center of gravity of the shoot and ear area, are closely related to height and yield respectively (Baker, 1995). It would be important for agronomists and breeders. The model is attempted to work for wheat (*Triticum aestivum* L.), because most is known about the lodging mechanism of this cereal species (Baker et al., 1998; Berry et al., 2000; Berry et al., 2003). As this model is developed for all small-grained cereals it would be necessary to test it for oat (*Avena sativa* L.) as well.

Because of the anatomical changes during vegetation period, several measured parameters in early milk stage (stem and panicle weight) will differ from over-ripe stage results in their values. The task of trial was to define approximate values of calculated parameters describing oat height at the center of gravity in early milk and over-ripe stages and determine connection with lodging risk.

Materials and Methods

The field trials were carried out at State Stende Cereals Breeding Institute in 2013. Five oat cultivars (int. al. four varieties – 'Stendes Darta', 'Laima', 'Arta', 'Kirovec'; and one breeding line – '33122')

Table 1

Meteorological data in the experimental period (Stende meteostation data, 2013)

Month	The mean daily temperature, °C			Sum of precipitation, mm		Percentage of monthly precipitation from long term average, %
	Monthly	Long term average	Long term average +/-	Monthly	Long term average	
April	4.0	4.3	-0.3	34.9	37.0	94.3
May	13.7	10.2	3.5	86.1	45.0	191.3
June	16.9	14.2	2.7	74.5	57.0	130.7
July	16.9	16.3	0.6	36.2	87.0	41.6
August	16.6	15.5	1.1	45.2	87.0	52.0

were used. The soil of the site was sod-podzolic, the humus content – 20 g kg^{-1}, the soil pH KCl – 6.6, the available for plants content of phosphorus P – 39 mg kg^{-1}, and that of potassium K – 53 mg kg^{-1}. The pre-crop was barley. All agro-technical operations were carried out at optimal terms according to the weather conditions during the vegetation period, depending on the plant development phases. Seed rate was 500 seeds per 1 m^2. Before cultivation of the soil a complex mineral fertilizer was applied: N – 51, P – 30, K – 42 kg ha^{-1}. Variants were arranged in four replications with a plot size 10 m^2 in a randomized block design.

The temperature and moisture conditions provided perfect oat field germination in 2013 and are represented in Table 1. The mean daily temperature changes were insignificant. Precipitations exceeding long term average and sufficient mean daily temperatures in May and June provided good conditions for germination and tillering. Low sum of precipitation and mean daily temperature close to long-term average in July and August ripened oat grains and gave excellent yield. However, strong wind gusts through all vegetation period provided good conditions for stem lodging.

Twice in vegetation period in principal growth stages – early milk (code 73 after BBCH-scale) and harvest-ripe (code 92 after BBCH-scale) – there were taken a bundle of examples containing 40 plants from each cultivar (10 from each replication). Plants were used for measurement from tillering node to the end of panicle. In experimental investigation the length and weight of stem and panicle for each plant were measured. All measured parameters were used for calculation the height at the center of gravity and gravity ratio of the height at the center of gravity and plant height, by setting them in formulas (1) by P.M. Berry, 1998 and (2) by authors.

$$X = \frac{\left(S_L S_W + 2S_L E_W + E_L E_W\right)}{2\left(S_W + E_W\right)} \quad (1)$$

$$k = \frac{X}{(S_L + E_L)} \quad (2)$$

where

X – height at the center of gravity (cm),
S_L – stem length (cm),
S_W – stem weight (g),
E_L – panicle length (cm),
E_W – panicle weight (g),
k – gravity ratio.

Before harvesting the lodging resistance was observed on field for each cultivar in 9 point system:
9 points – very high lodging resistance – all stems are on their upright position,
7 points – high lodging resistance – 25% of stems are laid down in 30°,
5 points – average lodging resistance – 50% of stems are laid down in 45°,
3 points – low lodging resistance – 75% of stems are laid down in 60°,
1 point – very low lodging resistance – all stems are laid down, harvesting is not possible.

The obtained results were statistically processed by MS Excel program package using the methods of descriptive statistics; arithmetic mean value and standard division were calculated for each measured and calculated parameter. ANOVA procedures were used for data analysis. P-values less than 0.05 were considered to be statistically significant.

Results and Discussion

The cultivars selected for study varied in their anatomical measurements and are represented in Table 2. Stem length varied among tested cultivars from 89.13 (‘Kirovec’) to 109.50 cm (‘Arta’) in GS 73 and from 81.56 (‘Kirovec’) to 103.57 cm (‘Arta’) in GS 92. Panicle length varied from 17.28 (‘Kirovec’) to 20.43 cm (‘Arta’) in GS 73 and from 14.48 (‘Kirovec’) to 17.34 cm (‘Arta’) in GS 92. Both stem and panicle length was significantly ($p<0.01$) lower in GS 92, compared with GS 73. By physiological processes in

plants, it is explainable with ripening process. Plant cells are still green and full with water and nutrients in milk development stage, but through dough and ripening stages plant cells are drying and dwindling. As cells are becoming smaller also full plant becomes shorter compared with milk development stage. Unfortunately, there is a lack of literature describing plant stem structure changes in ripening process.

Stem and panicle weight has significant ($p<0.01$) difference between growing stages as well. Stem weight varied from 5.73 ('Kirovec') to 9.25 g ('Laima') in GS 73 and from 1.17 ('Kirovec') to 2.04 g ('Stendes Dārta') in GS 92 (represented in Figure 1). The weight in GS 73 was approximately 3.5 to 4.9 times heavier than in GS 92.

Similar situation was observed with panicle weight, only the difference is 1.5 to 2.2 times (comparing with stem weight approximately four times). It is explained with grain filling process in scale. Panicle is drying and loses its weight, instead of grains in it which are ripening and getting heavier. Stems have no component (internode, nodes, and leaves) which would get heavier; moreover, part of nutrients from straw to grains is increasing their weight.

Plant height is depicted in Table 3. The statement that cultivars with higher plants have higher lodging risk is false. Results of this study showed that the cultivar 'Kirovec' whose characteristics is a shorter plant length; lodging was the highest – 3 points, observed on field. A mathematical model is given for calculating height at the center of gravity in literature (Berry et al., 2004), but unfortunately physiological processes and results are not mentioned. Height at the center of gravity describes the place of the plant, where the force line of gravity is going through. If this parameter is located lower, the risk of lodging is larger.

Table 2

Values of measured parameters used in the model of lodging risk determination, mean ± sd[1]

Cultivar	Stem length, cm		Stem weight, g		Panicle length, cm		Panicle weight, g	
	GS 73a[2]	GS92b	GS 73a	GS92b	GS 73a	GS92b	GS 73a	GS92b
Stendes Dārta	100.28 ±4.72	92.44 ±3.75	7.13 ±0.59	2.04 ±0.23	17.56 ±0.27	15.75 ±0.77	2.57 ±0.20	1.71 ±0.30
33122	96.23 ±4.91	87.27 ±4.93	8.64 ±1.91	1.90 ±0.39	19.06 ±1.04	15.65 ±1.31	3.73 ±0.59	1.71 ±0.58
Arta	109.50 ±2.66	103.57 ±2.84	8.02 ±0.87	1.84 ±0.27	20.43 ±0.60	17.34 ±1.49	3.56 ±0.43	1.68 ±0.27
Kirovec	89.13 ±5.20	81.56 ±5.82	5.73 ±1.29	1.17 ±0.16	17.28 ±1.43	14.48 ±0.78	3.18 ±0.57	1.62 ±0.28
Laima	102.83 ±1.46	97.38 ±7.35	9.25 ±0.49	1.95 ±0.37	18.48 ±0.86	15.33 ±1.68	3.53 ±0.41	1.57 ±0.50
$LSD_{0.05}$	3.01		0.54		0.71		0.28	
$LSD_{0.01}$	4.05		0.73		0.96		0.38	

[1]sd – standard deviation, [2]Trait means followed by different letters are significant by different growing stages at the level of $p<0.01$.

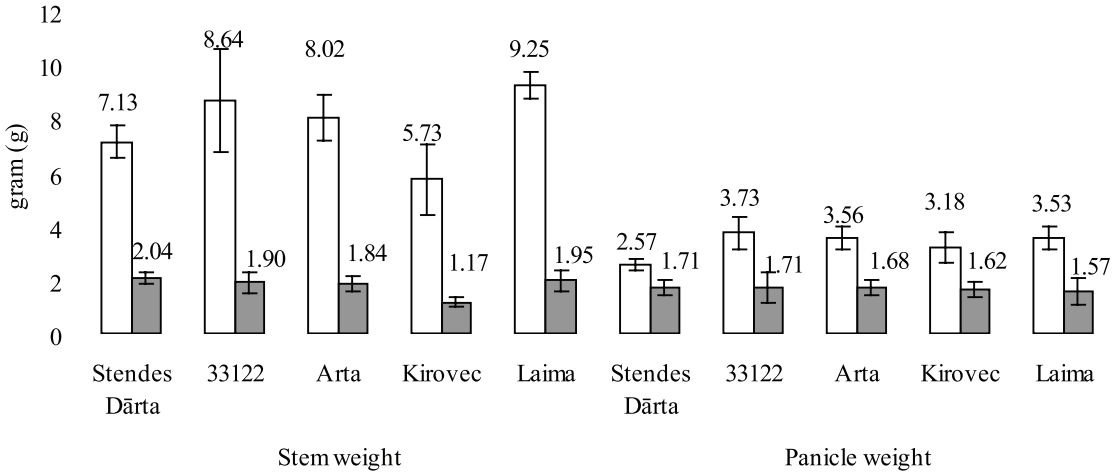

Figure 1. Stem and panicle weight (g) changes during vegetation period; where GS 73 □, GS 92 ■.

Table 3

Relationship between lodging resistance and calculated plant parameters

Cultivar	Lodging (points)	Plant height (cm)		Height at the center of gravity (cm)		Gravity ratio	
		GS 73a[1]	GS 92b	GS 73b	GS 92a	GS 73b	GS 92a
Stendes Dārta	9	117.84	109.02	65.65	70.47	0.56	0.65
33122	9	115.30	102.91	65.53	66.84	0.57	0.65
Arta	7	129.93	120.91	74.70	80.60	0.58	0.67
Kirovec	3	106.41	96.04	63.59	68.33	0.60	0.71
Laima	9	121.30	112.71	68.11	73.41	0.56	0.65
$LSD_{0.05}$		3.39		2.54		0.01	
$LSD_{0.01}$		4.56		3.43		0.01	

[1]Trait means followed by different letters are significant by different growing stages at the level of $p<0.01$.

Table 4

Correlation coefficients (r) between lodging resistance and calculated plant parameters ($n=5$; $r_{0.05}=0.878$; $r_{0.01}=0.959$)

GS 73	Plant length	Height at the center of gravity	Gravity ratio	Lodging resistance
Plant length	1.000	*	*	*
Height at the center of gravity	0.935[a]	1.000	*	*
Gravity ratio	-0.416	-0.055	1.000	*
Lodging resistance	0.524	0.203	**-0.955**[a]	1.000
GS 92	Plant length	Height at the center of gravity	Gravity ratio	Lodging resistance
Plant length	1.000	*	*	*
Height at the center of gravity	0.905[a]	1.000	*	*
Gravity ratio	-0.504	-0.088	1.000	*
Lodging resistance	0.491	0.075	**-0.998**[ab]	1.000

[a] Correlation is significant at the level of $p<0.05$; [b] Correlation is significant at the level of $p<0.01$.

As the height at the center of gravity is hardly related to plant height and plant height for various cultivars is different, it is not comparable parameter among tested cultivars. For accurate comparison a mathematically determined parameter – gravity ratio, which shows the proportion between height at the center of gravity and plant height was established. The gravity ratio in GS 73 for cultivars with low lodging resistance (9 points) was the lowest 0.56 ('Stendes Dārta' and 'Laima') – 0.57 ('33122'), but for the variety 'Kirovec' – 0.60, at it is characterized with low lodging resistance (3 points). For the cultivar 'Arta' the gravity ratio was 0.58, but lodging resistance 7 points. In addition, there was observed the fact that gravity ratio in GS 92 is higher than in GS 73. It is explainable with physiological changes in plants during ripening. As the measurement of the plant parts differs in growing stages used for calculations, calculated parameters are also different. Plant physiological changes during ripening make the gravity ratio higher; for example, for the cultivar 'Stendes Dārta' the gravity ratio in GS 73 was 0.56, but in GS 92 – 0.65.

Correlation among mathematically calculated parameters and lodging resistance is performed in Table 4.

Strong negative correlation ($r = -0.955 > r_{0.05} = -0.878$ in GS 73 and $r = -0.998 > r_{0.01} = -0.959$ in GS 92) was observed between the lodging risk and gravity ratio as it was described previously. Consequently, if the gravity ratio is higher, lodging resistance will be lower. As the correlation between lodging resistance and height at the center of gravity is only $r = 0.203$, it is necessary to improve it. Authors offer a model (Formula 3) for lodging resistance detection

combining model of P.M. Berry and their own formula of ratio calculation:

$$k = \frac{\frac{(S_L S_W + 2S_L S_W + E_L E_W)}{2(S_W + E_W)}}{(S_L + E_L)} = \\ = \frac{(S_L S_W + 2S_L E_W + E_L E_W)}{2(S_W + E_W)(S_L + E_L)} \quad (3)$$

By using such a model, it will be possible to detect lodging resistance for oat cultivars in both early milk and harvest-ripe growing stages. Furthermore, the gravity ratio could be useful for oat breeders at meteorological conditions, when lodging is not observed.

Conclusions

1. A significant ($p<0.01$) difference between the values of measured parameters in early milk and harvest-ripe stages, showing higher values in early milk stages was observed.
2. A strongly negative correlation ($r = – 0.998$) between the gravity ratio and lodging resistance for tested cultivars, meaning that lodging resistance could be calculated from height at the center of gravity was observed.
3. Gravity ratio changes were observed in both growing stages for all tested cultivars; for example, for the variety 'Kirovec' in early milk stage ratio was 0.60, but in over-ripe stage 0.71.
4. The formula for calculating gravity ratio could be used for oat lodging resistance determination in both early milk and over-ripe stages.

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FERTILISATION EFFECT ON BIOMASS FORMATION OF PERENNIAL GRASS USED AS ENERGY CROP

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Abstract
Bioenergy production growth rates in the recent years are leading to waste – digestate and wood ash amount increases, which are essential to be managed in the most efficient and safe way. In the formation of plant nutrient recycling these waste products are useful to be included in the energy crop fertilisation plan. In order to study the waste products application options for energy crops – reed canary grass (*Phalaris arundinacea* L.) and festulolium (×*Festulolium pabulare*) fertilisation trials were arranged in 2012 at the Skriveri Latvian University of Agriculture (LLU) Research Institute of Agriculture. In all fertiliser treatments: wood ash; digestate one time per season; digestate two times per season and mineral fertilisers the same doses of the main plant nutrients (N, P, K) were provided, the missing quantities of elements compensating with mineral fertilisers. To obtain the grass biomass, two cutting regimes were used – two-cut and one-cut harvest system. This article summarizes the findings on the productivity of the 1st year of use grassland swards and sward structure data. The productivity of perennial grass biomass was dependent on the type of applied fertilisers, grass species and cutting regime. In the first production year the highest average dry matter yield (7.30 t ha^{-1}) was produced by reed canary grass. The highest DM yields in average for both grass species were obtained by mowing once per season – in autumn at crop senescence (7.01 t ha^{-1}) and in fertilisation treatments of wood ash (WA) – 8.06 t ha^{-1} and mineral fertilisers (MF) – 7.24 t ha^{-1}.
Key words: by-products, energy crop, DM yield, festulolium, reed canary grass.

Introduction

The increase in proportion of alternative energy resources has become more pertinent in the recent decades, which are due to the concern of global warming and efforts to limit it through adoption of EU directives and international commitment for the limitation of fossil energy use and its partial replacement with renewable energy resources.

Biomass is the most common form of renewable energy, widely used in the third world. Energy can be obtained from biomass by direct combustion or fermentation (Prochnow et al., 2009). Lately much attention has been focused on identifying suitable biomass species, which can provide high energy outputs and to replace conventional fossil fuel energy sources (McKendry, 2002). The most promising crops for bioenergy are perennials: they can be harvested for several years in succession without reseeding and give high biomass yield with satisfactory biomass quality (Lewandowski et al., 2003; Jasinskas et al., 2008; Sanderson and Adler, 2008). Perennial grasses are environmentally friendly, pest resistant and less demanding in terms of soil conditions compared to other crops (Peeters, 2008). Using grasses as a source of bio-methane negates the need for tillage, and allows for carbon sequestration (Seppala et al., 2009; Murphy et al., 2013). In Latvia is provided suitable soil and climatic conditions for their growth as the amount of rainfall is sufficient, and distribution of precipitation is mostly favourable for high herbaceous biomass yield formation (Rancane et al., 2012). Perennial grasses may compete with maize in respect to the yield and yield stability (Techow et al., 2011).

An anticipated middle-term decrease of grassland use for feed is to be seen alongside an ever growing demand for sustainable energy from renewable energy resources (Wachendorf et al., 2009). Promissing alternative regarding the utilisation of biomass from grassland is using biomass low in digestibility for the generation of biogas (electrical power) and grass pellets for combustion purposes (Blumenstein and Möller, 2011).

Grass is an excellent source of bio-methane; it is a feedstock for anaerobic digestion with a high content of solids, and it has a high specific methane capacity. As a general rule, the specific methane yield of grass silage will increase the earlier the harvesting date, whereas the yield of biomass obtainable per unit grassland area will depend on the attainable growth of the crop (Prochnow et al., 2005; McEniry and O'Kiely, 2013). The increase in fibrious structure leads to a slower rate of degradation and also increases the required hydraulic retention time. However, reed canary grass has been shown to give higher specific methane yields with advancing crop maturity and decrease water content (Lehtomäki et al., 2008).

Reed canary grass (RCG) as a cool-season perennial grass is a promising bio-energy crop and a potential non-wood crop for industrial uses (e.g., papermaking) in Northern Europe (Saijonkari-Pahkala, 2001; Lewandowski et al., 2003; Kukk et al., 2011). RCG can be grown in a wide range of soils and environment and has been used as a pasture and hay

crop for many decades, and produce relatively high yields of total biomass (Wrobel et al., 2009; Tahir et al., 2010). Festulolium is a cross between fescue and ryegrass; therefore, it should have the winter-hardiness, persistence and stress tolerance of *Festucas* and the yield quality of *Lolium* (Bardule et al., 2013).

In the process of biomass direct combustion and fermentation waste products are formed – ash and digestate, the amounts of them tend to rise due to the increasing production levels. It is essential to manage their utilisation in the most efficient and safe way, providing the plant nutrient cycling. The ash is rich in potassium (K), calcium (Ca), sulphur (S), phosphorus (P), chlorine (Cl), and silicon (Si) (Insaam et al., 2009). Digestate, the residue from biogas processing, can be used as an organic fertiliser. In addition to energy generation, this route is a realistic choice for recycling plant nutrients, especially phosphorus, which will be a scarce plant production resource in the near future (Cordell et al., 2009).

The objective of this study was to determine the opportunities to use waste products as energy grasses fertilisers and to compare the effect of different type of fertiliser on reed canary grass and festulolium dry matter yield and biomass structure.

Materials and Methods

Experimental plots were established in the July of 2012, it was located in the central part of Latvia at the LLU Research Institute of Agriculture in Skriveri (56°41` N and 25°08` E). The soil of the experimental plot was classified as Endoluvic Epistagnic Phaeozem/ Stagnic Cutanic Albeluvisol (WRB, 2006), fine sandy loam.

Before establishing trials, the experimental area was divided into 40 plots, and from each plot soil samples were taken. The soil samples were prepared for agro-chemical analyses in accordance with LVS ISO 11464 (drying, crushing, sieving). Agrochemical parameters (pH KCl; pH H_2O; $C_{tot.}$, sulphur, organic matter, plant available P and K) were determined. The exchangeable soil acidity was measured in 1*M* KCl suspension potentiometrically (LVS ISO 10390/ NAC). The total carbon and sulphur was analysed using an ELTRA CS 530 element analyzer. The plant available phosphorus and potassium were determined using the Egner-Rheem (DL) method.

Two species of perennial grasses were included in the test: reed canary grass (*Phalaris arundinacea* L.) 'Bamse' and festulolium of tall fescue type (×*Festulolium pabulare*) 'Felina'. Following fertilisation treatments were used: C – control – not fertilised; MF – mineral fertilisers (ammonium nitrate, potassium sulphate and superphosphate); WA – wood ash; D 1 – digestate once per season; D 2 – digestate twice per season.

The fertiliser doses were chosen so that each fertilisation variant would provide for the same amount (kg ha^{-1}) of main plant nutrient elements per year: nitrogen (N); phosphorus (P_2O_5) and potassium (K_2O), accordingly – 100:80:160. The doses were decreased by approximately half in the sowing year (42 kg ha^{-1} N; 32 kg ha^{-1} P_2O_5 and 80 kg ha^{-1} K_2O), applying the correct amount of fertilisers in each variant. The fertilisers were applied manually before the perennial grasses were sown, after that they were incorporated into the soil with a soil tiller.

At the beginning of the vegetation period of the following year, the fertilisers were applied on the soil surface, in D2 variant the annual dose of digestate was split – one half was applied at the beginning of the vegetation period, the other – at the end, after the perennial grasses were cut. The missing amount of the nutrients was compensated using fertilisers in all fertilisation variants.

Perennial grasses were sown using a narrow row spacing with the drill 'Nordsten NS–1025'. Seeding rates: reed canary grass 12 kg ha^{-1} but festulolium 15 kg ha^{-1}. The size of one experimental plot was 43.2 m^2 (9.6 × 4.5 m), the total number of plots was 40. The variants of fertilisers were distributed randomly in 4 replications.

Three weeks after the experimental sowing, plants were sprayed with herbicide MCPA 750 to control dicotyledonous weeds. In September of the sowing year, swards were mowed.

In the 1st year of use the perennial grass sward productivity was established in both, intensive (two-cut harvest system) and extensive (one-cut harvest system – delayed cutting in autumn at crop senescence) mowing types. Both, swards and productive tillers, were measured in length, plants and tillers were counted in a 0.5 m long line, before the calculation of the 1st harvest dry matter yield. A sample sheaf was taken for botanic analysis and the establishment of perennial grass sward structures by cutting the perennial grasses with a sickle. All measurements and calculations were carried out 4 times for each fertilisation type.

Meteorological conditions during the trial years were different. The year 2012 was characterized as a rich with precipitation, the annual rainfall was 928 mm (it is 139% of a long-term average). Precipitation during the vegetation period in 2013 was slightly lower than the long-term average, and its distribution was not favorable for grass growth – hot and dry periods were interrupted by short and heavy rainfalls. Lack of moisture in July and August had a negative impact on the plant development. Precipitation distribution in Skriveri for years 2012 – 2013 compared with long-time average rates is shown in Figure 1.

The experimental data were statistically analysed using a two-way analysis of variance with grass species and fertiliser as factors and dry matter data assessed by using a three-way analysis of variance, with the cutting time as third factor. The differences among means at the 0.05 probability level (Excel for Windows 2003) were detected by LSD.

Results and Discussion

Soil analysis was performed to monitor their changes during the experiment. Some uniformity of soil properties was observed (Table 1). The average soil acidity pH KCl was 5.7 (ranged between 5.5 – 5.9); the average pH H_2O was 6.7 (ranged between 6.5 – 6.9). The average $C_{tot.}$ content was 24.3 g kg^{-1}, and ranged from 22.3 to 27.83 g kg^{-1} within individual plots. The average organic matter (OM) content was 41.9 g kg^{-1}, and ranged from 40.2 to 48.0 41.9 g kg^{-1}, but differences were not significant ($p<0.05$). In the test area plant available nutrients through the plots varied within the following limits: 75.3 – 117.7 mg kg^{-1} P_2O_5, 117.6 – 163.7 mg kg^{-1} K_2O and 13.2 – 44.8 mg kg^{-1} S.

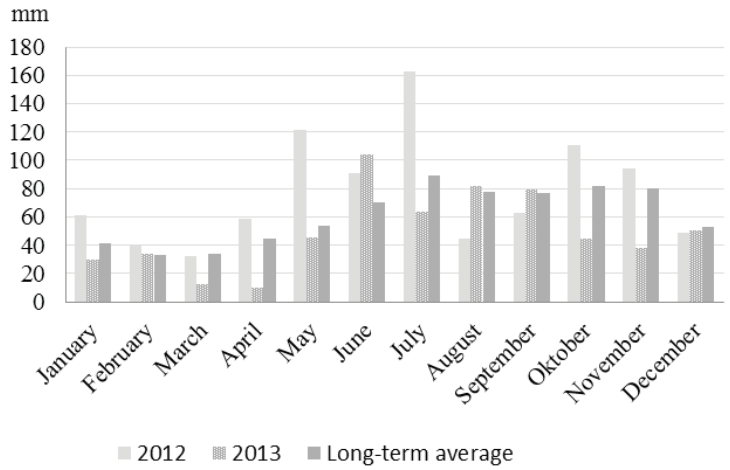

Figure 1. Precipitation over the months in 2012 and 2013 compared with long-term average rates in Skriveri.

Table 1

Soil properties before the experiment

Fertiliser variant	pH KCl	pH H_2O	$C_{tot.}$, g kg^{-1}	OM, g kg^{-1}	P_2O_5, mg kg^{-1}	K_2O, mg kg^{-1}	S, mg kg^{-1}
Reed canary grass							
Control	5.5	6.6	24.6	42.4	96.3	121.8	25.5
Mineral fertilisers	5.8	6.8	27.8	48.0	106.4	143.4	13.2
Wood ash	5.9	6.9	24.6	42.5	91.8	123.4	20.4
Digestate 1×	5.6	6.5	25.4	43.9	75.3	129.4	35.1
Digestate 2×	5.8	6.7	23.5	40.5	117.7	163.7	44.8
Average	5.7	6.7	25.2	43.5	97.5	136.3	27.8
$LSD_{0.05}$	*0.4*	*0.4*	*7.1*	*12.3*	*64.3*	*52.1*	*48.3*
Festulolium							
Control	5.7	6.6	22.5	38.9	102.0	127.5	37.8
Mineral fertilisers	5.7	6.9	24.8	42.7	92.7	122.1	18.3
Wood ash	5.7	6.7	23.3	40.2	82.5	117.6	17.8
Digestate 1×	5.7	6.8	24.0	41.4	89.5	128.7	23.7
Digestate 2×	5.7	6.8	22.3	38.4	105.2	124.7	14.7
Average	5.7	6.7	23.4	40.3	94.4	124.1	22.4
$LSD_{0.05}$	*0.41*	*0.4*	5.5	*9.6*	*70.2*	*47.7*	*33.3*

The statistical analysis shows that there were no significant differences among the mentioned values, all agrochemical parameters ranged within the significance limits ($p<0.05$); thus it can be concluded that the soil was homogeneous before the test set-up, and the natural background of soil will not significantly affect the results of the following experiments.

RCG and festulolium sward length measurements and the counting of plants/productive tillers in the field conditions were carried out to evaluate how fertilisers for the 1st year 1st sward yield affected the growth intensity, plant density and formation of productive tillers. The differences between the number of plants and productive tillers were not significant for different fertilisation variants of single species ($p<0.05$). Between species the RCG swards had the highest plant density, but festulolium swards had more productive tillers (Table 2). RCG has the tendency to tiller intensively in the 1st year of use, especially in sparse swards, just like the ones used in our experiment. The average number of productive tillers for a single RCG plant was 0.7, for festulolium – 2.7.

Results indicate that the average sward length of RCG in the beginning of June was 126.1 cm, which is significantly ($p<0.05$) higher than the average length of festulolium sward 96.1 cm (Table 2). The use of fertilisers for both perennial grass species ensured the growth of sward length. Growth of sward length for festulolium was observed in all fertilisation variants compared to control, the longest swards were in mineral fertiliser (MF) and wood ash (WA) variants, but differences were not significant ($p<0.05$). RCG had a significant growth in all variants. After evaluating the factor interaction effect, it was found that in the wood ash variant it was relatively lower for

Table 2

The field measurements of sward height and density before the mowing of 1st cut

Fertiliser variant (B)	RCG (A)		Festulolium (A)		Height of sward, cm	
	Plants in 0.5 m row	Tillers in 0.5 m row	Plants in 0.5 m row	Tillers in 0.5 m row	RCG (A)	Festulolium (A)
Control	18.3	6.5	26.0	11.0	106.0	85.8
Mineral fertilisers	15.0	16.0	30.3	9.3	140.6	94.4
Wood ash	13.8	13.0	27.0	12.0	138.4	93.3
Digestate 1×	14.8	8.5	32.0	12.3	123.3	89.3
Digestate 2×	15.5	10.5	34.3	11.3	122.2	95.1
$LSD_{AB\ 0.05}$	9.7	9.7	11.4	5.9	9.6	9.6
Average	15.5	10.9	29.9	11.2	126.1	91.6

Table 3

The 1st cut sward structure analysis

Fertiliser variant	Average length of tillers, cm	Productive tillers, %	Number of tillers per m^2	Length of ears, cm
RCG				
Control	102.3	10.2	62.7	5.5
Mineral fertilisers	131.0	22.0	182.3	8.0
Wood ash	135.0	13.0	125.4	8.8
Digestate 1×	122.9	14.1	89.2	6.7
Digestate 2×	119.9	16.7	138.1	6.4
$LSD_{0.05}$	8.6	10.5	94.8	0.9
Festulolium				
Control	72.1	91.7	117.4	14.5
Mineral fertilisers	81.8	83.9	170.8	16.9
Wood ash	80.3	89.3	217.9	16.8
Digestate 1×	78.8	82.3	160.2	16.0
Digestate 2×	83.9	100.0	158.4	17.6
$LSD_{0.05}$	6.8	19.6	52.0	1.9

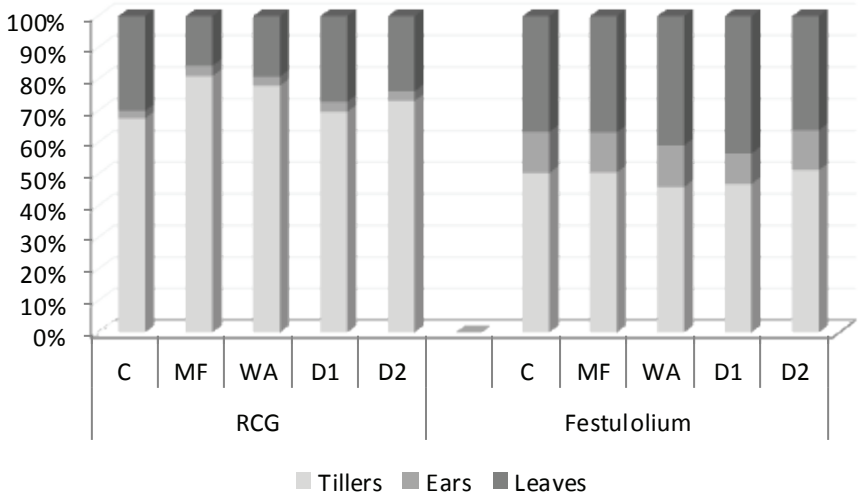

Figure 2. The structure (% from DM yield) of the 1st cut sward of RCG and festulolium.

reed canary grass, but relatively higher for festulolium compared to general tendencies (main effects).

Before the calculation of the 1st cut perennial grasses yield, sward sample sheaves were analysed in order to determine the average length of productive tillers and compare the sward structure in each fertilisation variant. The use of all fertiliser types promoted a significant growth of productive tillers for both, RCG and festololium, ensuring additional length of 17 – 33 cm for RCG and 6 – 11 cm for festulolium productive tillers compared to control. The largest increase in RCG sward height of the first cut was contributed by WA fertiliser use (Table 3).

After evaluating the fertiliser effect on the perennial grass sward development, it can be concluded that for RCG during the 1st year of use, the use of all fertilisers slightly increased the growth of the number of productive tillers in the 1st cut sward, but the number of productive tillers was significantly ($p<0.05$) higher only in MF variant (Table 3). For festulolium different fertilisers made no significant effect on the number of tillers in the 1st cut sward.

For RCG unproductive tillers had the proportionally highest number, which was 55 – 66%, the number of productive tillers on average made up 10 to 20%, whereas undeveloped tillers – 20 to 30%. For festulolium the sward was mostly made up of productive tillers (82 – 100%), there was a very small number of unproductive and undeveloped tillers. Some festulolium samples had no unproductive and/or undeveloped tillers at all. As a result of using any of the fertilisers, the length of ears was significantly higher ($p<0.05$) for RCG, as well as for festulolium. WA and MF variants had the strongest effect on the ear length of RCG, D1 and D2 fertilisation showed very good results for festulolium (Table 3).

Perennial grass sward analysis allows us to conclude that the development of RCG and festulolium takes place differently during the 1st year of use. In the 1st harvest sward of RCG the tillers made up 70 to 82%, but for festulolium the average number of tillers was only 50% (Figure 2). The number of ears in RCG sward was very small 5 – 9%, while in festulolium sward ears made up 14 –18% of the total mass. The greatest number of leaves (36 – 43%) was in festulolium sward; in RCG sward leaves made up 15 – 30%.

The use of fertilisers allowed both perennial grass species to develop a significantly ($p<0.05$) greater amount of dry matter yield. In two-cut harvest system for RCG 3.9 t ha^{-1} of dry matter was harvested in control variant and up to 8.4 t ha^{-1} in WA variant, which gave the best results in this season. Festulolium had similar tendencies – 2.5 t ha^{-1} of dry matter was harvested in the control variant, and the highest dry matter yield, 4.9 t ha^{-1}, was harvested in the WA variant (Figure 3).

The highest dry matter yields for both species were obtained by harvesting biomass once per season in autumn at crop senescence in comparison with obtained DM yields in the two-cut system: for RCG DM ranged from 6.29 to 9.92 t ha^{-1}; for festulolium – from 3.48 to 7.67 t ha^{-1}, depending on fertilisation treatment. RCG produced a significantly ($p<0.05$) higher dry matter yield when WA was used, while DM yields of festulolium were significantly higher compared with control in all fertilised variants. Using low and medium doses of fertiliser, frequent cutting may not produce the expected yield increase when water supply was as a limiting factor. The number and timing of harvests during the growing season can directly affect the biomass yield and biofuel quality (Tahir et al., 2010). The results of some investigation suggest declining anaerobic degradability (i.e.

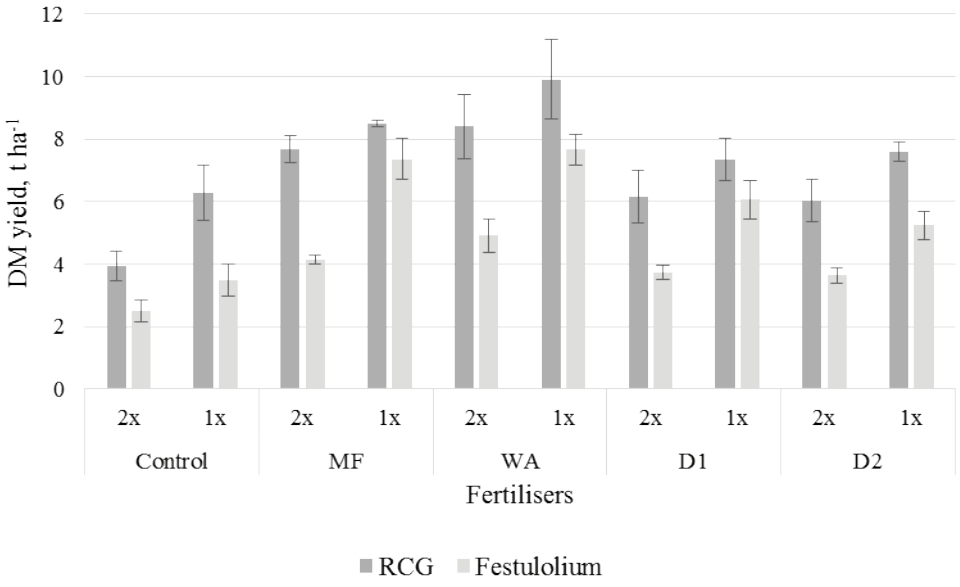

Figure 3. Dry matter yield of RCG and festulolium in intensive (2×) and extensive (1×) cutting regime; error bars indicate the standard errors.

methane production) and improving combustion properties (i.e. mineral composition) of the biomass with increasing sward maturity (Richter et al., 2011). Therefore, one-cut harvest systems provided biomass of better quality for direct combustion than two-cut systems (Kocourkova et al., 2006).

Analysis of variance showed that the influence of all three factors (cutting regime (A), grass species (B) and fertiliser type (C)) on dry matter yields was significant ($p<0.05$). The highest average DM yield (7.01 t ha^{-1}) was achieved by cutting once per season – at crop senescence in comparison with a two-cutting frequency (5.62 t ha^{-1}). The average DM yields of RCG were significantly higher than festulolium, 7.30 t ha^{-1} and 5.33 t ha^{-1}, respectively. In general, the use of all fertiliser variants contributed to a higher DM yield of grasses, in the 1st year of use higher DM yield was produced with WA and MF treatments, 8.06 t ha^{-1} and 7.24 t ha^{-1}, respectively. Interaction between the factors was not significant.

Relatively small yields of festulolium swards can be explained by sward formation in specific climatic conditions. In 2012, extensive amount of rainfall at the beginning of July was followed by a long period of insufficient rainfall. Perennial grasses were sown at the end of July, August was hot and dry, and the total amount of rainfall was lower by almost 50% of the average long-term values. In September, the soil also lacked moisture, and as a result of that festulolium did not germinate well and did not develop a dense sward till wintering. An unusually late spring of the following year delayed intensive tillering of the thinned sward; therefore, a large part of the potential 1st cut yield was lost.

The DM yield of the 2nd cut for both species was adversely affected by an insufficient amount of precipitation and unfavourable distribution of rainfall in the summer. As the grass lacked moisture at the important stages of development, including the early stages of re-growth of aftermath, it was negatively affected by the dry matter yield of intensively mowed grasses. Therefore, a higher total dry matter yield during the growing season was generally obtained from grasses in one-cut harvest system.

Conclusions

The results showed a significant dry matter yield dependence on the grass species, type of applied fertiliser and cutting regime. In the first production year the highest average dry matter yield (7.30 t ha^{-1}) was produced by reed canary grass. The highest average dry matter yields of reed canary grass and festulolium were provided by mowing swards in one-cut harvest system – in autumn at crop senescence (7.01 t ha^{-1}) and in fertilisation treatments with wood ash – 8.06 t ha^{-1} and mineral fertiliser – 7.24 t ha^{-1}.

Reed canary grass and festulolium 1st cut yield formation in the 1st year of use was different: the sward of RCG mostly (70 – 82%) consisted of tillers while the proportion of tillers in festulolium sward averaged only to 50%. Whereas the proportion of ears was higher for festulolium (14 – 18%), in the RCG sward it was only 5 – 9% of the total dry matter yield weight.

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POTATO MINITUBERS TECHNOLOGY – ITS DEVELOPMENT AND DIVERSITY: A REVIEW

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Abstract
The study consists of literature review on potato (*Solanum tuberosum* L.*)* initial seed material – minitubers production. This paper covers aspects of healthy potato microplants production techniques and subsequent greenhouse minitubers production methods. The diversity of conventional minituber growing techniques (on solid medium in greenhouses) is discussed. Review showed, that obtained minitubers number depends on growing methods and variety. Physical manipulation during *in vitro* phase could have positive effects on minitubers yielding capacities.
Key words: *Solanum tuberosum* L. *in vitro* plantlets, microplants, micropropagation, minitubers.

Introduction

Potato (*Solanum tuberosum* L.*)*, being vegetatively propagated crop, is prone to accumulation and further spread of several diseases affecting its yield and quality. Healthy planting material has essential role in potato production chain.

Clonal selection (a conventional seed production method), which had been used for decades, required intensive process control and seed programmes based on clonal selection could take 10 years and more.

Other propagation techniques have been developed and involved in seed production systems, thus decreasing time needed for seed multiplication These techniques include obtaining healthy stock material through virus eradication using meristem culture, rapid *in vitro* plants multiplication and increased number of individuals of the first year clones through minitubers production (Struik and Wiersema, 1999). Micropropagation techniques have widely been introduced in potato seed production systems (Jones, 1988; Struik and Wiersema, 1999) for more than three decades and development of *in vitro* multiplication system was a breaking point in the commercial production of high quality potato seed (Pruski, 2007).

Nowadays potato can be rapidly multiplied using nodal cuttings produced *in vitro* and involving following minitubers production. Methods, protocols and conditions to produce *in vitro* plantlets vary across laboratories, as well as methods for obtaining first generation potato seed tubers can be rather different, thus resulting in diverse outcomes.

The aim of this review is to cover aspects of the laboratory production of *in vitro* plantlets with an emphasis on the subsequent potato minitubers production. Aspects of microtubers (small tubers produced in *in vitro* conditions) production are skipped this time, as well as hydroponics and aeroponics techniques for minitubers production are noted without detailed study.

Materials and Methods

Monographic method has been used for this study. Available literature (journals, monographs, PhD thesis) have been studied with the aim to cover broad spectrum of methods developed for potato micropropagation and the following potato minitubers production. Additionally unpublished materials such as yearly reports since 1979 regarding potato minitubers production system establishment and development at State Priekuli Plant Breeding Institute have been studied in order to cover potato micropropagation and minitubers production techniques involvement in Latvia.

Results and Discussion

Microplant multiplication – obtaining of the stock material

Struik and Wiersema (1999) distinguish two major methods for obtaining a starting material in potato seed production: under semi *in vivo* conditions (greenhouses) using sprout, stem and leaf-bud cuttings and under artificial *in vitro* conditions.

In vitro produced plantlets are widely used as the base in potato seed programmes (Jones, 1988; Struik and Wiersema, 1999; Pruski, 2001; Tadesse, 2007) worldwide.

Jones (1991) claimed that large scale *in vitro* production of pathogen free plantlets was initiated in North America by New York State in 1978. In Latvia, Dr. Uldis Miglavs in 1974 was the first one who recommended including micropropagation methods in potato seed production programme (Миглавс, 1974). After that in 1979 newly developed laboratory at Priekuli Breeding and Experimental Station (now – State Priekuli Plant Breeding Institute) received its main objective of work – to develop potato seed production system in Latvia based on virus free plantlets (State Priekuli Plant Breeding Institute yearly reports 1979-1991).

Though liquid shaken cultures have been established (*in vitro* plantlets are cut into stem cuttings each with three to four nodes, and each stem piece is placed in liquid media, the flasks are shaken and after 2–3 weeks of rapid growth each flask contains 60 to 70 nodes (Dodds, 1988)), i*n vitro* single node cuttings are probably the most common multiplication method for mass propagation of *in vitro* plantlets (Roca et al., 1978; Ortiz-Montiel and Lozoya-Saldana, 1987; Ranalli et al., 1994; Grigoriadou and Leventakis, 1999; Struik and Wiersema, 1999; Pruski, 2001; Tadesse et al., 2001a; Gābere, 2004; Otroshy, 2006; Veeken and Lommen, 2009; Särekanno et al., 2010a; Asakaviciute, 2011; Ozturk and Yildirim, 2011; Milinkovic et al., 2012).

One cycle of multiplication using nodal cuttings takes about four weeks (Ranalli et al., 1994; Struik and Wiersema, 1999; Pruski, 2001; Asakaviciute, 2011; Milinkovic et al., 2012) and on average 3–5 new cuttings can be obtained from one plantlet (Rannali, 1997).

Murashige and Skoog (1962) inorganic salts and vitamin medium (known as MS medium) with added sucrose 30 g l^{-1} and agar 6–8 g l^{-1} is common nutrient medium for potato micropropagation *in vitro* (Pruski, 2001; Gābere, 2004; Otroshy, 2006; Corrêa et al., 2008; Asakaviciute, 2011; Milinkovic et al., 2012). Application of half strength MS medium has been reported by Ahloowalia (1994) and Ranalli et al. (1994). Several authors outline usage of plant growth regulators (PGR) such as kinetins (Kotkas and Rosenberg, 1999), gibberellins (Roy et al., 1994; Kotkas and Rosenberg, 1999), auxins (Grigoriadou and Leventakis, 1999; Ozturk and Yildirim, 2011). Adding auxins to medium has been reported as a promoter of rooting (Ozturk and Yildirim, 2011), but Rannali (1997) has stated that separate rooting phase is not required for potato microplants, as cultured shoots of potato quickly develop roots. Application of PGR Alar (daminozide) during the last subculture has been mentioned as promoter of microplants survival after their transfer to greenhouse (Lommen, 1995; Grigoriadou and Leventakis, 1999; Tadesse et al., 2001a; Veeken and Lommen, 2009). Additional inorganic salts tetrahydrated calcium nitrate and diammonium phosphate can be added to medium (Muro et al., 1997), but high grade sucrose can be successfully replaced by ordinary sugar (Ahloowalia, 1999).

Vast diversity of culture containers are in use for potato microplants propagation. Jones (1988) explored that in North America glass test tubers and Magenta polycarbonate boxes are used most frequently, while in Europe glass test tubes dominate. Glass test tubes had been and still are popular in Europe as containers for the plant micropropagation (Миглавс, 1974; Roca et al., 1978; Jones, 1988; Roy et al., 1994; Tadesse et al., 2001a; Veeken and Lommen, 2009; Särekanno et al., 2010a; Ozturk and Yildirim, 2011). Some authors mention plastic containers with 100 mL of medium (Milinkovic et al., 2012), 300 mL flasks (Corrêa et al., 2008), suitable container with 16–25 single nodes (Dodds, 1988) not specifying entire information about material, volume of container, number of explants per container. More clear data are given by Wattimena et al. (1983) who have reported the use of 3 to 5 shoots per 120 mL culture vessel containing 30 mL medium and sealed with parafilm. Ahloowalia (1994) writes about usage of 120 mL clear plastic containers with 20–25 mL medium, 10–15 explants per container, Veeken and Lommen (2009) outline plastic jars (10 cm diameter and 5 cm height), containing 75 mL of medium and 25 single node cuttings grown in jars. The authors state that bigger containers are used only in the last phase of multiplication. Pruski (2001) mentions usage of GA7 Magenta vessels (produced by Magenta Corporation, USA and having certain volume), where 16 explants are placed. It was already explored by Jones (1988) that in North America Magenta polycarbonate boxes were used more frequently than in Europe.

The utilization of glass test tubes has both advantages and disadvantages over the usage of bigger containers for cultivation of microplants. Glass test tubes are very costly, but once they are purchased, they can be reused almost for unlimited time. One of the main disadvantages of the usage of glass test tubes is a lot of labor required for washing the tubes, especially when laboratory has to produce tens of thousands of potato microplants.

Bigger containers can be both disposable and reusable. Reusable containers for microplants cultivations are usually costly, but the usage of disposable containers raises the question of sustainable development because a lot of waste is produced as a result of plants masspropagation. When contamination occurs, all plants grown in one bigger container are damaged unlike test tubes where only a single plant is lost per container.

The effect of culture container on potato microplants growth is rarely studied. Fal et al. (2002) claims that there are some facts often overlooked in tissue culture. Research on effects of culture vessel and its closure type on *in vitro* propagation of carnations (*Dianthus caryophyllus* L.) outlined the response of *in vitro* growth and morphogenesis of several *Dianthus caryophyllus* L. cultivars. It was dependent on the environmental differences inside various types of culture vessels. These findings were mainly related with the specific sensitivity of each cultivar to the gas exchange and medium desiccation determined

by the vessel type. It was stated that light inside the culture vessel depended on its type and was different from the light coming from the culture shelf, as well as various closures provided various gas exchange inside of culture vessels (Fal et al., 2002). The study on carnations could be possibly applied to potato micropropagation because both species are propagated through nodal cuttings. The study on influence of culture container volume, medium volume and culture density on the growth yields of lettuce (*Lactuca sativa* L.) and spearmint (*Mentha spicata* L.) shoots were conducted. In this study, culture vessel capacity greatly influenced the growth responses from lettuce and spearmint shoots (Tisserat and Silman, 2000). Concerning potato micropropagation it has been found that net photosynthetic rates per microplant and per leaf area were reduced at lower relative humidity rates in the culture container (Tanaka et al., 1992).

Further investigations on effects of tissue culture containers and their closures on potato microplants growth and quality as well as possible after effects of these treatments on minitubers formation in greenhouse might be necessary. New findings might contribute to improvement of efficiency of initial potato seed production.

A discussion about genetic stability of micropropagated plants has always been in the scope of researchers.

In the early years when *in vitro* multiplication of healthy stock material was introduced in potato seed production system, Roca et al. (1978) stated that no detectible changes due to *in vitro* procedures could be found using morphological and biochemical criteria. Plantlets derived from meristems could be more genetically stable that plantlets derived through other *in vitro* procedure - leaf discs etc. (Slack, 1980). On the contrary, it has been reported that mutations can arise when plants are derived from small explants such as meristems (Wright, 1983).

Ahloowalia (2000) has looked into phenotypic stability of microplants and minitubers by conducting the greenhouse experiment with thousands of plants. His findings showed only variegated leaves of one single branch of one single plant. Few off-type minitubers were observed, but they became true to type in the subsequent propagation (Ahloowalia, 2000).

Minitubers derived from microplants have been tested for genetic variation under field conditions (Rosenberg et al., 2007). No genetic variation has been reported; however, the authors stated that meristem clones differed in the intensity of flowering, height of stems and in the uniformity of plants.

There is still a different point of view whether the initial material for micropropagation should be renewed every year (by meristems, shoot tips cuttings and other methods) or it can be obtained from repositories where stock plants are maintained for a longer time.

Acclimatization (hardening) of microplants

Various authors have different views on the necessity of the acclimatization phase of *in vitro* plants before they are planted into production containers in greenhouses.

Potato microplants can be planted in small containers (e.g. paper pots (Muro et al., 1997), plastic rolls (Miglavs, 1987; Rosenberg et al., 2007; Särekanno et al., 2010a), in transplanting trays with small cells (Tadesse et al., 2001a)) filled with certain growing medium (e.g. in fertilized peat (Muro et al., 1997)). Plants can be kept under reduced light (Muro et al., 1997; Corrêa et al., 2008). After acclimatization plants are usually disturbed and replanted to other growing trays and growing medium.

On the other hand, it has been reported that microplants can be planted directly to the greenhouse without passing acclimatization phase (Ahloowalia, 1994; Grigoriadou and Leventakis, 1999).

Minitubers growing methods

Two major traits by which minitubers can be distinguished from microtubers and conventional seed tubers can be derived from literature.

The first trait is the way of obtaining minitubers. A majority of authors agree that minitubers are produced from *in vitro* derived potato plantlets under greenhouse conditions (Lommen and Struik, 1992a; Ahloowalia, 1994; Rannali, 1997; Struik, 2007) either on a soil or in soil-less systems such as hydroponics and aeroponics or under field conditions (Jones, 1991; Särekanno et al., 2010a). The way of production distinguishes minitubers from microtubers, which are produced under *in vitro* conditions. Some authors mention that minitubers can also be produced from microtubers (Ahloowalia, 1994).

The second trait is the size of minitubers and this trait is less unambiguous. Struik and Wiersema (1999) summarize that the size of minitubers may be in the range from 5–25 mm although in many potato seed production systems larger minitubers are also common.

Some seed production systems involve growing of minitubers directly under field conditions (Wattimena et al., 1983; Särekanno et al., 2010a; 2010b; 2012) regardless the delicate planting material (Wiersema et al., 1987) and careful handling that is required (Lommen and Struik, 1992b).

Most seed programs already more than two decades ago involved greenhouse minituber production (Miglavs, 1987; Dodds, 1988; Jones, 1991). Regardless large-scale development of soil-

less systems worldwide, growing of potato minitubers in greenhouses in normal potting substrates (soil, peat etc.) is still considered a simple and cheap way of production; therefore, it can be called a conventional minitubers production system.

The main purpose of initial potato seed production is obtaining as many medium sized minitubers with good health status per one *in vitro* plantlet or per area unit of greenhouse as possible.

Lommen and Struik (1992a) have stated five main parameters which can be manipulated in minitubers production phase: '(1) the number of minitubers per *in vitro* plantlet, (2) the number of minitubers per unit area, (3) the average weight per minituber, (4) the minituber yield per plantlet, and (5) the minituber yield per unit area'.

Many crop husbandry techniques have been utilized in order to manipulate minituber yield parameters. These techniques include planting density, growing medium, fertilizing, growing container used and others. In many cases all these treatments can interact; therefore, when one of them is changed, other yield parameters can be obtained.

A wide diversity of planting densities is described in literature covering densities from 24–25 plants per m^{-2} (Wiersema et al., 1987; Roy et al., 1994; Veeken and Lommen, 2009) to even 800 plants per m^{-2} (Lommen and Struik, 1992a). Authors provide information about planting densities of 40–48 plants per m^{-2} (Wiersema et al., 1987; Gābere, 2004; Dimante, 2013), 100 plants m^{-2} (Dodds, 1988; Roy et al., 1994), 200 plants m^{-2} (Grigoriadou and Leventakis, 1999). In the study of Veeken and Lommen (2009), three planting densities were compared – 25 plants m^{-2}, 62.5 plants m^{-2}, 145.8 plants m^{-2}. Similarly, Roy et al. (1994) compared minituber yield at three planting densities 25 plants m^{-2}, 49 plants m^{-2}, 100 plants m^{-2}. The density of 280 plants per m^{-2} was authorized by the Netherlands General Inspection Service of Agricultural Seeds and Seed Potatoes (Struik and Wiersema, 1999). On average 200–400 plantlets per m^{-2} can be planted, when a repeated harvesting method is used (Lommen and Struik, 1992a).

The most popular growing medium mentioned in literature is peat (Miglavs, 1987; Kotkas and Rosenberg, 1999; Gābere, 2004), various mixtures containing peat such as 1:1 mixture of sand and peat (Wiersema et al., 1987), 2:1 peat sand mixture (Muro et al., 1997), 2:1:1 soil, vermiculite and sand substrate (Ranalli et al., 1994), 5:1 peat – perlite mixture (Roy et al., 1994). 1:1 peat – perlite (Grigoriadou and Leventakis, 1999), peat-clay mixture 1:1 (Veeken and Lommen, 2009). Commercial ready-made substrates can be used for minitubers production as well, e.g. mixture of perlite and potting soil 1:1 is mentioned by Loomen and Struik (1992a), ready-made potting compost obtained from commercial company (Ahloowalia, 1994), nutrient rich potting soil without any specification is described by Ostroshy (2006).

Regrettably few authors specify composition of fertilizers used for enrichment of growing medium, as well as there is a lack of clear information about additional feeding during growing season promoting good tubers set. Moreover, fertilizer composition is a production secret for private enterprises producing potato minitubers.

Very common situation is when only description of either fertilizing of growing substrate or additional feeding can be found.

Application of Nitrofoska® at the rate of 3.3 kg m^{-3} of peat is mentioned in literature (Miglavs, 1987). Wiersema et al. (1987) specify usage of NPK 1:1:1 40 g m^{-2} three times per season. Roy et al. (1994) describe application of NPK 14:14:14 100 mg l^{-1} N until four weeks before harvest. Rannali et al. (1994) indicate a weekly application of solution containing Nitrofoska® fertilizer N:P:K:Mg (12:5:14:1.5). Gābere (2004) describes composition of peat mixture indicating the usage of N 0.14 kg, P 0.07 kg and K 0.07 kg per m^{-3} of peat, as well as the usage of $CaNO_3$ solution at rate 2 g l^{-1} as foliar applications two times per season. In addition, Lommen and Struik (1992a, 1992b) specify that 131.4 mg l^{-1} of N is added to the mixture of perlite and potting soil. The authors describe a complete composition of nutrient solution including macro and micro salts. The solution is used at a low concentration (which is not specified) twice a week with respect of 100 to 200 ml per six plants. Struik and Wiersema (1999) introduce the procedure followed by the Netherlands General Inspection Service for Agricultural Seeds and Seed Potatoes, which include the usage of potting soil with nutrients for the first 2 months. When tubers are initiated, two types of fertilizers are used – NPK 17:17:17 applied by hand at the amount of 1g per m^{-2}. NPK 18:18:18 including trace elements is dissolved in water and applied in quantity 1 g of fertilizer per m^{-2} every 2 weeks until irrigation is stopped.

Various growing containers have been in the use for minitubers production. Pots made from paper, plastics and other materials with various diameters (e.g. 25 cm (Miglavs, 1987), 13 and 19 cm (Vanaei et al. 2008), 20 cm (Milinkovic et al., 2012) etc.) are very common. Bigger nursery beds could be considered as very suitable containers for masspropagation of minitubers. In the study of Wiersema et al. (1987) 1 m wide nursery beds are mentioned. Roy et al. (1994) describe wooden beds, which are 1 meter wide and 50 cm high. In addition, other dimensions of planting beds are described in literature – 25 m long and 1.25 m wide benches (Muro et al., 1997), plastic beds 50×360×20 cm (Gābere, 2004). The usage of plastic

boxes is reported as well (Grigoriadou and Leventakis, 1999; Gābere, 2004; Veeken and Lommen, 2009).

According to Otroshy (2006), 100 days is a normal production cycle for minitubers. Time for destructive harvest can be from 70 days after planting (Ahloowalia, 1994) to even 121 days after planting (Roy et al., 1994).

Alternative method in conventional minitubers production system – repeated harvesting

Lommen and Struik (1992a; 1992b) have developed a distinct approach to minitubers growth. This technology involves non-destructive harvesting of minitubers, and thus is called – repeated harvesting.

The authors describe procedure clearly 'Plants were lifted carefully from the soil mixture, tubers > 0.3 g were removed and plants were replanted into the soil mixture. Whether the weight of the removed tubers was > 0.3 g had to be estimated, using a diameter of approximately 8 mm as a criterion. Plants were always replanted deeper than before. Replanting depth was not recorded but depended on the harvest date, and increased as the length of the stem part without leaves increased. Care was taken not to damage stems and stolons. Damage of roots, however, could not be avoided. Removing tubers > 0.3 g in a non-destructive harvest, many new tubers were initiated on existing stolons, newly formed stolons and directly on the below-ground part of the main stem' (Lommen and Struik, 1992b). Such harvesting procedure was used at the first two harvests; the third harvest was destructive (Lommen and Struik, 1992a). Later Veeken and Lommen (2009) summarize that repetitive harvesting results in relatively small tubers, and in addition has a high labor demand. Therefore, repetitive harvesting method may be less interesting and effective for commercial production.

Minitubers yield (numbers)

Ahloowalia (1994) has stated that obtained tuber number is the most important parameter in the production of minituber for seed.

Struik (2007) summarizes that the number of minitubers is usually in the range from 2–5 tubers per planted plant. Average data obtained by many authors across varieties fall in the frame marked by Struik (2007). The varietal differences in terms of tuber number per plant have been confirmed by authors (Hagman, 1990; Ahloowalia, 1994; Gābere, 2004; Otroshy, 2006; Struik, 2007; Dimante, 2013).

Very small average tuber number per plant of only 0.26–3.07 tubers was reported by Aahlowalia (1994), 1.85–2.52 tubers per plant reported by Grigoriadou and Leventakis (1999), but Corrêa et al. (2008) reported average yields of 7.00–8.31 minitubers per plant

Authors investigating the influence of planting density on minitubers yield have relevant finding that increasing of planting density decreases minituber number per plant and average minituber size, but increases tuber number per area unit (Roy et al., 1994; Veeken and Lommen, 2009). The study of Roy et al. (1994) and Veeken and Lommen (2009) overlaps in the smallest planting density, which is 25 plants per m^{-2}. Nevertheless, the authors have got significantly different results. Roy et al. reported the average yield of 11.1 minitubers per planted plant, but Veeken and Lommen mentioned the yield of 5.4 minitubers per plant as average 10 weeks after planting. These differences could be explained by several factors: thus, confirming the potential significance of various treatments. Different varieties, different planting containers were used (plastic boxes by Veeken and Lommen and relatively large propagation beds by Roy et al.). The layer of planting substratum was respectively 10 cm and 18 cm for Veeken and Lommen and Roy et al., as well as substratum mixture and fertilizing was different. Roy et al. performed additional treatments, providing supplementary irradiation and performing plant hilling during the season.

Effects of in vitro phase on subsequent minitubers production

Several efforts have been attempted in order to understand possible manipulation with plant status during *in vitro* phase and acclimatization and its effect on subsequent minituber yield parameters.

It has been reported that minitubers yield in the greenhouse could be improved by modifying *in vitro* growing conditions (Seabrook et al., 1995; Tadesse, 2000; Pruski, 2001; Otroshy, 2006; Milinkovic et al., 2012).

Ahloowalia (1994) reported that a longer duration of *in vitro* phase has a negative effect on minituber production. Milinkovic et al. (2012) reported contrary results – significantly positive effect of extended *in vitro* growing period on subsequent minitubers yield resulting in up to 97% higher number of minitubers in comparison with control.

Decreasing of photoperiod during the last subcultures of *in vitro* plant multiplication could have positive effect on subsequent minitubers yield in greenhouses (Seabrook et al., 1995). Milinkovic et al. (2012) did not confirm this finding, reporting that minitubers number per plant did not change significantly when a shorter photoperiod than standard 16 hours daylight during *in vitro* phase was used.

According to the study of Otroshy (2006), lower *in vitro* temperature subsequently resulted in larger minitubers, but it did not affect minitubers number. These results are opposite to Tadesse's et al. (2001b)

findings that lower temperature during *in vitro* phase did not affect minitubers average weight significantly.

Further studies are necessary to understand the influence of various physical treatments during *in vitro* phase on subsequent microplants yielding capacities in a greenhouse, especially on stable production of large enough number of minitubers.

Conclusions

1. Methods, protocols and conditions to produce *in vitro* plantlets vary across laboratories, as well as methods for obtaining first generation potato seed tubers can be rather different.
2. No common opinion about a necessity of hardening phase of potato microplants can be found.
3. Minitubers are obtained from *in vitro* grown microplants. The way in which minitubers are obtained is the main trait, which distinguishes them from microtubers and conventional potato seed tubers.
4. A wide diversity of planting densities, fertilizing protocols and other growing techniques are reported in literature. The influence of a variety on minitubers number has been approved.
5. Physical manipulation during *in vitro* phase could have positive effects on subsequent minitubers yielding capacities, but further investigations are required.
6. Not all of the minitubers growing methods described in literature can be suitable for commercial production. Seed producers have to adapt techniques, which are the most effective for their capacity.

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TABLE POTATO VARIETY 'TEELE' WITH HIGH YIELDING AND LATE BLIGHT RESISTANCE

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Abstract
The characteristics of the new potato cyst nematode (*Globodera rostochiensis*, pathotype R_O1) resistant, medium ripening potato variety 'Teele' ('Cinja' ´ 'Paola') developed at the Estonian Crop Research Institute (ECRI) is discussed in this paper. The variety 'Teele' was included in Estonian and European Variety List in 2013.The variety 'Teele' passed the preliminary, dynamic and final trials in 2008 - 2012 at the ECRI, where it was compared with standard varieties 'Maret' (early), 'Piret' (medium) and 'Anti' (late). The official trials in Estonia and the technical examination (DUS test) in Czech Republic were carried out in 2011 – 2012. Potato cyst nematode (Ro1) and wart (*Synchytrium endobioticum* (Schilbersky) pathotype 1 (D 1)) resistance of the new variety were determined in Poland in the Plant Breeding and Acclimatization Institute. Tuber yield and tuber weight of the variety 'Teele' were equal to 'Maret' and 'Anti' but higher than 'Piret'. The number of tubers per plant was equal in all standard varieties. Tuber yield and yield of marketable tubers of dynamic trials present the medium ripening of the variety 'Teele'. The potato variety 'Teele' had good quality characteristics (shallow eyes, regular shape) and good culinary traits of table potato variety. It had a relatively good resistance to late blight (*Phytophthora infestans*) (field resistance), overcoming standard varieties 'Maret' and 'Piret'.
Key words: potato variety, tuber yield, late blight resistance, cooking quality.

Introduction

Breeding process of potato, the fourth most important food crop in the world (FAOSTAT, http://faostat.fao.org), is very important. In the cold temperate climate zone, potato is one of the essential vitamin sources throughout the year. Potatoes contain 15 mg of vitamin C per 100 g of fresh tuber. The challenge is to produce more food on limited available land resources and with less available water under the changing climate conditions (Struik, 2008). Potato breeders constantly study new genotypes because potato is strongly vulnerable to both biotic and abiotic stresses (Bonnel, 2008). According to International Union for the Protection of New Varieties of Plants (UPOV), new potato variety needs to be described by 42 characteristics. New variety has to be sustainable in modern changing agriculture and maintain stability under changing climate conditions (Brown, 2011). People's preferences and consuming habits have changed, thus changing food industry's demands, setting up additional requirements for new potato varieties. In the breeding process, selection moves towards these requirements. The most important characteristics for food industry are tuber yield, starch content and yield, the colour and quality of chips and fries. Last properties show the concentration of reduced sugars. Cooking type, texture firmness and taste are also important (Tsahkna, 2004). Tuber yield, starch and sugar content form a complex characteristics, which are influenced by numerous genetic and environmental factors (Li et al., 2010; Gebhardt et al., 2007, 2011), thus making it difficult to achieve the desired level of these characteristics. Changed environmental factors have caused diseases to become more aggressive and pests to multiply faster and more extensively (Cooke et al., 2012; Runno-Paurson et al., 2011, 2012, 2014). Especially great damage is caused by potato late blight; therefore, varieties with race-specific resistance were bred throughout years to fight this disease, but the rapid development of the pathogen has overcome existing races. Results from foliar resistance blight evaluation trial indicated that current western European potato varieties are too susceptible to late blight to be grown without chemical control under North-East European conditions (Runno-Paurson et al., 2013). More attention has been drawn towards breeding varieties with non-race-specific resistance (horizontal, partial, quantitative or field resistance) (Landeo et al., s.a.; Cooke et al., 2011).

Breeding of new potato varieties is a continuing process, during which ecological conditions should be considered (Baciu et al., 2012). Being a part of the European Union (EU), it is allowed to import every potato variety on the list of EU varieties. But the practice has shown that varieties do not act accordingly to their description in the countries far from the origin of a variety. An imported variety from different soil and weather conditions may act totally different in Estonian conditions. Therefore, potato varieties bred in Estonia are better adapted to Estonian climatic conditions and could be cultivated in other Eastern-European countries with similar conditions.

The aim is to breed potato varieties with high yielding and more permanent late blight resistance.

The main goal of potato breeding of the ECRI is to breed medium and medium late disease resistant high yielding table and processing potato varieties. This paper gives information about agronomical and biological characteristics of a new variety 'Teele' in 2008-2012, when it was tested in preliminary (2008), final (2009 - 2012) and dynamic trials (2008 - 2012).

Materials and Methods

A new nematode and wart resistant medium potato variety 'Teele' (breed J 1490-03) was developed at the Estonian Crop Research Institute (ECRI). The variety 'Teele' was selected from the cross between German potato cyst nematode resistant varieties 'Cinja' and 'Paola'. The official trials in Estonia and technical examination (DUS test) in Czech Republic were carried out in 2011 - 2012.

Experimental fields of the ECRI are located on sandy loam (*Calcaric Luvisol*) soil by FAO/UNESCO classification. Fields were deeply shredded, cultivated and complete chlorine free mineral fertilizer (containing 80 g kg^{-1} N, 50 g kg^{-1} P and 190 g kg^{-1} K) by 650 kg ha^{-1} was used locally in spring. Chemical control of weeds was carried out with mixture of herbicides Sencor 70 WP (a. i. metribuzin 700%) 250 g ha^{-1} and Titus 25 DF (a. i. rimsulfuron 25%) 25 g ha^{-1}. During the growth period, plants were hilled up three times and harrowed once. The variety 'Teele' was compared with Estonian standard varieties 'Maret' (early), 'Piret' (medium) and 'Anti' (late). Conventional breeding method, crossing with different hybrid varieties or hybrids, and repeated selection of followed hybrids tuber generations were used in breeding. Crossing and growing of seedlings were carried out in the greenhouse and the next generations were tested in the field. The preliminary, final and dynamic trials were planted in 5 replications. Tuber yield was weighed, the analysis of the yield structure was carried out and starch content was estimated with Reimann scales (on the basis of special weight). Yield structure contains tubers per plant and tuber weight. The dynamics of the tuber yield was determined three times at 7 – day intervals in mid July. Each sample consisted of 10 plants harvested by hand from the test plots. The planting material was pre-sprouted.

Late blight infection was assessed according to the 0 - 100% scale (EPPO, 1989). The estimation of late blight was carried out three times, and the first estimation depended on the beginning of the infection. The resistance to potato cyst nematode pathotype Ro1 of the variety 'Teele' was assessed in biological tests according to UE protocol (EPPO 2000) in 9-degree scale. There was no new developed cyst on roots of the variety 'Teele', and it was classified as highly resistant to potato cyst nematode pathotype Ro1 with 9 degrees. The resistance to wart of variety 'Teele' was assessed by The Glynne-Lemmerzahl method (laboratory tests). According to Diagnostic Protocol of EPPO PM 7/28 (OEPP/EPPO, 2004), the results indicated that variety 'Teele' was weakly resistant to wart in laboratory conditions (reaction type 1, 2, and 3). The assessment of the resistance of variety was carried out in the laboratory of Department of Plant Pathology of Plant Breeding and Acclimatization Institute in Poland.

Estimations of the cooking type and other quality traits tests were carried out by the workers of the potato breeding section of the ECRI in autumn and

Table 1

Weather conditions of potato vegetation period in 2008 - 2012

		Average of air temperature, °C*					Summary of precipitation, mm*					Average of relative humidity, %*				
Month	Decade	2008	2009	2010	2011	2012	2008	2009	2010	2011	2012	2008	2009	2010	2011	2012
June	I	13.4	13.5	13.2	19.8	11.2	0	52	28	0	29	55	78	72	61	73
	II	14.5	14.4	13.7	16.0	15.1	73	42	35	28	39	80	79	76	76	74
	III	15.4	15.4	16.0	16.5	13.8	28	3	25	10	42	81	76	75	75	77
July	I	16.4	16.3	20.1	20.8	19.3	33	17	31	14	16	75	76	73	75	75
	II	16.9	16.9	23.2	19.3	15.5	21	50	3	11	58	74	82	71	76	81
	III	16.9	16.8	22.8	21.5	19.1	2	13	10	9	11	78	82	71	77	75
August	I	16.4	16.4	21.2	16.4	16.0	95	29	19	14	22	84	84	76	73	80
	II	15.4	15.4	19.6	16.2	14.7	33	51	26	47	76	86	85	77	86	83
	III	14.1	14.1	13.9	16.3	13.5	67	30	46	13	32	90	87	84	62	87
Sept.	I	13.0	15.4	10.7	13.4	12.2	39	24	23	13	24	93	93	84	87	84

*according to ECRI weather station

Table 2

Average of tuber yield characteristics and late blight infection of the potato variety 'Teele' at the ECRI in 2008 – 2012

Character	Unit	LSD_{05}[1]	'Maret'	'Piret'	'Anti'	'Teele'
Tuber yield	t ha^{-1}	2.2	44.0	37.2	44.9	47.1
Tubers per plant	Number	0.5	9.9	9.5	10.0	10.6
Tuber weight	g	4.0	80.0	71.8	81.6	80.0
Late blight:						
1st estimation	%[2]	3.7	13.2	7.4	0.9	5.9
2nd estimation	%[2]	5.8	54.2	42.4	9.2	34.3
3rd estimation	%[2]	3.6	91.5	68.4	32.6	44.1

[1] – least significant differences

[2] – percentage of infested foliage surface

spring in every testing year (Tsahkna, 2004). The taste was estimated on a 9 – point scale where 9 – excellent taste and 1 – unsuitable, with a strong flavour. Mealiness was estimated on a 5 – point scale, where 1 – watery, mellow and moist, 5 – dry and very mealy. Disintegration (destruction or disintegration during boiling) was also estimated on a 5 – point scale, where 1 – whole (no disintegration) tuber and 5 – tuber had boiled into pieces (complete disintegration). Two methods to estimate the suitability of potato varieties for chips were used: colour test after frying the tuber slices (1 - 9 points, where 8 and 9 points are preferred) and determination of the content of reducing sugars by colorimetric method. The suitability for chips was estimated both in autumn and spring. To estimate the quality of chips in spring, samples were kept in +10 °C for 4 weeks (Tsahkna, 1995). The trial was designed and analysed by NNA (Nearest Neighbour Analysis) method using AGROBASE 20 computer package. Data were analysed by the analyses of variance and Agrobase was used for correlations. The analyses of tuber yield characteristics, tuber yield and yield of marketable tuber of dynamic trials, the analysis of late blight infection and the estimates of the components (determination coefficient) due to environment R_E^2 (growth year), genotype R_G^2 were calculated and were expressed as % of total variance. The least significant differences (LSD_{05}) among mean values were calculated.

Data of weather conditions during the trial years is presented in Table 1.

Results and Discussion

June precipitation fell short from the precipitation demand for potato growth (70 – 85 mm) only in 2011 (Table 1). The July precipitation demand of potato is 120–150 mm and all sample years were below that at the Jõgeva weather station. The July precipitation shortage may affect both tuber number and total yield per plant. August precipitation failed to satisfy the potato growth demand (90 – 115 mm) (Jõudu, 2002) in 2011. The interannual differences in precipitation explain weather-induced variation (Tables 3, 4, 5).

All sample years featured late blight infection (Table 2) at the beginning of August when average air humidity was high, precipitation was within the normative and daily average air temperature remained above 10 °C (Table 1).

The morphological description of the variety 'Teele' is given in accordance with UPOV characteristics. The height of the plant is tall and growth habit upright. Leaf is medium, with light intensity green colour. Flower corolla is medium, white and intensity of anthocyanin coloration on inner side is absent or very weak. Tuber shape is short-oval, depth of eyes shallow, colour of skin yellow and colour of flesh yellow. The maturity of variety 'Teele' is medium.

The most important yield characteristics (tuber yield, number of tubers per plant, tuber weight) and the yield of new potato variety 'Teele' are compared with standard varieties ('Maret', 'Piret' and 'Anti') and presented in Table 2. Tuber yield of the variety 'Teele' exceeded significantly the standard varieties 'Piret' and 'Maret' and was equal with 'Anti'. The variety 'Teele' had the highest number of tubers per plant, exceeding the standard varieties. The new variety 'Teele' exceeded only the variety 'Piret' in tuber weight (Table 2). Potato tuber yield, weight and number of tubers per plant are influenced by environmental conditions (temperature in air-soil, moisture regime) and genotype (Jõudu, 2002). A variability of yield and tuber weight was mostly influenced by environment R_E^2=54.6 and R_E^2 =38.8, respectively; a variability of tubers per plant was significantly influenced by the combined effect of genotype and environment R_{GxE}^2 =24.2 (Table 3).

The rapid development of late blight (Runno-Paurson et al., 2009; Cooke et al., 2012) has given

Table 3

Analyses of variance of tuber yield characteristics. Components of variation due to environment (R_E^2), genotype (R_G^2), genotype by year (R_{GxE}^2) and residuals in percentage of the total sum square

Source of variation	Tuber yield	Tubers per plant	Tuber weight
Environment	54.6***	21.3***	38.8***
Genotype	13.8***	6.7*	6.8***
Genotype by year	12.3***	24.2***	23.4***
Residual	13.2	33.1	19.5
R2	0.81	0.52	0.69

*** – significant at p<0.001

* – significant at p<0.01

Table 4

Analyses of late blight infection. Components of variation due to environment (year) (R_E^2), genotype (R_G^2), genotype by year (R_{GxE}^2) and residuals in percentage of the total sum square

Source of variation	Of 1st estim.[1] of LB inf.[2]	Of 2nd estim. [1] of LB inf. [2]	Of 3rd estim. [1] of LB inf. [2]
Environment	8.4**	33.6***	37.5***
Genotype	13.5***	22.5***	34.7***
Genotype by environment	43.0***	32.5***	24.3***
Residual	25.6	7.4	2.3
R2	0.65	0.89	0.97

*** – significant at p<0.001

** – significant at p<0.01

* – significant at p<0.05

estim.[1] – estimation, inf.[2] – infection, LB – late blight

the reason to pay more attention towards breeding varieties with horizontal resistance in breeding programs (Landeo, 2002). It leaves no other choices but to assess the existence of field resistance in new genotypes during the breeding process (Wulff et al., 2007).

The variety 'Teele' had relatively good resistance to foliage late blight, significantly exceeding standard varieties 'Maret' and 'Piret'. These results are shown in Table 2. Variations of the infection with late blight were different in every observation. The most significant combined effect of genotype and environment occurred in the first observation – R_{GxE}^2=43.0; the environmental influence was significantly higher in the second and third observation R_E^2= 33.6 and R_E^2=37.5, respectively. Genotype had a significant influence in the second and third observation 22.5% and 34.7%, respectively (Table 4).

Results of dynamic trials characterize earliness of the variety 'Teele'. Tuber yield and yield of marketable tubers, determined at three different times, are presented in Figure 1. The average tuber yield of variety 'Teele' exceeded significantly the variety 'Anti' and was equal with the variety 'Piret' at the first and second harvest. Variation depended mainly on the differences of varieties (Figure 1 and Table 5). Marketable tuber yield of the variety 'Teele' was also higher than the variety 'Anti' and equal with the variety 'Piret' at the same harvest, even though the variation was influenced by environment and genotype, which was 61.7% (environment variation 30.8% + genotype variation 30.9%) at the second harvest (Figure 1 and Table 5). It shows the great influence of these factors to the formation of tuber yield (Jõudu, 2002).

The average tuber yield and marketable tuber yield of the variety 'Teele' exceeded significantly the varieties 'Piret' and 'Anti' in the third harvest. Influence of these three factors to the variation of tuber yield was significantly 68%. Thus, the results of the dynamic trials show clearly that the new variety 'Teele' belongs to medium varieties.

The cooking quality traits of the variety 'Teele' are very important, because it was bred as a table potato variety. Table 6 presents the data of taste, starch content, mealiness, disintegration, chips colour and reducing sugar content of the variety 'Teele' compared with standard varieties.

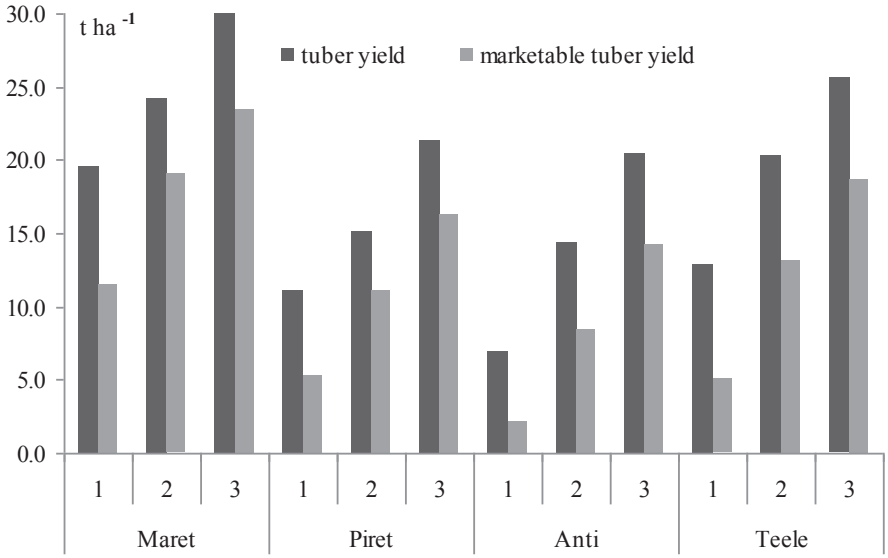

Figure 1. Average of tuber yield and yield of marketable tuber of dynamic trial in 2008 - 2012
LSD_{05} tuber yield 1.8 and marketable tuber yield 1.1 of 1st harvest
LSD_{05} tuber yield 2.1 and marketable tuber yield 2.2 of 2nd harvest
LSD_{05} tuber yield 2.2 and marketable tuber yield 2.1 of 3rd harvest
1 – first harvest; 2 – second harvest; 3 – third harvest.

Table 5

Analyses of tuber yield and yield of marketable tubers (market yield) in dynamic trials. Components of variation due to environment (R_E^2), genotype (R_G^2), genotype by year (R_{GxE}^2) and residuals in percentage of the total sum square

Source of variation	Tuber yield of 1st harvest	Tuber yield of 2nd harvest	Tuber yield of 3rd harvest	Market. yield of 1st harvest	Market. yield of 2nd harvest	Market. yield of 3rd harvest
Environment	14.1***	24.3***	29.1***	40.3***	30.8***	40.8***
Genotype	53.2***	35.1***	20.6***	37.4***	30.9***	18.9***
Genotype by year	12.1**	9.1ns	11.3**	9.6***	6.6ns	7.9*
Residual	16.0	19.7	14.1	8.5	20.6	13.9
R^2	0.79	0.69	0.61	0.87	0.68	0.68

ns - non-significant
*** – significant at p<0.001
** – significant at p<0.01
* – significant at p<0.05

The content of dry matter, including starch content, is influenced by the variety (Van der Zaag, 1992; Tsahkna and Tähtjärv, 2007). The starch content of the variety 'Teele' was lower than in standard varieties. The taste of potato is not very important trait in the breeding programme at the moment, but due to the fact our consumers use potato as a table potato, we still estimate this. The assessment of taste of the variety 'Teele' is lower compared to standard varieties. Texture is a complex factor of cooking quality, which is very important for consumer acceptance. Texture has been shown to be genetically determined and breeding for a certain texture is possible. There is a positive correlation between starch content and mealiness and disintegration (Hallikainen, 2001). Mealiness and disintegration of the variety 'Teele' are lower than those of varieties 'Maret' and 'Piret' and equal to the variety 'Anti'. It gives the possibility to use a new variety as salad and table potato (cooking type B). The trial of reducing sugar content and the chips colour indicate that the variety 'Teele' is not suitable for production chips (Table 6). Reducing sugars is one of the most important factors to determine whether the variety is suitable for production of chips, because the content of reducing sugars depends on the variety (Hendrickx and Vleeshouwers, 2005).

Table 6

Cooking quality of the potato variety 'Teele' at the ECRI in 2008 – 2012

Character	Unit	'Maret'	'Piret'	'Anti'	'Teele'
Taste	1-9 points[1]	7.8	7.8	7.7	7.5
Mealiness	1-5 points[2]	3.4	2.4	1.9	1.9
Disintegration	1-5 points[2]	2.9	1.8	1.3	1.4
Starch content	g kg^{-1}	169.0	146.0	143.0	128.0
Chips colour: in autumn	1-9 points[1]	9.0	8.4	5.6	6.8
in spring	1-9 points[1]	8.8	8.4	5.8	6.4
Reducing sugars content*	g kg^{-1}	3.0	3.0	7.0	5.0

[1] – 9 the excellent taste or preferred chips colour
[2] – 5 max mealiness or disintegration
*– average of 2008 - 2010

Conclusions

The new nematode resistant medium maturity potato variety 'Teele' (breed J 1490-03) was developed at the Estonian Crop Research Institute and included in Estonian and European Variety List in 2013.

The tuber yield of new variety exceeded significantly the standard varieties 'Piret' and 'Maret', and was equal with the variety 'Anti'. The new variety 'Teele' had the highest number of tubers per plant, exceeding the standard varieties. The variety 'Teele' exceeded only the variety 'Piret' in tuber weight. 'Teele' exceeded varieties 'Piret' and 'Anti' in total and marketable tuber yield in different harvest time in dynamic trials. Starch content of the variety 'Teele' was lower than in standard varieties. Mealiness and disintegration of the variety 'Teele' are lower than for varieties 'Maret' and 'Piret' and equal to the variety 'Anti'. Due to this, the variety 'Teele' can be used as salad and table potato. Reducing sugar content and chips colour assessment prove that the variety 'Teele' is not suitable for chips production.

The variety 'Teele' had relatively good resistance to foliage late blight, significantly exceeding the standard early variety 'Maret' and medium variety 'Piret'.

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FERTILIZATION VALUE OF EARLY RED CLOVER, WASHINGTON LUPIN AND CRIMSON CLOVER AS GREEN MANURE CROPS

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Abstract
Field trials were carried out at Jõgeva Plant Breeding Institute in 2008 – 2011 to identify the possibilities of using early red clover (*Trifolium pratense* L.) 'Jõgeva 433' (diploid), Washington lupin (*Lupinus polyphyllus* Lind.) 'Lupi' and crimson clover (*Trifolium incarnatum* L.) as green manure. Fresh material of the above species was ploughed into the soil in autumn of the sowing year. Fertilizer value was quantified through yield and grain quality of spring wheat 'Vinjett' and barley 'Inari'. The composition and amount of ploughed biomass were recorded. By the time of ploughing, Washington lupin had produced the most abundant biomass. From studied species crimson clover had the lowest fertilizer value – only by 6–7% extra yield of spring wheat in the following year. Crimson clover had no residual effect of fertilization in the second year. The fertilizer values of red clover and Washington lupin were approximately equal. Their effect on yield increase of spring wheat and barley lasted for three years, on grain quality for two years. Red clover, ploughed into the soil in the year of sowing, resulted in maximum spring wheat yield increase of 23.9%, compared with N 0 treatments; that of Washington lupin was 21.3%. The residual positive effect in the second year quantified as barley production increased by 6.2% in Washington lupin and 7.9% in red clover. The yield increase in the third year was 3.9% and 12.1%, respectively. Green manure increased the contents of crude protein and gluten in spring wheat and crude protein content in barley.
Key words: Washington lupin, red clover, crimson clover, green manure, fertilization value.

Introduction

After Estonia regained its independence, great changes have occurred in agriculture. Former collective and state farms were practicing both plant and animal husbandry in a single unit. Now, the numerous specialized plant producers have taken their place. Since manure has become scarce, alternatives must be found to preserve and enrich the humus content of soil. Attention has been paid to green manures. In north Estonia, soil is favourable for white sweet clover (*Melilotus albus* L.) cultivation (Kõrgas, 1963) while in the southern part, sandy and acid soil is more appropriate for red clover (*Trifolium pratense* L.). In the past, Washington lupin (*Lupinus polyphyllus* Lind.) was cultivated there. The species has caught the attention of researchers in neighbouring countries because of its great nitrogen fixation – 250–350 kg ha^{-1} (Heinsoo et al., 1986; Kurlovich et al., 2007) and high protein concentration (Aniszewski, 1993). Washington lupin cultivars containing alkaloids are used as raw material in chemical industry, low alkaloid content lupin is used as protein-rich forage (Aniszewski, 1992; Kurlovich et al., 2008). Latvian researchers have reported that green biomass of Washington lupin is the appropriate raw material for biogas production (Dubrovskis et al., 2011). Lithuanian researchers have reported that high alkaloid Washington lupin has qualifications of good raw material for bioethanol production (Kryževiciene, 2006). In Russia, varieties 'Pervenets' and 'Truvor' (Kurlovich, 2002) and variety 'SF/TA' in Finland (Aniszewski, 1993) have been bred. Latvian national variety list includes a perspective domestic breed. In 1990, to meet the local demand, Jõgeva Plant Breeding Institute initiated breeding of species, too. A perspective candidate variety was submitted to national testing in 2004. It passed the trials and was registered as the cultivar 'Lupi' in 2013. The main objective of the current research was to compare fertilization value of Washington lupin and early red clover. We also investigated possibilities of using crimson clover (*Trifolium incarnatum* L.) as a green manure crop. According to J. Frame (2005), the species is appreciated for its great nitrogen fixation ability (up to 155 kg nitrogen ha^{-1}) and is recommended in northern areas for green manure (Foveland and Evers, 1995).

Materials and Methods

A field trial was established at the Jõgeva Plant Breeding Institute (58° 45' N, 26° 24' E) in the spring 2008. Early red clover 'Jõgeva 433' (diploid, seed rate 12 kg ha^{-1}, row space 15 cm), Washington lupin 'Lupi' (30 kg ha^{-1} and 30 cm), and crimson clover (20 kg ha^{-1} and 15 cm) were sown without a cover crop. Fertilizer value of ploughed in biomass was evaluated on the basis of yield and grain quality of spring wheat (*Triticum aestivum*) 'Vinjett' (2009) and barley (*Hordeum vulgare*) 'Inari' (2010, 2011). The field plots seeded with timothy (*Phleum pratense*) were used as a control (sown at the same time as green manure crops). Timothy plots were regularly mown with a lawn mower in the summer 2008. Green manure crops and timothy were ploughed in during the autumn of the year of sowing (2008). Ammonium nitrate was applied prior to the last tillage procedures

before sowing the cereals. A nitrogen rate was 0, 60 and 120 kg ha^{-1} in spring wheat and 0, 60 and 90 kg ha^{-1} in barley. The treatments had five replications. The trial was conducted on calcaric cambisol (K_0), with following characteristics: pH_{KCl} 5.8, P 27, K 67, Ca 2150, Mg 159 mg kg^{-1} and C_{org} 24 g kg^{-1}. Phosphorus and potassium fertilizers were applied just once before the establishment of the trial (in 2008) at a rate of P 19 and K 67 kg ha^{-1}. Besides the yield of grain at 12% moisture content, the following quality indicators were measured on both cereals: volume weight, 1000 kernel weight; crude protein content and gluten content in spring wheat.

The vegetation period of 2008 was one month longer than long-term average, extremely rainy while air temperature was lower, and that established very favourable growing conditions for green manure crops. The vegetation period of 2009 was similar to long-term average of air temperature, amount and proportion of precipitation. Vegetation periods of 2010 and 2011 had a low precipitation level with high air temperature. In both years, barley matured extremely early in the trial. Microbiological activity of soil was inhibited because of the serious drought. It could have had an effect on the rank of green manure residual.

Aboveground biomass was determined using a forage plot harvester Hege 212. Samples of fresh material (approximately 1 kg) from each plot were taken to measure dry matter. After weighing, the samples were dried at 105 °C to a constant weight and the dry matter yield was calculated. The monoliths (surface 15x30 cm) from up to 25 cm in depth were taken to determine the root and stubble biomass. The roots were washed, then dried and weighed. Chemical analyses of biomass were conducted in the laboratory of Agricultural Research Centre. Nitrogen and carbon were determined by using TUMAS ISO/TC 16634-2:2009 method**.** To determine moisture content of grain yield, volume weight and crude protein content, Draminski Grain moisture meter PN-EN ISO 9001:2001 was used. Gluten content of spring wheat was measured by Glutomatic 2200.

The software AGROBASE 20™ was used for statistical data analysis. The significance of the differences of the variants was calculated using the LSD test.

Results and Discussion

The whole formed biomass was ploughed in mid-October (on 15th October 2008) of the sowing year.

Only roots and stubble of the timothy, equivalent to 3.18 t dry matter ha^{-1}, containing 7 g kg^{-1} N, were ploughed in. Crimson clover (Italian origin), which was supposed to be a very late cultivar on the basis of shipping documents, proved to be an entirely early variety in Estonian conditions. Its seeds matured in the beginning of September, were harvested for seed and the straw was removed from the field. Roots and stubble of crimson clover were ploughed in (0.32 t dry matter ha^{-1}, 12.6 g kg^{-1} nitrogen). Washington lupin had produced 12.98 t dry matter ha^{-1} of which roots formed 6.27 t ha^{-1}, nitrogen content 23.5 g kg^{-1}; stems and leaves together 6.71 t ha^{-1}, nitrogen content 20.8 g kg^{-1}. Red clover produced 7.98 t dry matter ha^{-1}, of which roots 3.70 t ha^{-1}, nitrogen content 19.1 g kg^{-1}; stems and leaves combined 4.28 t ha^{-1}, nitrogen content 21.2 g kg^{-1}. Total amount of nitrogen ploughed into the soil with green manure was 287 kg in case of lupin, 161 kg in red clover and 4 kg nitrogen ha^{-1} in crimson clover (Table 1).

In the first year (2009) of residual effect, Washington lupin and red clover ensured approximately equal increase of spring wheat yield compared to control variant (21.3 and 23.9%, respectively, Table 2). The yields were similar with fertilization treatment N 120 kg ha^{-1}. The 1000 kernel weight decreased in both cases compared to the control variant but the gluten content increased. As a result of residual effect of crimson clover, the spring wheat yield increased by 5.9%, which was statistically significant. All

Table 1

Biomass ploughed into the soil and its nitrogen content

	Dry matter t ha^{-1}		N g kg^{-1}	C/N ratio	N kg ha^{-1}	N total kg ha^{-1}
Phleum pratense (control)	roots and stubble	3.18	7.0	47.7	22.3	22
Lupinus polyphyllus	roots and stubble	6.27	23.5	20.9	147.3	
Lupinus polyphyllus	green mass	6.71	20.8	19.7	139.6	287
Trifolium pratense	roots and stubble	3.70	19.1	21.3	70.7	
Trifolium pratense	green mass	4.28	21.2	18.1	90.7	161
*Trifolium incarnatum**	roots and stubble	0.32	12.6	33.0	4.0	4

*seed harvest

Table 2

Spring wheat 'Vinjett' yield and grain quality in the first year of residual effect (2009)

No	Variant 2008	Grain yield kg ha^{-1}	%	Volume weight, g l^{-1}	TKW g	CP g kg^{-1}	Gluten content g kg^{-1}
1	*Phleum pratense* N 120	5,434	126.2	706	28.68	135.8	313.8
2	*Lupinus polyphyllus*	5,220	121.3	756	35.62	133.8	272.4
3	*Phleum pratense* N 0 (control)	4,305	100.0	765	36.00	136.8	214.6
4	*Trifolium pratense*	5,332	123.9	747	32.82	136.0	248.0
5	*Trifolium incarnatum**	4,560	105.9	777	37.04	143.2	197.4
6	*Phleum pratense* N 60	4,980	115.7	765	32.70	133.2	240.0
	LSD $_{0.05}$	219		12	0.35	4.2	9.6

*seed harvest

Table 3

Residual effect of green manure crops on the yield and grain quality of barley 'Inari' in the second (2010) and third year (2011)

No	Variant 2008	Grain yield kg ha^{-1}	%	Volume weight. g l^{-1}	1000 seed weight. g	Crude protein content g kg^{-1}
Residual effect on the second year (2010)						
1	*Phleum pratense* N 90	4,576	154.8	583	39.78	126.0
2	*Lupinus polyphyllus*	3,140	106.2	650	42.84	115.2
3	*Phleum pratense* N 0 (control)	2,957	100.0	655	41.16	107.8
4	*Trifolium pratense*	3,192	107.9	658	41.60	109.8
5	*Trifolium incarnatum* *	2,682	90.7	656	40.62	109.6
6	*Phleum pratense* N 60	4,879	165.0	625	40.38	110.2
	LSD $_{0.05}$	196		7	0.94	2.1
Residual effect on the third year (2011)						
1	*Phleum pratense* N 90	3,516	153.5	660	46.68	119.2
2	*Lupinus polyphyllus*	2,381	103.9	675	46.72	114.2
3	*Phleum pratense* N 0 (control)	2,291	100.0	678	47.50	112.6
4	*Trifolium* pratense	2,569	112.1	680	47.88	116.2
5	*Trifolium incarnatum* *	2,188	95.5	675	47.16	111.8
6	*Phleum pratense* N 60	3,285	143.4	668	46.74	115.0
	LSD $_{0.05}$	242		4	1.12	3.2

*seed harvest

seed quality properties were improved except gluten content, which remained below the control treatment.

Grain yield of barley 'Inari', formed as a result of the residual effect of Washington lupin in the second year (2010), was by 6.2% higher than in the control treatment, although it was not statistically significant (Table 3). However, the concurrent increase of 1000 kernel weight and crude protein content was significant. The residual effect of red clover resulted in barley 'Inari' yield increase of 7.9%, which was also statistically significant. There were no major changes in grain quality. Starting from the second year (2010) there was no residual effect of crimson clover.

Residual fertilizer effect of red clover continued in the third year (2011). Grain yield of barley 'Inari' was by 12.1% higher than in the control treatment. The residual effect of Washington lupin expressed by the increase of grain yield in the third year (2011) was only 3.9% higher than in the control treatment, being statistically not significant. There was no residual effect on the quality in the third year (2011).

From the studied green manure crops, Washington lupin, sown without a spring cover crop, produced the highest amount (12.98 t ha^{-1}) of biomass by the end of the very long and favourable growing season, containing 287 kg of nitrogen. The respective values

for red clover were 7.98 t ha^{-1} and 161 kg at the same time. The residual fertilizer effect of the above species was comparable in the first year. They produced extra grain yield at nitrogen 120 kg ha^{-1} compared to unfertilised treatment. The residual effect of Washington lupin was approved by the increase of wheat gluten content by 5.8%. In the second and third year, the residual effect of Washington lupin increased grain yield of barley, though not significantly. In the second year, 1000 kernel weight and protein content of barley increased. Significant positive residual effect of red clover to the yield of barley appeared even in the third year.

Most of nitrogen was ploughed in with the biomass of Washington lupin, but its advantage was not affirmed by the estimated fertilization value. The problem may lie in the fact that the roots of Washington lupin are vigorous, remain alive in the soil longer and do not decompose. Secondly, growing seasons of 2010 and 2011 were droughty but at the same time, high temperature regime predominated. These conditions decreased soil microflora activity.

Only a modest amount of crimson clover biomass, which contained 4 kg ha^{-1} of nitrogen was ploughed in with the roots and stubble. Crimson clover as annual species has a weaker root system in comparison with perennials (red clover, timothy, Washington lupin). Its root system is formed mainly of fibrous roots. The crimson clover had finished its growth cycle for the middle of September and its ploughing took place in mid-October. For that time one part of the fibrous roots could have been decomposed in the soil during the intermediary one and a half month. Despite of modest amount of biomass, there was a significant residual effect to yield and quality of spring wheat after the first year. Yield increase was probably not solely the result of better nitrogen nutrition. Enhanced soil microbiological activity could also have an impact. The residual effect of fertilisation did not last till the second and third year in crimson clover.

Conclusions

Washington lupin has high nitrogen fixing ability. It can be used as a replacement of red clover in South-Estonian lighter texture acid soils as green manure. The replacement makes it possible to save red clover seeds for producing valuable forage for livestock.

The fertilizer value of Washington lupin was similar to early red clover during the first two years of the residual effect. Spring wheat yield increased by 21.3% after Washington lupin and by 23.9% after red clover in the first year of the residual effect. During the second year of the residual effect, barley grain yield increased by 6.2% after Washington lupin and by 7.9% after red clover. During the third year of the residual effect, the fertilizing impact of Washington lupin decreased to 3.9%, while the fertilizer value of red clover was still 12.1%

Nevertheless, additional research to exploit its full properties is needed. The research should focus on the application of herbicides before ploughing and/or chopping the roots with tillage equipment. As long as the risk of the species turning into weed in following crops exists, it cannot be recommended to organic farmers.

Crimson clover reaches its full bloom by the middle of July. In Estonia, it can be cultivated as green manure for winter crops. Fertilizer value of crimson clover is lower compared to red clover, and residual effect is limited only to the first year. Also, it is necessary to continue trials with these species.

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THE EFFICIENCY OF BIOGAS DIGESTATE ON GRASSLAND COMPARED TO MINERAL FERTILIZER AND CATTLE SLURRY

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Abstract
Biogas production from organic wastes is gaining popularity, especially in agriculture, which produces high quantities of organic wastes suitable for anaerobic digestion. Digestate is the valuable by-product of the biogas production that is considered as a valuable fertilizer. The objectives of the experiment conducted from 2012 to 2013 at the Estonian University of Life Sciences were to compare the impact of biogas digestate, undigested (raw) cattle slurry, and inorganic nitrogen fertilizer on grass yield and to assess the fertilizer value of digestate produced from different feedstock. Fertilizers were applied to the grassland rich in low grasses by broadcasting in quantities according to the nitrogen rate of 180 kg ha^{-1} in three split applications. The application rate of organic fertilizers was calculated based on NH_4-N content. Grassland yield was determined on four treatments: (i) control (no fertilizer was applied), (ii) mineral N-fertilizer (NH_4NO_3), (iii) cattle slurry and (iv) cattle slurry digestate. Grass yield was measured three times during the growing period. Our research showed that digestate when applied based on its NH_4-N content is effective fertilizer in grassland. It could be used as a substitute for mineral-N fertilizer, but its efficiency is slightly lower when compared to cattle slurry, due to its lower application amount resulting in lower nutrient and organic matter amount applied to the grassland. Co-digestion of cattle slurry with solid manure, hay and silage does not decrease digestate fertilizer value in grassland, because the addition of other substrate increases digestate DM content.
Key words: cattle slurry, digestate, grassland yield.

Introduction

Recycling of organic materials has an essential role for the protection of the environment (Makádi et al., 2012). Therefore, the production of methane-rich biogas has increased considerably in European countries in recent years. As agriculture produces high quantities of organic wastes and residues, which are rich in nutrients, biogas production from it is an efficient way of obtaining energy, but it is also an ecological way for the disposal of agricultural wastes (Ondrejčíková et al., 2009). Anaerobic digestion in livestock production systems is especially appealing as the wastes generated are suitable for digestion and hence can provide an additional source of income and reduce costs (Demirer and Chen, 2005).

The biogas yield depends on the feedstock used for anaerobic digestion, especially on its energy density and biodegradability (Weiland, 2003). In anaerobic digestion process different organic materials could be used alone or they could be co-digested. Cattle manure is an excellent substrate for biogas production in anaerobic digesters, although the gas yield from a single substrate is not high (Yohaness, 2010), because the majority of energy-rich substrates (i.e. carbohydrates and proteins) have been eliminated through the digestive tract of the animal (Weiland, 2003; Lehtomäki et al., 2007). However, mixing cow manure with other kind of waste materials in co-digestion can optimize the production of biogas (Yohaness, 2010). It has been found that co-digestion of slurries with grass silage increases yields of methane (Koch et al., 2009; Wang et al., 2009). This is due to the positive synergy effect of the co-substrates (Lehtomäki et al., 2007; Jagadabhi et al., 2008), which provides the missing nutrients and balances substrate composition (Umetsu et al., 2006).

Undigested residues leaving the biogas reactor, the digestate, is the by-product of methane production, coming from organic wastes (Makádi et al., 2012). It is considered as a valuable fertilizer due to the increased availability of nitrogen and the better short-term fertilization effect (Weiland, 2010). In addition, it reduces the need for applying mineral nitrogen and PK fertilizers, which are becoming increasingly expensive due to their energy-intensive production nature.

Comparison of digestate application with mineral N fertilizers have shown that N from mineral fertilizers is physiologically better exploited (Pospíšil et al., 2009) and digestate has lower fertilizer N values (Quakernack et al., 2012), but also that N recoveries of digestate and mineral fertilizers are similar (Gunnarson et al., 2010; Fouda, 2011). When comparing it with undigested slurry some research have showed a slightly lower N removal from anaerobically fermented slurry (Messner and Amberger, 1987), while others have found similar (Möller et al., 2008) or even higher N uptake from digested slurry compared to undigested slurry (Morris and Lathwell, 2004).

The objectives of this study were to compare the impact of biogas digestate, undigested (raw) cattle slurry, and inorganic nitrogen fertilizer on low grasses grassland yield and to assess the fertilizer value of digestate produced from different feedstock.

Materials and Methods

The experiment was conducted at the Erika Experimental Station, Estonian University of Life Sciences (58°23'32" N, 26°41'31" E; elevation 60 m) in 2012 and 2013 on the soil type classified as a *Stagnic Luvisol* (World Reference Base for Soil Resources). Sward, which was established in 2008, consisted of smooth meadow grass (*Poa pratensis*), red fescue (*Festuca rubra L.*), perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). In 2012, white clover had been lost from the sward due to the poor wintering conditions. In 2013 the same happened to perennial ryegrass, which is a short-term grass species in Estonian conditions. Treatments were in four replicates in randomized complete block design: (i) control (no fertilizer was applied), (ii) mineral N-fertilizer (NH_4NO_3), (iii) cattle slurry and (iv) cattle slurry digestate. NH_4NO_3 and organic fertilizers were applied in quantities according to the nitrogen rate of 180 kg ha^{-1} in three split applications (60 kg N ha^{-1}). The application rate of organic fertilizers was calculated based on NH_4-N content. Fertilizers were applied by broadcasting three times in the growing period in 3.05., 12.06. and 3.08. in 2012 and in 3.05., 11.06. and 30.07. in 2013. Cattle slurry was applied in quantities of 67 t ha^{-1} (23+22+22 t ha^{-1}) and 76 t ha^{-1} (25+29+22 t ha^{-1}) in 2012 and 2013 accordingly and digestate 57 t ha^{-1} (19+19+19 t ha^{-1}) and 72 t ha^{-1} (19+29+24 t ha^{-1}) in 2012 and in 2013 accordingly.

Grass yield and dry matter (DM) were determined by using the methodology described by Mannetje (2000). Grass yield was determined from experimental plots of 8.8 m^{-2} (2.2 × 4 m) three times (5.06.12., 23.07.12., 23.09.12., 4.06.13., 23.07.13., 23.09.13.) in the growing period with experimental plot harvester Haldrup equipped with an electronic weighing device. The DM content (%) of biomass from the subsamples (100 g) was determined by drying the sample in force-drough oven for six hours at 105 °C and calculated using the following formula:

$$DM=(M_d * 100)M_f$$

where M_d is the weight of dry material (g), and M_f is the weight of fresh material (g).

The meteorological conditions during the experimental period were monitored with a Metos Compact (Pessl Instruments) electronic weather station. The growing period of 2012 was cooler than that in 2013. The temperatures in 2013 exceeded long term average temperatures. Still the grass growing conditions were more favourable in 2012, when the sum of precipitation during the growth period was similar to the long-term average. Better moisture conditions in 2012 probably increased also the efficacy of used fertilizes. In 2013, the precipitation was considerably lower than in 2012 and long-term average, inhibiting also the plant growth. The average air temperatures during the study period and long-term observations are shown in Table 1.

Table 1

Meteorological conditions in experimental period

Month	Decade	Average temperature, °C			Total precipitation, mm		
		2012	2013	1985-2013 mean	2012	2013	1985-2013 mean
April	I	0.0	-1.7	2.7	6.4	0.0	9.3
	II	5.1	6.0	5.0	25.8	1.6	9.2
	III	9.9	6.1	8.2	9.8	15.2	8.0
May	I	10.4	12.3	10.3	2.4	3.4	12.2
	II	12.3	16.4	11.4	43.6	38.4	22.3
	III	12.2	15.6	12.9	35.6	18.8	23.1
June	I	11.5	19.5	14.9	13.8	2.2	20.1
	II	15.2	15.2	15.2	42.2	29.8	27.6
	III	14.1	19.9	16.3	44.6	20.4	28.3
July	I	19.3	18.3	17.7	15.8	14.6	19.0
	II	15.3	17.3	17.9	43.8	20.8	25.3
	III	19.6	17.9	18.1	14.2	27.2	26.1
August	I	16.6	19.8	17.6	35.6	12.8	32.7
	II	15.2	16.3	16.4	27.8	61.8	29.6
	III	14.1	14.7	15.0	24.0	0.0	26.9
September	I	12.3	13.0	12.9	24.6	9.0	20.0
	II	13.7	13.5	10.8	8.2	1.2	17.4
	III	10.6	6.6	9.4	24.8	17.2	19.8
May-September	I-III	13.9	15.1	12.9	377.2	270.2	376.8

Chemical composition of cattle slurry and digestate was determined in Estonian University of Life Sciences at the Laboratory of Plant Biochemistry. The pH of cattle slurry and digestate was determined in 1 N KCl, dry matter (DM) by oven drying for 2 hours at 135 °C. Total Nitrogen (N_{tot}) content was determined by the Kjeldahl method (Tecator ASN 3313). Ammonium nitrogen (NH_4-N) in samples was extracted with 2 M KCl using the flow injection analysis (Tecator ASN 65-32/84) and nitrate nitrogen (NO_3-N) with 2 M KCl using the flow injection analysis (Tecator ASN 65-31/84). The plant material was converted to Kjeldahl digest. Total phosphorous (P) was analyzed by stannous chloride method (Fiastar 5000 AN 5242, ISO/FDIS 15681), calcium (Ca) with *o*-cresolphthalein complexone (Fiastar 5000 AN 5260), and magnesium (Mg) by titan yellow method (Fiastar 5000 ASTN90/92). For potassium (K) determination flame photometry was used (AOAC 1990). The organic matter (OM) content was determined by loss on ignition at 360 °C.

The composition of the cattle slurry did not differ significantly between experimental years (Table 2), but there was a difference in digestate. The feedstock of the digestate in the first year was only slurry from dairy cattle (the average age of animals five to seven years) housed indoors the whole year round. In the second year, dairy cattle slurry was co-digested with solid dairy manure, hay from natural grasslands and silage residues. Therefore, the DM (dry matter) content of the digestate was higher in 2013 when compared to 2012, and it was comparable to DM content in raw cattle slurry.

Significant differences among treatments were determined using one-way analysis of variance (ANOVA). The comparison of treatment means was done using the least significant difference (LSD) test. All calculations were performed using the statistical package Statistica 12 (StatSoft.Inc). The probability level was set at 0.05.

Results and Discussion

Our results showed that in two-year average fertilization with organic fertilizers increased the grassland yield significantly ($p<0.05$) when compared to control (Table 3). It was probably due to the organic

Table 2

Chemical composition of the cattle slurry and digestate used in the experiment

Factor	Sampling time						Mean	
	2012			2013			2012	2013
	26.04.	30.05.	30.08.	24.04.	03.06.	16.07.		
Cattle slurry								
pH	6.9	7.4	7.2	7.4	6.8	7.3	6.9-7.4	6.8-7.3
DM %	8.7	8.2	8.0	8.4	8.0	8.1	8.3	8.2
N_{tot} kg t^{-1}	4.7	4.7	4.4	3.9	4.0	3.9	4.6	3.9
NH_4-N kg t^{-1}	2.6	2.8	2.7	2.2	2.6	2.8	2.7	2.4
NH_4-N in N_{tot} %	55.0	55.8	62.0	57.0	64.0	59.0	58.0	60.0
NO_3-N kg t^{-1}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P kg t^{-1}	0.9	0.8	0.8	0.8	0.5	0.7	0.8	0.7
K kg t^{-1}	2.3	2.2	2.2	2.4	2.9	2.6	2.2	2.6
Ca kg t^{-1}	1.5	1.2	1.4	1.1	0.8	0.9	1.4	0.9
Mg kg t^{-1}	0.7	0.7	0.7	0.6	0.9	0.7	0.7	0.7
OM in DM %	69.0	72.0	69.0	72.0	67.5	66.0	70.0	68.5
Digestate								
pH	8.3	8.1	8.0	7.9	7.7	8.4	8.0-8.3	7.7-8.3
DM %	3.5	3.8	3.2	6.9	8.7	8.8	3.5	8.1
N_{tot} kg t^{-1}	4.3	4.4	4.3	4.0	4.5	4.5	4.3	4.3
NH_4-N kg t^{-1}	3.1	3.2	3.3	2.5	2.6	2.5	3.2	2.5
NH_4-N in N_{tot} %	73.0	73.0	76.0	62.0	57.0	55.0	74.0	58.0
NO_3-N kg t^{-1}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
P kg t^{-1}	0.8	0.8	0.8	0.7	0.8	0.9	0.8	0.8
K kg t^{-1}	2.2	2.2	2.4	3.4	3.8	3.3	2.3	3.5
Ca kg t^{-1}	1.1	0.8	1.0	1.3	1.8	1.3	1.0	1.3
Mg kg t^{-1}	0.6	0.6	0.5	0.6	0.9	1.0	0.6	0.9
OM in DM %	56.0	57.0	54.0	68.0	62.5	59.5	56.0	63.0

matter in those fertilizers that contribute to the soil organic matter turnover (Makádi et al., 2012). In two-year average the use of mineral N-fertilizer did not have a significant ($p<0.05$) impact on the yield, although it was slightly higher when compared to control. We found out that digestate was as good as inorganic fertilizer N sources for improving yields. Similar results have been reported by Gunnarsson et al. (2010) and Fouda (2011), who found comparable NH_4-N recoveries of digestate and mineral fertilizers.

There was no significant difference between the two-year average yield in digestate and cattle-slurry treatment. However, the yield was a bit higher when using cattle slurry. The same tendency was seen in the yields of different cuts; there was no significant difference between organic treatments, but still the yield in cattle slurry treatment was a bit higher. The average yield difference between cattle slurry and digestate treatment was biggest in the first cut, when the use of cattle slurry increased the yield by 620 kg when compared to digestate, the difference in the second and third cut was 210 kg and 10 kg accordingly. The reason for this was probably the lower amount of other plant nutrients and also the lower amount of organic matter (Table 2) applied to the grassland with digestate, as organic fertilizer amount was calculated based on their NH_4-N content and the total cattle slurry amount applied exceeded the amount of digestate. In two-year average cattle slurry was applied 71.5 t ha^{-1} and digestate 64.5 t ha^{-1}.

In both experiment years the yields in cattle slurry treatment were higher when compared to digestate treatment, although in the first year the difference was not statistically significant ($p<0.05$). Cattle slurry increased the yield by 1.02 t ha^{-1} and 0.64 t ha^{-1} more than digestate in 2012 and 2013 accordingly. When compared to mineral N-fertilizer treatment, the digestate increased ($p<0.05$) the yield by 1.83 and 0.92 t ha^{-1} and cattle slurry by 2.85 and 1.56 t ha^{-1} in 2012 and 2013 accordingly. The amount of cattle slurry applied exceeded the application amount of digestate by 10 and 4 t ha^{-1} in 2012 and 2013 accordingly, as there was more NH_4-N in the N_{tot} in digestate (3.2 and 2.5 kg t^{-1}) when compared to cattle slurry (2.7 and 2.4 kg t^{-1}). However, the yields between two treatments were significantly different ($p<0.05$) only in the second year. We hypothesize that this could be due to the carry-over effect from the first year. When compared to digestate the amount of applied cattle slurry was higher. Therefore, the sward fertilized with cattle slurry received more N_{tot} (63.1 kg ha^{-1}) in 2012, which could have increased the second year yield due to the mineralization of the organically bound nitrogen.

The grassland yield depended on the experimental year ($p<0.05$). When comparing experiment years, average yields in all treatments were higher ($p<0.05$) in 2012 (9.62 t ha^{-1}) when compared to 2013 (5.93 t ha^{-1}), because the second year meteorological conditions were not as favourable for grass growth when compared to the first year. This

Table 3

Dry matter yields of 2012 – 2013

Treatment	Cut			Total	DM increase in yield, t ha^{-1}
	I	II	III		
DM t ha^{-1} mean of 2012-2013					
Control	2.73^{A*}	1.65^{A}	1.72^{A}	6.11^{A}	-
NH_4NO_3	3.02^{A}	1.90^{A}	2.21^{A}	7.14^{AB}	1.03
Cattle slurry digestate	3.37^{A}	2.36^{B}	2.78^{B}	8.52^{BC}	2.41
Cattle slurry	3.99^{A}	2.57^{B}	2.79^{B}	9.35^{C}	3.24
DM t ha^{-1} 2012					
Control	4.47^{A}	1.13^{A}	1.91^{A}	7.51^{A}	-
NH_4NO_3	4.59^{A}	1.60^{B}	2.56^{B}	8.76^{B}	1.25
Cattle slurry digestate	4.93^{AB}	2.28^{C}	3.38^{C}	10.59^{C}	3.08
Cattle slurry	5.83^{B}	2.31^{C}	3.46^{C}	11.61^{C}	4.10
DM t ha^{-1} 2013					
Control	1.00^{A}	2.17^{A}	1.53^{A}	4.70^{A}	-
NH_4NO_3	1.46^{B}	2.20^{A}	1.86^{B}	5.52^{B}	0.82
Cattle slurry digestate	1.81^{C}	2.43^{A}	2.20^{C}	6.44^{C}	1.74
Cattle slurry	2.15^{D}	2.83^{B}	2.10^{C}	7.08^{D}	2.38

*Within the same column, values with different letters are significantly different ($p<0.05$).

is also demonstrated by higher yield increases in the first year when compared to the second year (Table 3). Although the average temperatures in 2012 were lower when compared to 2013, in 2012, there was more precipitation than in 2013 (Table 1). From the first decade of April until the third decade of September the precipitation was 377 mm and 270 mm in 2012 and in 2013 accordingly. In addition, considerably lower first cut yields in 2013 were probably due to the longer snow cover and lower amount of precipitation in the spring, which strongly slowed the grass growth at the beginning of the vegetative period. Another reason why the yields in 2013 were lower than in 2012 could be that in the second year the sward consisted predominantly of smooth meadow grass and red fescue, as perennial ryegrass had disappeared from the sward. The average yield of fertilized swards was 10.32 t ha^{-1} and 6.25 t ha^{-1} in 2012 and 2013 accordingly.

It should be taken into account when assessing the yields in both years that in 2012 the digestate was the result of only cattle slurry anaerobic digestion, but in the year 2013 the cattle slurry was co-digested with solid manure, hay and silage. The composition of the digestate used in the second year was similar to raw cattle slurry, because the addition of solid manure, hay and silage to the digester increased the DM content of the digestate (Table 2). Digestion of co-substrates is common practice in the biogas plants, because the digestion of cattle slurry as the only substrate is not economical (Weiland et al., 2003) due to lower biogas yield and also due to limited availability of animal wastes. Comparison between the yield increases in digestate treatments when compared to cattle slurry between the years showed that the yield in digestate treatment was 75.12% and 73.11% from the yield increase in cattle slurry treatment in 2012 and 2013 accordingly. This result leads to the conclusion that co-digested cattle slurry is as effective in grassland as the digestate from only cattle slurry anaerobic digestion.

Conclusions

In conclusion it can be said that the digestate is an effective grassland fertilizer, and it could be used as a substitute for mineral fertilizers. When applied based on their NH_4-N content, the digestate efficiency is little lower when compared to cattle slurry. Swards fertilized with digestate get less other plant nutrients due to the lower application rate of the digestate, which reduces its efficiency when compared to cattle slurry. Co-digestion of cattle slurry with manure, silage and hay does not decrease the digestate fertilizer value when used in the grassland.

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EFFECT OF FARM SIZE ON THE PRODUCTIVITY AND LONGEVITY OF LATVIAN BROWN COWS

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Abstract
One of most important traits in dairy farming is cow (*Bos primigenius taurus*) longevity. In last few years the length of productive life in Latvian dairy cow population significantly decreased. Cow longevity depends on a large amount of genetic and non-genetic factors. Data from 1037 excluded Latvian brown (LB) breed cows were included in the analysis. Cows were born in the period from the year 2002 to 2006 and a farm size was 9 – 163 cows per farm. Farms were dislocated in all main regions of Latvia. Average life length of cows excluded from the herd was 2463.0 days in small farms, 2234.6 days in medium size farms and 2089.5 days in large farms. Cows' productivity in one life day in small farms was 5.9 kg energy corrected milk (ECM), in medium size farms 6.2 kg ECM and in large farms 6.5 kg ECM. Large farm cows had longer life length in days ($p<0.05$), but higher productivity in one productive day ($p<0.05$) and productivity in one life day.
Key words: cows; lifetime productivity; lifespan.

Introduction

The term 'longevity' refers to duration of life that ends in natural death. Most farm animals have no opportunity to achieve longevity, because they are slaughtered long time before their natural death. Longevity in cows (*Bos primigenius taurus*) can be measured by their lifespan (time from birth to culling from herd) and length of productive life (time from first calving to culling from herd) (Arthur et al., 1992).

Dairy cattle have the potential to live up to the age of 20; however, most modern farm cows are culled even before reaching the sixth lactation. The fact that cows are removed from the herd prematurely is affected by various global factors. One of them is milk or beef purchase price rapid increase, but mostly cow culling happens due to low fertility, mastitis and udder problems as well as milk productivity loss and great age. In the UK an average cow on a farm was kept about 3 lactations until problems with fertility, health and milk quality (Ojangoa et al., 2005; Brickell and Wathes, 2011) occurred. The reasons for culling in farms are different. In farms by first two lactations average are culled 47.9 cows (in different farms from 20.0 – 67.9%). In farms there are culled not only lactating cows, but also heifers in rearing process. In farms with biggest herds most common culling reasons was low fertility, insemination problems, but the third most common reason was increased somatic cell count in milk (Brickell and Wath, 2011). Culling decisions are the result of cow factors such as health, milk production, and reproductive status, but also of factors such as the availability of replacement heifers, parlour capacity or land availability, and prices. With a good availability of heifers farmers might not be able to see the opportunities that lie in keeping healthy cows in the herd, to avoid the hidden costs behind the culling.

Milk production depends on various factors – the main of them are housing and feeding conditions, farm management system, region and season. Cows with low productivity level are culled from herds earlier, because of low productivity level, which is not profitable, but high-yielding cows are often culled because of problems with udder health, metabolism and other (Kalantari et al., 2010).

European farm income from culled cows constitutes an average of 10 - 15% of the total farm budget. Studies in Romania showed that a cow comes profitable reaching 5.85 years of age (Bognar et al., 2009), by increasing cow age, farmers can get more profit from one cow, avoiding costs of rearing new heifer. Housing and feeding conditions are the main factors that affect cow health and productivity. Housing and keeping conditions vary not only in different regions and countries, but in different farms as well. The aim of the study was to evaluate productivity and longevity traits of Latvian brown cows in small, medium and large size farms.

Materials and Methods

In the study data on 1037 Latvian Brown breed cows that started at least first lactation and were culled from the herd in the period from the year 2000 – 2013 were analysed. The farms were located in every Latvian region (Kurzeme, Zemgale, Latgale and Vidzeme). All farms were characterized with different housing, feeding and breeding systems.

Data used in this study were obtained from Latvian Agricultural data centre. Information about cow productivity, the date of cows' birth, date of first calving and date of culling were obtained from the data base. The previously mentioned traits were used to calculate cows' lifespan, length of productive

life, lifetime productivity, and productivity in one productive day.

To characterize milk productivity used energy corrected milk (ECM), which is calculated by the following formula:

$$ECM = milk\ yield \times \times \frac{[(0.383 \times fat, \%) + (0.242 \times protein, \%)]}{3.14}$$

Cows were selected from fifteen different farms of which five are added to the group of small farms (<25 cows), five to the average farm groups (25 - 100 cows) and five farms at large farm group (over 100 cows). Cows distribution in farms and farm groups are shown in Table 1.

For farm privacy reasons we gave them group and consecutive code where – S – small, M – medium and L – large.

Data in tables are represented as mean ± standard error. The factor of farm impact on cow longevity and productivity traits was determined by analysis of variance. Pairwise comparisons between farms occurred by using Bonferroni test. Significant differences in the tables were marked with different superscripted letters of the alphabet (A, B, C, etc.). The mathematical processing was performed using the SPSS program package (Næs et al., 2011).

Results and Discussion

On average, there wre no significant differences between the lactation count in small, medium and large farms, but cows stayed longer on small farms - on average 3.8 lactations. In different conditions cows show different productivity and lifetime results. High rates of dairy cows culling are due to all kinds of diseases and injuries (25.7% of cases), sudden death (23.9% of cases), udder problems and mastitis (16.8% of cases), problems with reproductive traits (16.0% of cases) and low productivity (8.3% of cases) (Dechow et al., 2012; Novaković et al., 2010).

In M size farms some of cows (4.45% or 15 cows) had the maximum 11 lactations if compared with small farms, the maximum lactations were only 7 (Table 2).

There is a significantly longer lifespan in S farms (2463.0 days) than in M farms (2234.6 days) as well as there is a significantly shorter lifespan in L farms (2089.5 days) ($p<0.05$). All cow life length traits are in large amplitude with coefficient of variation from 23.6

Table 1

The number of milk cows in research farms

Farm	Region*	Cows in farm	Farm	Region*	Cows in farm	Farm	Region*	Cows in farm
1S	V	21	1M	K	64	1L	Z	120
2S	V	10	2M	L	76	2L	L	163
3S	Z	9	3M	Z	85	3L	V	113
4S	L	9	4M	K	60	4L	K	100
5S	K	16	5M	V	52	5L	K	139
Count		65	Count		337	Count		635

*K – Kurzeme, Z – Zemgale, V – Vidzeme, L – Latgale

Table 2

Longevity traits depending on farm size

Farm group	Traits		Min	Max	V%
S	Lactation	3.8 ± 0.17	1	7	37
	Lifespan, days	2463.0 ± 72.34^{A}	1271	4203	23
	Length of productive life, days	1584.5 ± 74.78^{A}	25	1584	38
M	Lactation	3.7 ± 0.09	1	11	48
	Lifespan, days	2234.6 ± 37.15^{B}	983	4879	28
	Length of productive life, days	1387.9 ± 38.28^{B}	244	4022	50
L	Lactation	3.5 ± 0.07	1	10	36
	Lifespan, days	2089.5 ± 30.10^{C}	779	4403	36
	Length of productive life, days	1248.5 ± 29.25^{C}	6	3538	59

^{ABC}-traits with different superscriptions are significantly different ($p<0.05$)

Table 3

Longevity traits in different farms

Farm code	Lactation	Lifespan, days	Length of productive life, days
1S	3.2 ± 0.29^{A}	2365.2 ± 123.87^{A}	1459.7 ± 145.20^{B}
2S	4.6 ± 0.37^{B}	2564.8 ± 153.30^{B}	1766.8 ± 155.15^{C}
3S	4.9 ± 0.51^{B}	2548.9 ± 263.94^{B}	1719.3 ± 208.29^{C}
4S	3.4 ± 0.34^{A}	2319.0 ± 168.27^{A}	1380.4 ± 171.02^{AB}
5S	3.8 ± 0.34^{AB}	2587.8 ± 79.74^{B}	1673.3 ± 153.98^{B}
1M	4.5 ± 0.22^{B}	2584.8 ± 74.91^{B}	1699.5 ± 80.84^{B}
2M	3.1 ± 0.20^{A}	2112.0 ± 81.83^{A}	1150.3 ± 84.83^{A}
3M	3.3 ± 0.17^{A}	2001.7 ± 69.48^{A}	1241.0 ± 69.59^{A}
4M	3.4 ± 0.20^{A}	2125.2 ± 71.75^{A}	1313.6 ± 79.97^{B}
5M	4.4 ± 0.27^{B}	2488.8 ± 101.28^{BC}	1677.7 ± 101.99^{B}
1L	3.8 ± 0.60^{AB}	2651.6 ± 60.31^{C}	1558.5 ± 63.44^{B}
2L	2.9 ± 0.12^{A}	1759.0 ± 49.68^{A}	1020.9 ± 50.9^{A}
3L	2.6 ± 0.13^{A}	1707.0 ± 48.91^{A}	909.5 ± 49.13^{A}
4L	5.3 ± 0.23^{C}	2639.8 ± 88.45^{C}	1884.8 ± 88.57^{C}
5L	3.1 ± 0.11^{A}	1906.1 ± 41.35^{A}	1065.7 ± 40.97^{A}

^{ABCD}-traits with different superscriptions are significantly different ($p<0.05$)

Table 4

Lifetime productivity traits depending on farm size

Farm group	Traits		Min	Max	V%
S	Lifetime productivity, kg ECM	15278.6 ± 843.20	386.0	28218.6	44
	Productivity in one life day, kg ECM	5.9 ± 0.23	0.3	9.0	32
	Productivity in one productive day, kg ECM	9.6 ± 0.26^{A}	3.1	15.4	20
M	Lifetime productivity, kg ECM	15226.4 ± 577.51	1593.0	67845.2	69
	Productivity in one life day, kg ECM	6.2 ± 0.16	1.0	14.7	49
	Productivity in one productive day, kg ECM	10.3 ± 0.20^{A}	1.5	20.8	35
L	Lifetime productivity, kg ECM	15034.2 ± 411.48	54	50616.8	68
	Productivity in one life day, kg ECM	6.5 ± 0.11	0.1	11.4	44
	Productivity in one productive day, kg ECM	11.9 ± 0.15^{B}	2.3	25.3	32

^{ABC}-traits with different superscriptions are significantly different; $p<0.05$

to 59.0%. The same tendency occurred in the length of productive life, where it was significantly longer in S farms (1584.5 days), but in L farms it was only 1248.5 days ($p<0.05$). In S farm group the first calving was at the age of 879 days, M farm group – 847 days and in L farm group – 841 day, which makes approximately 29.3 months in S farms 28.3 months in M farms and 28.0 months in L farms.

On different farms there are different conditions and different solutions to farming problems. Also, there is a difference between smaller farms and farms with larger number of cows in them. According to our investigation, there are significant differences between lifespan and productive life length as well as in the count of closed lactations (Table 3).

Lifespan was significantly lower for cows in L size farms (1707.0 and 1759.0 days). Longer lifespan occurred on 2L and 3L farms, but it was significantly higher on 1L and 4L farms (2651.6 and 2639.8 days; $p<0.05$). In S farm group there are overall similar lifespan readings that vary from 2319.0 (4S farm) to 2587.8 (5S farm) days. In M farm group there is a significant difference between 2M, 3M, 4M farms and 1M and 5M farms, respectively, between the lowest and highest lifespan readings ($p<0.05$). Length of productive life keeps on the same tendencies – significantly longest length of productive life was on 4L farm (1884.8 days), but on L farms there are the lowest productive life performances too (in 2L, 3L and 5L farms). As well as

Table 5

Lifetime productivity traits in different farms

Farm code	Lifetime productivity, kg ECM	Productivity in one life day, kg ECM	Productivity in one productive day, kg ECM
1S	13975.9 ± 1462.68^{AB}	5.6 ± 0.47^{B}	9.9 ± 0.53^{C}
2S	17117.8 ± 1716.26^{BC}	6.6 ± 0.35^{B}	9.6 ± 0.29^{C}
3S	15736.1 ± 2028.44^{B}	6.0 ± 0.46^{B}	9.1 ± 0.37^{BC}
4S	10975.9 ± 1920.57^{A}	4.6 ± 0.58^{AB}	7.9 ± 0.75^{B}
5S	18001.5 ± 1910.01^{BC}	6.7 ± 0.48^{BC}	10.6 ± 0.44^{C}
1M	26306.5 ± 1459.60^{C}	9.8 ± 0.34^{D}	15.1 ± 0.36^{D}
2M	8966.5 ± 853.11^{A}	3.8 ± 0.22^{A}	7.3 ± 0.26^{A}
3M	15437.4 ± 1173.18^{B}	6.8 ± 0.38^{BC}	11.4 ± 0.45^{CD}
4M	15437.4 ± 1173.18^{B}	6.8 ± 0.38^{BC}	11.4 ± 0.46^{CD}
5M	14943.7 ± 1221.33^{AB}	5.6 ± 0.26^{B}	8.5 ± 0.29^{AB}
1L	24223.9 ± 1171.65^{C}	8.5 ± 0.29^{C}	15.1 ± 0.34
2L	9732.7 ± 529.24^{A}	4.9 ± 0.16^{AB}	9.5 ± 0.16^{B}
3L	13523.8 ± 837.05^{AB}	7.1 ± 0.28^{C}	14.4 ± 0.24
4L	15304.6 ± 873.91^{B}	5.3 ± 0.19^{B}	7.6 ± 0.19^{A}
5L	14350.7 ± 710.87^{ABC}	6.9 ± 0.22^{BC}	12.9 ± 0.25

^{ABC}-traits with different superscriptions are significantly different ($p<0.05$)

in lifespan traits, the highest length of productive life was in S farm group.

In comparison, on farms where longer lifespan and length of productive life occurred, higher milk yield not only in one lifespan day, but also in one productive life day occurred, too (1L and 1M farms). On 4L farm there was the longest lifespan, but one of the lowest lifetime productivities, which led to lower productivity in one lifespan day and productive life day.

The average cows lifetime productivity on S farm was 15278.6 kg, on M farm 15226.4 kg and on L farm 15034.2 kg. All cow lifetime productivity traits are in a large amplitude with coefficient of variation from 20.8 to 69.6%. The L farms cows had significantly higher productivity in one productive day ($p<0.05$), the differences between S and L are 2.3 ECM kg per one productive day, but productivity in one life day does not significantly differ between different size farms. Productivity in one life day and productivity in one productive day increase with farm size and it means that in M and L farms the average cows' age at first calving is smaller.

As lifetime productivity and productivity in one life day in every farm group is not significantly different, but there are significant differences between productivity in one productive life day, we can assume that on S farms there are main problems with heifer rearing. In studies it was found out that optimal first calving age for dairy heifers is 23 – 27 months, if the first calving age is under 23 months, then the length of productive life decreases by 12%. A similar tendency was observed when first calving age comes over 27 months. (Ajili and Rekik, 2010) The results of our paper shows that for cows with shorter lifespan and length of productive life productivity in one productive day is significantly higher than for cows with longer lifespan and productive life.

The lifetime productivity traits on different farms can be different, mainly because of significant difference in cow lifespan and productive life length. The cow lifetime productivity is shown in Table 5.

Higher lifetime productivity (26306.5 kg ECM) was observed on 1M farm in which there were 64 dairy cows. It was noticed that farm cows had the highest productivity in one life and one productivity day, but the lowest average lifetime productivity occurred on 2M farm (8966 kg ECM). In one life day significantly largest amount of productivity – 9.8 kg ECM – was on 1M farm as well as on 1L farm (8.5 kg ECM) ($p<0.05$). The smallest amount of productivity in one life day was on 2M farm – 3.8 kg ECM in one lifespan day. In one productive life day the largest amount of productivity was on 1M and 1L farms (15.1 kg ECM), but significantly lower productivity in one productive life day (7.3 – 7.9 kg ECM in one day) was on 2M, 4L and 4S farms. As shown in different studies, on farms with cow count form 20 – 100 productive life is significantly higher than on farms with cow count <20 or >100. In farms with cow count 20 – 100 lifespan was 5.7 years and length of productive life was 3.34 years, but within smaller farms it was respectively 6.14 and 3.24 years but in larger farms 5.48 and 3.15 years (Sawa and Bogucki, 2010; Gandini et al., 2012).

Conclusions

1. The largest lifetime milk productivity (15278.6 kg in lifetime) occurred on S (Small) farm group, so productive life (2463.0 days) was the largest, but there wasn't significant difference between other groups.
2. Higher lifetime productivity 26306.5 kg ECM was observed on 1M farm in which there were 64 dairy cows and on this farm cows had the highest productivity in one life (9.8 kg ECM) as well as in one productive day (15.1 kg ECM).
3. On the 4L farm cows were kept on average 5.3 lactations and the number of lactations is significantly higher than on other farms. 20% of investigated farms kept cows over 4 lactations.
4. There is no significant difference between lifetime productivity on different size farms, but there is difference between productivity in one productive day. L farms cows have higher productivity in one productive day ($p<0.05$) and productivity in one life day.

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RELATIONSHIP BETWEEN BODY CONDITION SCORE, MILK PRODUCTIVITY AND LIVE WEIGHT OF DAIRY COWS

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Abstract
Live weight and body condition are indicators for dairy cow's (*Bos taurus*) health, milk productivity and reproduction. Live weight and body condition are defined by genetic and non-genetic factors. These factors are dependent on dairy cows growing and welfare. The aim of research was to analyze body condition relationship with milk productivity and live weight. Data were collected from 49 different breed and lactation dairy cows. Research location was Latvia University of Agriculture Research and Study farm 'Vecauce'. Data were collected from October 2013 to January 2014. Body condition score of all cows decreased from 2.8 ± 0.05 to 2.5 ± 0.04 points in research period. Milk yield increased from 35.6 ± 0.79 kg in the 1st recording to 40.9 ± 1.12 kg in the 2nd recording. Milk yield decreased in the 3rd recording ($p<0.05$). Fat content was the lowest in the 2nd recording (35.5 ± 0.09 g kg^{-1}). Protein content was significantly different in the 1st and 2nd recordings ($p<0.05$). Somatic cell changes were not significant. Body condition decreased of older lactation cows, but milk yield increased at the same time. Milk yield was significantly the greatest in red breed group, compared with Holstein black and white cows (51.1 ± 3.21 kg vs. 41.4 ± 0.78 kg; $p<0.05$). Body condition score significantly affected live weight in such body condition score groups: <2.5 points, 2.75 to 3.0 points. Effect was not significant on live weight in body condition score 3.25< group. Milk productivity and quality traits were not affected by the body condition score ($p<0.05$).
Key words: body condition score, live weight, milk yield.

Introduction

Metabolic processes increase if milk productivity increases. It promotes an increase of metabolic stress. Milk productivity and reproduction traits decrease then. Indicators, which characterize dairy cows metabolic processes, are body condition score (BCS) and live weight. It is very important to evaluate the changes of these indicators. BCS is a visual parameter, which characterizes backfat thickness. Dairy cows cannot intake enough feed in an early stage of lactation and the result is a negative energy balance. According to other researchers, BCS and live weight start to decrease after the 30th lactation day (Banos et al., 2004; Bewley et al., 2008; Yamazaki et al., 2011). Locker S. et al (2011) found out that BCS is the lowest from the 40th to 80th lactation day. In this period BCS decreases to 2.45 points. According to previous studies, the risks to become ill with milk fever, ketosis and fatty liver increases if BCS is greater than 3.5 points after calving and the loss of BCS is great. BCS has been researched since 1970. Researchers started to search for BCS relationship between animal health and milk productivity (Roche et al., 2013). BCS is defined by genes, and heritability coefficient of BCS is average (mean h^2 is 0.26). Genetic correlation between BCS and mastitis is negative. The risk to become ill with mastitis and metabolic diseases is greater for thinner cows (Loker et al., 2012). BCS is a visual indicator, but changes in BCS are related to changes in blood content. Glucoses and triglycerides decrease, if BCS decreases. Cholesterol content increases in this case (Mouffok et al., 2013). BCS has higher influence on milk productivity and reproduction traits for high yielding cows compared to low productivity cows. It is explained with metabolism intensiveness of high yielding cows (Pryce et al., 2002).

The aim of research was to analyze body condition relationship with milk productivity and live weight.

Materials and Methods

Research location was Latvia University of Agriculture Research and Study farm 'Vecauce'. Data was collected from October 2013 to January 2014. Data were analyzed from 49 dairy cows. By a breed factor dairy cows were grouped in 3 groups – Holstein Black and White (HBW, n=12), Red breeds (Latvian Brown, Danish Red, Holstein Red and White; RB, n=30) and milk breed-crosses (F1 HBW×RB; XP, n=7). By lactation cows were grouped in 3 groups – the 1st lactation (n=17), 2nd lactation (n=12), 3rd and older lactation (3rd<, n=20). Cows were kept in a loose housing farm. Cows had *at libidum* access to total mixed ration (TMR). TMR ingredients were 20.0 kg grass silage (*Leguminoseae*, *Phleum pretense* L., *Lolium perenne* L., *Poa pratensis* L., *Dactylis glomerata* L.), 20.0 kg maize silage (*Zea mays* L.), 1.0 kg hay (*Leguminoseae*, *Phleum pretense* L., *Lolium perenne* L., *Poa pratensis* L., *Dactylis glomerata* L.), 6.5 kg grains (*Hordeum vulgare* L), 2.0 kg rapeseed meal (*Brassica napus* L.), 2.0 kg sunflower meal (*Helianthus annuus* L.), 2.0 kg soybean meal (*Glycine max* L.), 0.5 kg sugar beet pulp (*Beta vulgaris* L.), 1.0 kg molasses, 0.2 kg Biotin plus, 0.15 kg baking soda, 0.08 kg salt, 0.07 kg living yeast, 0.07 kg chalk. BCS was evaluated 3 times for each cow in milk recording days. The 1st recording

was on average 14 ± 0.66 day after calving, the 2nd recording was on average 47 ± 0.57 day after calving, 3rd recording was on average 80 ± 0.63 day after calving. Body condition was evaluated in 5 points system (1-thin, 5-fat). Live weight was measured at the same time. Live weight was measured with a special printed tape (with values of live weight). Measures were done at heart girt.

Recording data was collected from Agricultural data center database from the heard recording data. Monthly control milk samples were analyzed for fat, protein and somatic cells count. All of these parameters were analyzed in accredited milk quality laboratory SIA 'Piensaimnieku Laboratorija' with FOSS instrument CombiFoss FC.

Somatic cell count was calculated to somatic cell score (SCS) by formula:

$$SCS=\log_2(\text{Somatic cell count}/100000)+3 \quad (1)$$

Dairy cows were grouped by BCS in 3 groups – the 1st BCS group from 2.0 to 2.5 points (n=94), the 2nd BCS group from 2.75 to 3.0 points (n=47) and the 3rd BCS group from 3.25< points (n=6).

For data analysis SPSS and MS Excel software were used. For traits characterization mean values and standard error, minimal and maximal values were used. To examine BCS, live weight and milk productivity changes according to recording time and BCS group, ANOVA single factors were performed. To analyze breed and lactation influence on these traits, ANOVA Two factors were performed. Bonferroni test was performed to determine significance. The factor was significant if $p<0.05$. Significant differences were marked by different letters ($^{a;b;\ c}$ and $^{A;B;C}$) with superscript.

Results and Discussion

The mean live weight after calving was 639 ± 8.76 kg, but the lowest live weight was on the 47th lactation day, analyzed live weight changes in 90 lactation days. The lowest live weight was 612.6 ± 8.84 kg. Live weight increased in the 3rd recording on average by 9.0 kg, but BCS decreased if compered changes between recordings. Mean of BCS was 2.8 ± 0.05 points in the 1st recording. BCS decreased to 2.5 ± 0.04 points in the 3rd recording (Table 1). A change of live weight was not significant, while BCS varied significantly when compered the 1st and 3rd recording ($p<0.05$). According to researchers of the United Kingdom, BCS characterized dairy cows reproduction traits. The lowest live weight by the UK researchers was found in the 12th lactation week (average 84th lactation day). The UK researchers found out that mean BCS Holstein cows decreased from 2.60 points (after calving) to 2.39 points (Pryce et al., 2001). We obtained similar results. In our case the lowest BCS was on the 80th lactation day. Somatic cell count is an indicator of udder health. Somatic cell count was from 99 ± 32 to 219 ± 104 thousands mL^{-1}.

Foreign scientists concluded that the lowest live weight is from the 10th to 50th lactation day. Live weight increases after this period. BCS changes are similar to those of live weight (Berry et al., 2011). We obtained similar results.

Table 1

Live weight, body condition score (BCS) and milk productivity changes in recordings (n=49)

Trait	1st recording			2nd recording			3rd recording		
	$\bar{x} \pm s_{\bar{x}}$	min	max	$\bar{x} \pm s_{\bar{x}}$	min	max	$\bar{x} \pm s_{\bar{x}}$	min	max
Live weight, kg	616.6 ± 7.47	496	721	612.6 ± 8.84	478	750	621.3 ± 8.39	486	742
BCS	2.8 ± 0.05 ^{a}	2	4	2.7 ± 0.05 $^{a\ b}$	2.0	3.5	2.5 ± 0.04^{b}	2.0	3.5
Milk yield, kg	35.6 ± 0.79 ^{a}	23.6	47.5	40.9 ± 1.12^{b}	22.8	56.4	40.4 ± 0.92 ^{b}	21.1	55.0
Fat, g kg^{-1}	45.4 ± 0.09 ^{a}	31.6	62.9	35.5 ± 0.09^{b}	24.3	50.5	39.2 ± 0.11^{c}	21.9	55.0
Protein, g kg^{-1}	33.1 ± 0.03^{a}	29.6	40.2	34.1 ± 0.03^{b}	29.9	37.7	33.9 ± 0.03 ^{ab}	28.2	39.9
Somatic cell score	2.5 ± 0.22	-0.1	7.6	1.8 ± 0.22	-1.1	6.9	2.0 ± 0.28	-0.8	8.43
Somatic cell count, thousands mL^{-1}	154 ± 50	12	2422	99 ± 32	6.0	1513	219 ± 104	7	4317

$^{a;b;c;}$ – traits with different letters significantly different by recordings ($p < 0.05$)

Milk yield was significantly lower in the 1st recording (35.6 ± 0.79 kg) compered the 2nd (40.9 ± 1.12 kg) and 3rd (40.4 ± 0.92) recording results ($p<0.05$). Other scientists found out that correlation between milk yield and BCS is negative, respectively, milk yield decreases if BCS increases. Correlation is positive between milk yield and live weight. It is due to the fact that cows with greater live weight can intake more feed (Berry et al., 2003). According to results of foreign researchers, negative energy balance period is from calving to the 9th week of lactation. Milk yield increased to the 6th week of lactation, but BCS and live weight decreased in this period (Gross et al., 2011). We found out that phenotypic correlation was negative between milk yield and BCS (r_p = -0.150), while positive correlation was between live weight and milk yield (r_p = 0.191).

Milk fat content was significantly greater in the 1st recording (45.4 ± 0.09 g kg^{-1}) compared with the 2nd and 3rd recording, but protein content was greater in the 2nd recording (34.1 ± 0.03 g kg^{-1}).

Data was analyzed from different lactation HBW, red breeds and cross-breed cows. Significantly greater live weight (643.8 ± 7.15) was in the 3rd< lactation HBW cows group. Live weight of HBW was the lowest in the 2nd lactation group. We found out similar trend also in red breed and crossbreed groups, too. The greatest live weight was found in red breed 3rd< lactation group (690 ± 29.49 kg; Table 2).

BCS was the greatest in HBW 1st lactation and cross-breed 3rd < lactation groups (3.0 ± 0.13 points in both groups). According to previous studies, BCS is greater in the 1st lactation if compared with the 2nd and 3rd lactations. BCS decreases after the 4th lactation to the same level as in the 1st lactation. This process may be affected by cows' age (Kadarmideen, 2004).

Milk yield was significantly greater in all breed groups for the 3rd < lactation dairy cows; the greatest milk yield was 51.1 ± 3.21 kg in red breed group. We found out that milk yield of older dairy cows increased. Foreign scientists got similar results, milk yield of older dairy cows increases. This tendency gets stronger if dairy cows have high genetic potential to produce milk (Short et al., 1990). Genes are factors of breed, respectively, majority genes, which are responsible for milk productivity traits, are located in the 14th chromosome. Milk productivity depends on these genes polymorphisms and interaction (Grisart et al., 2001). Genotype, which is responsible for high milk yield, is mostly in Holstien cow's genome. This genotype encodes lower fat and protein content in milk (Thaller et al., 2003).

Fat content varied from 29.3 ± 0.43 to 43.6 ± 0.10 g kg^{-1} and was significantly different between lactations in HBW and red breed groups ($p<0.05$). Protein varied from 31.7 ± 0.12 to 34.3 ± 0.09 g kg^{-1} and difference was not significant. We obtained inconsistent results if compared with other scientists, because in our research fat content was greater for Holstein breed cows.

BCS affects metabolism processes of dairy cows; respectively, metabolism stresses sensitivity changes by BCS. The level of BCS changes can affect metabolism processes, because body condition is connected with metabolism of lipids. Metabolism affected dairy cows' milk productivity (Bernabucci et al., 2005). A metabolism imbalance is greater for primiparous if compared with multiparous, because primiparous have difficulties to balance metabolism processes (Meikle et al., 2004). Fat cows were metabolically challenged during early lactation due to intense mobilization of body fat. Thin cows were associated with increased plasma indicators of body

Table 2

Live weight, body condition score (BCS) and milk productivity changes between breeds and lactations

Breed	Lactation	Live weight, kg	BCS	Milk yield, kg	Fat, g kg^{-1}	Protein, g kg^{-1}	Somatic cell score
HBW	1st	605.3 ± 20.85^{a}	3.0 ± 0.13$^{a; A}$	31.9 ± 2.27 ^{a}	41.9 ± 0.30^{ab}	34.3 ± 0.09	3.4 ± 0.69^{a}
	2nd	582.6 ± 8.89 ^{a}	2.7 ± 0.06 $^{ab; A}$	39.9 ± 0.97 ^{b}	38.7 ± 0.13$^{a; A}$	33.9 ± 0.04	1.7 ± 0.30^{b}
	3rd<	643.8 ± 7.15 ^{b}	2.7 ± 0.05$^{b; A}$	41.4 ± 0.78 $^{b; A}$	43.6 ± 0.10 ^{b}	34.0 ± 0.03	1.8 ± 0.24^{b}
RB	1st	601.9 ± 9.33^{a}	2.6 ± 0.06$^{a; B}$	34.4 ± 1.01^{a}	36.4 ± 0.14^{ab}	33.3 ± 0.04	2.3 ± 0.21
	2nd	551.7 ± 29.49^{a}	2.3 ± 0.19$^{ab; B}$	42.6 ± 3.21^{b}	29.3 ± 0.43$^{a: B}$	32.9 ± 0.12	1.8 ± 0.98
	3rd<	690.0 ± 29.49^{b}	2.0 ± 0.19$^{b; B}$	51.1 ± 3.21$^{b; B}$	37.6 ± 0.43^{b}	31.7 ± 0.12	2.7 ± 0.98
XP	1st	619.1 ± 13.19	2.8 ± 0.08^{AB}	33.0 ± 1.44^{a}	39.8 ± 0.19	33.9 ± 0.06	2.4 ± 0.44
	2nd	–	–	–	–	–	–
	3rd<	651.8 ± 20.85	3.0 ± 0.13 ^{A}	49.6 ± 2.27$^{b; B}$	41.5 ± 0.30	32.0 ± 0.09	3.1 ± 0.69

^{a,b} – traits are significantly different between different lactation groups in the same breed ($p<0.05$)

$^{A, B}$ – traits are significantly different between different breeds in the same lactation group ($p<0.05$)

– data were not found

protein mobilization during the first weeks of lactation, and lower milk protein secretion (Pires et al., 2013). Sirotkin et al. (2013) found out that indicators of blood were significantly different if compared two dairy cows research groups. Ca^{2+}, P_i, Mg^{2+}, Fe^{2+}, Cu^{2+}, leptin and insulin contents were different. These parameters of blood are connected with ovulation process.

Analyzing BCS influence on live weight and milk productivity, we found out that BCS affected live weight. Live weight was the greatest (636.3 ± 7.40 kg) for the dairy cows whose BCS was 2.75 to 3.0 points. Milk yield was the greatest (39.3 ± 0.72 kg) for the dairy cows with BCS 2.0 to 2.5 points (Table 3).

According to Hungarian scientists, milk fat, protein and lactose decreased from the week 2nd to 8th. Those milk content parameters are affected by lactation and BCS and are very important for reproduction traits – first ovulation and pregnancy parameters (Adrien et al., 2012).

Milk fat decreased in research group with the highest BCS, and it could be the result of lipid metabolism. Lipid metabolism is a cumbersome process. Researchers of Serbia indicated the importance of environment conditions: high temperature and humidity are important factors, which affected BCS. Adaption process is difficult for dairy cows with BCS 4.0 and more points. Milk yield, fat content decreases, when temperature and humidity are too high. Milk yield, fat content and protein content could be lower of our research in the summer time. Body temperature was the highest for dairy cows with the highest BCS, because stress level of metabolism was high when humidity increased (Cincovic et al., 2011).

Other researcher found out that BCS does not affect somatic cell count. Phenotypic correlation was low between somatic cell count and BCS. It could be explained by the fact that somatic cell count is affected by environment factors and udder form (Kadarmideen, 2004).

We analyzed live weight relationship with milk yield and fat according to BCS and recording (1st R, 2nd R, 3rd R – 1st, 2nd, 3rd recordings; Figure 1.).

Milk yield was greater in the 2nd recording 1st and the 2nd BCS groups and in the 3rd recording 3rd BCS

Table 3

Body condition score (BCS) influence on live weight and milk productivity traits

Traits	BCS group		
	2.0 – 2.5	2.75 – 3.0	3.25 <
BCS	2.4 ± 0.02	2.9 ± 0.03	3.7 ± 0.11
Live weight, kg	606.0 ± 6.10	636.3 ± 7.40	633 ± 16.87
Milk yield, kg	39.3 ± 0.72	38.6 ± 1.06	36.5 ± 2.76
Fat, g kg^{-1}	40.0 ± 0.08	40.5 ± 0.11	37.8 ± 0.35
Protein, g kg^{-1}	33.7 ± 0.02	33.5 ± 0.03	34.9 ± 0.11
Somatic cell score	2.0 ± 0.17	2.2 ± 0.26	2.3 ± 0.69

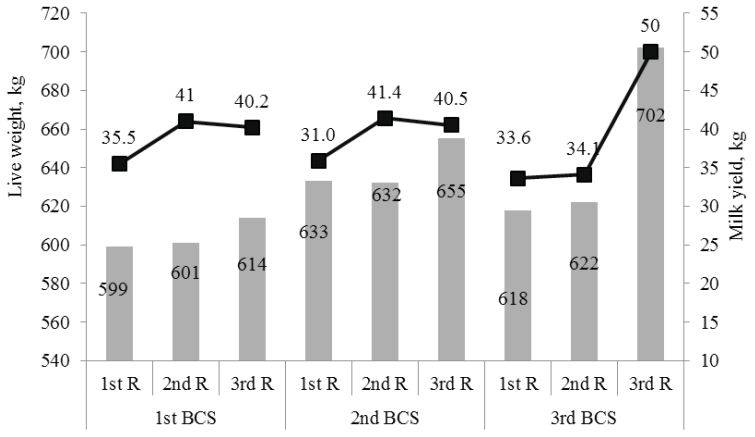

Figure 1. Live weight relationships with milk yield according to body condition score (BCS) in 1st recording: ■ live weight, kg; ▬ milk yield, kg.

Table 4

Fat and protein content changes according to body condition score (BCS) in 1st recording

BCS group	Recording	Fat, g kg^{-1}	Protein, g kg^{-1}	Fat-protein ratio
1st BCS	1st	45.6	33.3	1.41
	2nd	33.2	33.9	0.97
	3rd	38.1	34.0	1.09
2nd BCS	1st	45.0	32.4	1.39
	2nd	35.3	34.4	1.03
	3rd	38.4	34.8	1.10
3rd BCS	1st	39.6	36.1	1.10
	2nd	37.7	34.0	1.11
	3rd	32.3	33.0	0.98

group. Live weight increased in all groups. Analyzing results of the 1st and 2nd recordings, we concluded that our results are similar to those of foreign scientists. Milk yield was the greatest by cows with average BCS value – 2.5 points (Kadarmideen, 2004). Live weight and BCS changes are connected with the length of dry period. Live weight changes were greater for dairy cows with a longer dry period. Dry period affects milk yield and milk content (Gulay et al., 2003).

Fat, protein and fat-protein ratio (FPR) were different between recordings and BCS groups (Table 4).

Analyzing milk fat and protein content, we found out that these parameters were the lowest in the 2nd recording. Fat and protein content was greater in the 1st recording, but FPR was the lowest in the 2nd recording 1st and the 2nd BCS groups (0.97 and 1.03). FPR increased in the 3rd recording 1st and 2nd BCS groups. FPR of the 3rd BCS group increased between the 1st and 2nd recordings, but in the 3rd recording decreased. According to scientists of Czech Republic, FPR of Holstein cows decreased during the whole lactation time. FPR value was 1.91 on the 25th lactation day, but on the 45th lactation day it was 1.45. Fat and protein content was the lowest on the 45th lactation day (Čejna and Chládek, 2005). FPR value was the greatest in the first lactation week; it is connected with milk chemical content (Toni et al., 2011). Optimal FPR value of red breed cows is 1.25 to 1.45 in lactation time (Negussie et al., 2013). FPR was lower of the 2nd lactation dairy cows compared with the 1st lactation cows (Reksen et al., 2002). Low values of FPR usually are connected with low fat content in milk. Farmers can avoid low fat syndrome, by feeding dairy cows on TMR which contains high level of fat acids and neutral detergent fiber. Positive effect is by linseeds that are used in TMR; respectively, fat content increased when linseeds (Suksombat et al., 2013) were used.

Body condition is an, important factor which affected milk productivity and reproduction. Dairy cows cannot be very thin or fat. Incomes of dairy business decrease if cows are thin or fat because BCS affects milk yield, reproduction. Lactation gets longer, but daily milk yield decreases and the period between parturitions becomes longer. Farmers cannot grow next dairy cows – heifers (Kashfi et al., 2011; Mushtaq et al., 2012; Jaakson et al., 2013). Feed for high productivity dairy needs to be balanced. Body condition does not decrease to feeding cows with balanced feed (Tamadon et al., 2011). Body condition is a trait with good heritability by a sire. Making a selection, the body condition is a trait by which farmers can select sires. Body condition needs to be in harmony with milk productivity and reproduction (Kadarmideen and Wegmann, 2003).

Conclusions

Body condition score decreased from 2.80 ± 0.05 to 2.54 ± 0.04 points in 90 days after calving, live weight increased from 616.6 ± 7.47 to 621.3 ± 8.39 kg, but milk yield was the greatest in the 2nd recording or the 47th lactation day (40.9 ± 1.12 kg per day).

Body condition score of the 1st lactation of Holstein Black and White was 3.0 ± 0.13 points, and it was the greatest if compared with red breed and cross-breed cows ($p<0.05$). Milk yield was the greatest in red breed 3rd< lactation group (51.1 ± 3.21 kg; $p<0.05$). Body condition score significantly affected live weight, but other traits did not affect ($p<0.05$) it.

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THE QUALITY OF LATVIAN WARMBLOOD BROODMARES AND THEIR PROGENY DEPENDING ON TYPE AND ORIGIN

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Abstract
In breeding of Latvian Warmblood breed carriage type horses *Equus caballus* one of major breeding objectives is producing of typical purebred animals with minimal influence of modern warmblood breeds. The aim of the study was to analyze the quality of Latvian Warmblood horse breed carriage and sport type mares included in the register of Latvian Horse Breeding Association broodmares and differences between types in valuation and quality of progeny. The data included 135 records from Latvian Warmblood horse breed mares having valuation both of conformation and performance. The data from 79 sport type and 56 carriage type broodmares were collected. The majority of mares in population (31.9%) had proportion 0 – 24.9% of Latvian warmblood purebred ancestors. The proportion of Latvian Warmblood purebred ancestors in the group of carriage type mares was high – 72.2%, while sport type mares – only 23.4%, a difference between groups was significant ($p < 0.01$). Sport type mares showed significantly ($p < 0.05$) better results in conformation and performance quality whereas carriage type broodmares had significantly higher count of daughters registered in Stud book. Comparison of breed types showed that groups did not differ significantly by height of withers and quality of progeny. Lower quality of conformation and performance of carriage type had to be explained by pedigree data as the main criteria for acception, also with older age of broodmares. For future the main goal has to be censorious licensing of best carriage type stallions and using young broodmares with higher quality of gaits and conformation for breeding purpose.
Key words: Latvian Warmblood, carriage type, sport type, broodmares.

Introduction

The main breeding objective for the Latvian Warmblood sport type horse *Equus caballus* is to produce horses suitable and competive for show jumping or dressage, for carriage type horse – produce purebred horses with a steady temperament, suitable for tourism, hobby class riding, driving, reittherapy. Historically, Latvian Warmblood horses had been used for universal purpose, both riding and farm work. Due to the use of several warmblood breeds for improving jumping and gait characteristics, Latvian Warmblood horse became lighter. There was a small number of horses with high proportion of purebred Latvian Warmblood ancestors in their pedigree. Preservation of carriage type started in 2004 based on determination of preserving genetic resources of livestock. The horses accepted as appropriate for the breed's genetic resources must conform to several criteria. The most important criteria is an origin of horse. Currently, one of major breeding objectives in breeding of carriage type horses is producing of typical purebred animals with minimal influence of modern warmblood breeds. A steady temperament, an easiness of handling and a strong body conformation are desirable features for carriage type horses (Rozitis et al., 2008). Recently the count of carriage type horses has been increasing and also a demand for well trained purebred carriage type horses has been growing.

There are stallions of related breeds widely used to reach the breeding objective of breed's sport type. Breeding Programme of Latvian Warmblood Horse determines also criteria for quality of broodmares appropriate to breeding objective and registering in Stud Book. Nevertheless, mares are used for breeding purpose based on the owners' preference and knowledge without external control. Especially selection of Latvian Warmblood sport type females are totally breeder dependant likewise in majority of warmblood breeds (Dubois et al., 2007). Certainly, the selection of stallion is important for the genetic progress in population although the mare contributes as much as the male to the individual foal (Viklund et al., 2011) The selection of females can contribute 1/4 of genetic response (Dubois et al., 2008). Currently, Latvian Horse Breeding Association with the help of a special prize and support payments, promotes breeders to include the best mares in stud herd.

The aim of the study was to analyze a quality of Latvian Warmblood horse breed carriage and sport type mares included in the register of Latvian Horse Breeding Association broodmares and differences between types in valuation and quality of progeny.

Materials and Methods

Data from Latvian Horse Breeding Association broodmares regist of 2013 were analyzed. The data included 135 records from Latvian Warmblood horse breed mares having valuation both of the conformation and the performance. The data from 79 sport type and 56 carriage type broodmares were collected. Based on methodology of an assessment of the broodmares' quality each mare had records of:

a) age;

b) proportion of Latvian Warmblood purebred ancestors in pedigree;
c) height of withers;
d) valuation of conformation;
e) percentage from maximal conformation valuation;
f) points for limb conformation faults;
g) points for conformation;
h) valuation of performance;
i) percentage from maximal performance valuation;
j) points of performance;
k) points for participating in competitions or horse shows.

Each mare also had records of her progeny. The quality of progeny was unified in total score that included points for:

a) reproducing licensed stallions;
b) reproducing broodmares registered in Stud book;
c) high quality progeny;
d) progeny participated in competitions or horse shows.

The Latvian horse breed broodmares were evaluated in accordance with Breeding Programme of Latvian Warmblood Horse. Conformation valuation included seven conformation criteria – type, top line of horse (head, neck, withers, shoulder, back, loins, croup),width of body, conformation of forelimbs, conformation of hind limbs, correctness of movement and temperament.

The performance valuation included four performance criteria evaluated in field-tests – quality of walk, trot, canter and free-jumping.

Some mares accepted as appropriate for the breed's genetic resources had valuation without scoring of jumping quality or without both scoring of jumping quality and canter quality. Each criteria should be valuated in ten point scale and summed for valuation of conformation and valuation of performance.

The information about pedigree of broodmares and their conformation and performance valuation was found out from Stud Book and public horse database of Latvian Horse Breeding Association, available at: www.lwhorse.lv.

The method of points for limb conformation faults, conformation, participating in competition or horse shows, reproducing licensed stallions, reproducing broomares included in Stud book and the progeny participated in competitions or horse shows were used for investigation. The points for the listed traits were given according methodics for detecting quality of broodmares registered in Latvian Horse Breeding Association with aim to range them by quality.

For further analysis the mares were divided in four groups by proportion of Latvian Warmblood purebred ancestors in their pedigree, expressed in percentage:

a) 0 – 24.5%, n = 43;
b) 25 – 49.5%, n = 32;
c) 50 – 74.5%, n = 27;
d) 75 – 100%, n = 33.

The mares also were divided in four groups of age – 5 – 9 years (n = 32), 10 – 14 years (n = 35), 15 – 19 years (n = 47) and 20 and more years old (n = 21).

The points for limb conformation faults were deducted if the mare had score 6 or lower for forelimbs, hind limbs or correctness of movement, 1 point for each trait. The points for the conformation were given according to total valuation of conformation (percentage from maximum – 90 – 100% – 4; 80 – 89% – 3; 70 – 79% – 2; 50 – 69% – 1 point) and points for faults of limbs and correctness of movement were deducted.

The points for performance were given according to total valuation (percentage of maximum) likewise in conformation scoring. For broodmares of carriage type having no valuation of canter or/and jumping quality, percentage was calculated from sum of 2 or 3 performance traits.

The points for participating in competition or horse shows were given: 4 points to the mares participated in competitions and 2 points – in horse shows. Additional 2 points were given to the mares, who won prizes in show jumping up to 130 cm or dressage up to Advanced class. The additional 4 points were given to the mares, who won prizes in show jumping over 130 cm or dressage over Advanced class.

The total score of quality of progeny consisted of four criteria. The points for producing licensed stallions and mares included in Stud book were given for every licensed son (4 points for each) and every daughter registered in Stud book (2 points for each). The count of progeny with sum of conformation and performance valuation over 80 or performance valuation over 28 points were pointed out for each mare; 1 point was given for every offspring of high quality. The points for participating in competition or horse shows were given to progeny in the same way as to broodmares. Additional 4 points were scheduled to carriage type progeny, who won prizes in driving.

The statistical analysis was performed using IBM SPSS Statistics 20. The comparision of both groups were carried out by Independent Samples T-test. The data in groups of proportion of Latvian Warmblood purebred ancestors were analyzed using nonparametric nominal data descriptive statistic method Crosstabs. The significance of the differences between the groups was assessed using Chi-square ($p<0.05$). Pearson correlation was calculated.

Results and Discussion

The quality of Latvian Warmblood broodmares were showed in Table 1 and Table 2. The broodmares had different origin - the proportion of Latvian Warmblood ancestors in origin in all population varied from 0 to 100%.

The mares of investigation group were large enough, reached average 166.97 cm in height of withers. The conformation and performance quality ranged widely. Low coefficient of variation in height of withers purported that population was uniform. It is also the result of criteria of Breeding Programme of Latvian Warmblood Horse, limiting height of withers for both type horses. Sport type horses only above 160 cm in withers can be included to breeding (licensing, registering in Stud book). The optimal height is determined as 162 cm for mares. There were strongly limited requirements for carriage type – small horses are undesirable, limitation also includes width of chest and cannon bone circumference. Only mares higher than 160 cm of withers can be accepted as appropriate for the breed's genetic resources, with some exceptions. There was only 1 mare below 160 cm included in the register and also in the investigation due to high quality of traits. An information from Breeding Programme of Latvian Warmblood Horse suggests that average height of withers in all population is 164.8.

The difference between carriage type mares and sport type mares is shown in Table 2. According to the breeding objective to include mares to breed's genetic resources based on the origin, proportion of Latvian Warmblood purebred ancestors in the group of carriage type mares was high – 72.2%, while sport type mares – only 23.4%, difference between groups was significant ($p<0.01$). A significant difference ($p<0.05$) between groups was found in age, all scores of conformation, included limb conformation faults, performance (expressed as percentage from maximum) and points for daughters registered in Stud book. Sport type mares showed better results in conformation and performance quality whereas carriage type broodmares had significantly higher count of daughters registered in Stud book. It should be noted that valuation of conformation and performance in ten point scale and summing could have a low objectivity due to evaluation in different periods and various appraisers, and it also did not describe certain conformation and performance traits (Orbidane and Jonkus, 2013). Many traits in sport horse breeding are not easy to measure and have to be defined in a subjective way (Koenen et al., 2003). The objectivity could be increased with using evaluation of each trait, introducing of linear profiling using descriptions of trait expressions and regular training of appraisers in order to ensure the collection of suitable information for population analysis (Sanchez et al., 2012; Duensing et al., 2013). Certainly, evaluation of mares is essential, the increased proportion of tested broodmares gives opportunity for higher selection intensity of mares (Viklund et al., 2011).

The majority of mares (31.9%) had proportion 0 – 24.9% of Latvian Warmblood purebred ancestors. Respectively, mares with proportion 25 – 49.9% were 23.7%, with 50 – 74.5% were 20%, with 75 – 100% were 24.4%. The influence of foreign stallions increased in last 25 years. The similar trend was observed in Swedish Warmblood breed, where the proportion of mares covered by foreign stallions has increased dramatically in past decades, especialy since early 1990s (Thoren Hellsten et al., 2008).

Relatively high percentage of broodmares with so large proportion of Latvian Warmblood purebred ancestors in their pedigree explicable with high interest in the preservation and breeding of breed's genetic resources by breeding organizations. Corresponding to it, large numbers of mares accepted as appropriate for the breed's genetic resources were included in registers of broodmares for evaluating and ranging.

Division in age groups showed that more than a third part of the broodmares were 15 – 19 years old (34.5%). The 5 – 9 years old young mares were 23.7%, 10 – 19 years old – 25.9% and older than 20 years – 15.6%. This proportion of age did not describe all population of mares; however, it suggests that there is a trend to use horses in breeding for too many years.

Table 1

Description of traits of Latvian Warmblood broodmares

Parameter	Minimum	Maximum	Average	Standart error	Coefficient of variation, %
Height of withers, cm	158	177	166.97	0.30	2.1
Proportion of Latvian Warmblood purebred ancestors, %	0	100	43.62	2.79	74.3
Valuation of conformation	42	70	52.37	0.37	8.2
Performance, % from maximum	35	90	71.71	0.70	11.3

Table 2

Comparison of carriage and sport type Latvian Warmblood broodmares

Parameter	Group	Average	Standart deviation	Standart error	p value
Age, years	carriage type	15.61	6.10	0.82	0.01**
	sport type	13.28	4.39	0.49	
Proportion of Latvian warmblood purebred ancestors, %	carriage type	72.21	20.36	2.72	0.00***
	sport type	23.35	22.59	2.54	
Heigh of withers, cm	carriage type	166.68	3.37	0.45	0.41
	sport type	167.18	3.52	0.40	
Valuation of conformation	carriage type	51.26	3.72	0.50	0.01**
	sport type	53.16	4.47	0.50	
Conformation, % from maximum	carriage type	73.05	5.26	0.70	0.01**
	sport type	75.90	6.40	0.72	
Points for limbs conformation faults	carriage type	0.80	1.10	0.15	0.04*
	sport type	0.46	0.84	0.10	
Points for conformation	carriage type	1.84	0.53	0.07	0.02*
	sport type	2.09	0.66	0.07	
Performance, % from maximum	carriage type	69.82	6.93	0.93	0.02*
	sport type	73.05	8.64	0.97	
Points for performance	carriage type	1.79	0.71	0.09	0.24
	sport type	1.94	0.74	0.08	
Points for competitions/horse shows	carriage type	1.13	2.37	0.32	0.32
	sport type	0.79	1.61	0.18	
Points for licensed stallions	carriage type	0.93	2.29	0.31	0.62
	sport type	0.76	1.70	0.19	
Points for mares registered in Stud book	carriage type	1.18	0.90	0.25	0.04*
	sport type	0.61	1.21	0.14	
Points for high quality progeny	carriage type	1.70	1.84	0.25	0.15
	sport type	2.18	1.96	0.22	
Points for competitions/horse shows (progeny)	carriage type	2.11	4.03	0.54	0.26
	sport type	1.47	2.54	0.29	
Total score of progeny	carriage type	5.91	7.36	0.98	0.42
	sport type	5.01	5.45	0.61	

*Difference is significant at the 0.05 level.
** Difference is significant at the 0.01 level.
*** Difference is significant at the 0.001 level

Other authors recomended to replace old broodmares by younger ones with higher genetic values for achieving a large annual genetic progress (Viklund et al., 2011).

Finding out proportion of age of mares in each type and each group of origin verified significant difference between groups ($p<0.01$). It could be explained by a fact that the structure of genetic resources was made from older carriage type mares, they also had higher proportion of Latvian Warmblood purebred ancestors in pedigree. The percentage of proportion is shown in Table 3.

An occurence of traits was found in each group of different proportion of Latvian Warmblood purebred ancestors in pedigree. Figure 1. shows the scattering of valuation of conformation in groups with different proportion of purebred ancestors in pedigree.

Table 3

Frequencies of interaction between Latvian warmblood broodmares' age and groups of type and origin

Age, years	Group, within frequencies calculated	Type		Proportion of Latvian Warmblood purebred ancestors in pedigree, %			
		Carriage	Sport	0 – 24.9	25 – 49.9	50 – 74.9	75 – 100%
5-9	age group	0.438	0.562	0.281	0.250	0.188	0.281
	type/origin	0.250	0.228	0.209	0.250	0.222	0.273
10-14	age group	0.257	0.743	0.543	0.200	0.114	0.143
	type/origin	0.161	0.329	0.442	0.219	0.148	0.152
15-19	age group	0.319	0.681	0.319	0.298	0.213	0.170
	type/origin	0.268	0.405	0.349	0.438	0.370	0.242
>20	age group	0.857	0.143	-	0.143	0.333	0.524
	type/origin	0.321	0.380	-	0.094	0.259	0.333

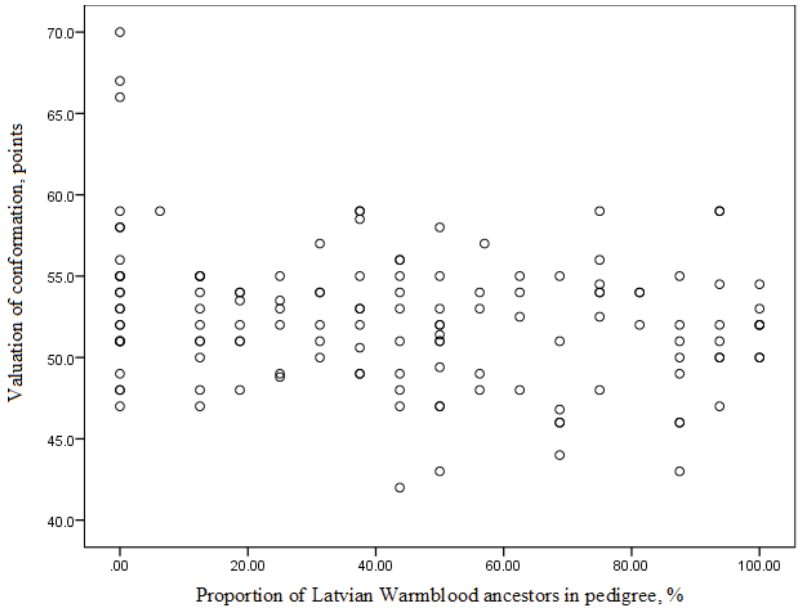

Figure 1. Valuation of conformation of Latvian Warmblood breed mares with different proportion of purebred ancestors.

Significant difference in groups of origin was detected to points for performance ($p<0.01$) and points for competitions and horse shows ($p<0.05$). The broodmares with larger proportion of Latvian Warmblood ancestors had exhibited in horse shows (in this case) more often than sport type mares. Certainly, high influence from other highly productive warmblood breeds also means higher quality of gaits and jump that results showed. The investigation of Swedish Warmblood showed that differences of jumping quality between stallions of different breeds have become smaller over time although foreign populations have considerably affected the show jumping performance in the past (Thoren Hellsten et al., 2008). Currently, Breeding Programme of Latvian Warmblood Horse has not accepted the mares from related breeds as appropriate to the breeding programme and their offspring, born in Latvia, could not be registered as Latvian Warmblood as previously. Possibility the use of imported mares of related breeds for breeding purpose in the past, not only stallions, led to the situation that a mare with a lack of Latvian Warmblood breed presence in her pedigree and also her progeny in many generations were registered as purebred Latvian Warmblood.

Calculated fenotipic correlations showed connection between some traits (Table 4). Low and negative correlation was found between proportion

Table 4

Correlations of traits of Latvian Warmblood broodmares

Parameter	Proportion of Latvian Warmblood ancestors in pedigree, %	Valuation of conformation	Age, years	Performance % from maximum	Points for high quality progeny	Points for competitions and horse shows of progeny
Proportion of Latvian Warmblood ancestors in pedigree, %		-0.217*	0.188*	-0.261**	-0.358**	-0.088
Valuation of conformation			-0.019	0.257**	0.247**	0.017
Age, years				-0.268**	0.375**	0.381**
Performance, % from maximum					0.082	-0.056
Points for high quality progeny						0.496**
Points for competitions and horse shows of progeny						

*Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

of Latvian Warmblood ancestors in pedigree and points for high qualitative progeny, also negative (r_p = -0.261) – with performance, expressed as a percentage from maximal valuation.

Connection between the age of mares and points for progeny are perspicuous due to larger number of offspring. Positive and close to moderate correlation also was calculated between high quality progeny and their participation in competitions and horse shows.

Conclusions

Comparison of Latvian Warmblood carriage and sport type broodmares using horse breeding organisation's data showed that groups did not differ significantly by height of withers and quality of progeny while a significant difference among the types was found in age and valuation of conformation and performance. Significant difference ($p<0.05$) of types in points of limb conformation faults had to be explained with uncritical acceptance of mares as appropriate for the breed's genetic resources due to remaining of small number of Latvian Warmblood purebred animals with low influence of other breeds in their origin. The mares of carriage type also were older, and recently there has been a high number of progeny from them gained by a realization of breeding programme. The main recommendation is a provident selection of high quality carriage type stallions for breeding purpose to reproduce young broodmares with higher quality of gaits and conformation for reproducing offspring more suitable for driving and youth sport. The analysis of data did not show a significant difference in quality of progeny between types, except in points for daughters registered in Stud book where carriage type mares had a better result.

There was insuffucient evaluation data of broodmares, especially born before 2007, without the description of traits that is defined in the methodology of the recent breeding programme. The next step should be a reevaluation of broodmares to clarify trends of the population that is essential for achieving set breeding objectives.

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DAIRY COW BEHAVIOUR AT INDIVIDUAL FEEDING BINS, CAN WE ESTIMATE INTAKES FROM BEHAVIOURAL OBSERVATIONS?

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Abstract
Estimating feed intakes of individual cows in a loose-housed system is difficult and unreliable. It is known that estimating intakes from the number of bites taken at grazing is unreliable. Feeding from a total mixed ration (TMR) is likely to have fewer confounding variables. All cows were over 30 months old, of three breeds: Holstein Friesian, Estonian Red and Estonian Native. There were 30 feeding bins and 66 cows. Cows were observed over the whole 24 hour period, from 1st July to October 22nd 2011, in Märja experimental farm, of the Estonian University of Life Sciences, and data from 37 cows were collected. Measures taken included: time spent at the feed bin, number of bites taken at each visit, weight lost from the bin, and evidence of stealing. Mean time spent at each visit to the feed bin with standard error was 4.4 ± 0.68 minutes. The mean number of bites per visit was 7.1 ± 1.01. There was a significant correlation between the number of bites and total time cows spent feeding ($p<0.001$), with a regression R^2 value of 79.8%. No correlation was found between the number of bites and feed removed at each visit ($p=0.851$) nor between the time per visit and amount of feed removed ($p=0.681$). Therefore, there is no evidence that either the number of bites taken per visit or the time spent at bin per visit are related to intakes. There was no effect of age. Stealing was evident and widespread.
Key words: feeding behaviour, feed intake, dairy cow feeding.

Introduction

Cattle behaviour observations began in the 18th century in Europe (Porzig, 1969; reviewed by Mölder, 1971). The subject of interest at that time was feeding behaviour at pasture: in particular which plants cattle preferred and which were avoided. More recently, efforts have been made to estimate intakes at grazing, but such estimates are too variable to be of any use. The parameters of bite rate, bite number per visit, but not bite mass, of same-sex, similar bodyweight cows are interpreted as being as a result of different herbage type and quality over the grazing area (Hirata et al., 2010).

It might be expected that there would be less variability when feeding from a TMR (total mixed ration), where variation due to herbage factors would be much reduced. If intake estimates from feeding behaviour parameters should be reliable this would be of great benefit in the management of the dairy cow. B. J. Tolkamp et al., (2000) reported that meal length is less variable than the number of meal bouts, although the number of daily visits and intakes per visit were "very variable", while T. J. De Vries et al. (2003) reported similarly that the number of hits (approaches to feed by a cow) were most repeatable and the number of meals per day least (of five other measures). However, these authors also reported high variability between cows. In addition, although repeatability for a behavioural measure can be demonstrated within-cows this does not indicate a robust use for this measure in reliably predicting intakes.

For nutritional, management, health and welfare reasons it is of interest to producers to be able to estimate the intakes of individual cows.

Using individual feeding bins, which record visit time and weight loss from the bins at each visit by individual cows, could test the assumption that intakes can be estimated from behavioural observation. One further confounding factor could be the stealing of feed from bins by illegitimate cows, as identified by J. Mononen et al. (2011). The extent of stealing might be related to breed, and this will also be considered. The aim of this experiment was to evaluate if the time spent at the feeding bin and the number of bites taken is related to feed intakes and further, to estimate if age has any effect on those parameters. A supplementary aim was to evaluate stealing behaviour by observing how frequently cows successfully visited feed group bins that were not intended for their use, the "incorrect" bins for their nutritional needs.

Materials and Methods

The trial was carried out on Märja experimental farm of the Estonian University of Life Sciences, Tartu, Estonia. The cows were cubicle housed. There were 30 feeding bins for 66 cows, and data was collected from 37 cows, selected at random. All of the sampled cows were over 30 months old (a mean of 55 months). The heard comprised three breeds: Holstein Friesian (n=29), Estonian Red (n=6) and Estonian Native (n=2).

They were milked twice daily (at 5 am and 3 pm) in a milking parlour adjacent to the housing area. All cows had access to individual feeding bins through a gate opening in response to a transponder around each cow's neck. Cows were divided into three feeding groups according to milk yields and

fed different portions of a TMR comprising grass and clover silage and concentrates. Water consumption was also recorded. Feeding groups are based upon lactation, milk yields, breed and milk urea concentration.

Cows were observed from a gantry over the feeding area. The following parameters were automatically recorded at each visit to the feeding bin: time and duration of the visit, mass of feed removed. Behavioural observations of the cows were made throughout the whole 24-hour period, on eleven separate occasions, from July 1st to October 22nd. Every time a cow came to the bin her ID number, bin number, time and amount of feed taken were recorded by the observer. Parameters recorded were the following: the time spent at the feeding bin, the number of bites taken at each visit, the weight lost from the bin and the evidence of stealing from the bin by another cow.

Statistics

Descriptive statistics for each parameter were calculated: namely the mean, median, standard error and range. Time of feeding bouts, number of bites and consumed feed were compared with Spearman's Rank correlation and regression analyses.

T- test and ANOVA were used to analyse data within breed and age groups. Normality of the data was tested with the Anderson- Darling test. If the data were not normally distributed, and could not be normalised with log-transformation, nonparametric tests were used (Kruskal-Wallis or Mann-Whitney). All statistical analyses were made with the Minitab statistical program, Minitab 13.

Results and Discussion

Overall, for all sampled cows, the mean time spent eating was over four minutes per visit, a median about three minutes (Table 1). The median number of bites per visit was 5.5 and mass of feed removed from the bins per visit was 114 g.

Table 1

Descriptive statistics for all cows

	Mean	Median	Standard error	Range
Time (min)	4.4	2.8	0.68	0-16
Bites, per visit	7.1	5.5	1.01	1-28
Feed removed per visit (g)	2,285	1,136	411	48-10,784

There was a significant correlation between the number of bites taken and time spent at the bin ($p<0.001$).
$R^2 = 0.798$ (Figure 1).
Regression equation: $y = 0.65 + 1.63x$
Where y = no. of bites per visit and x = time spent eating, in minutes

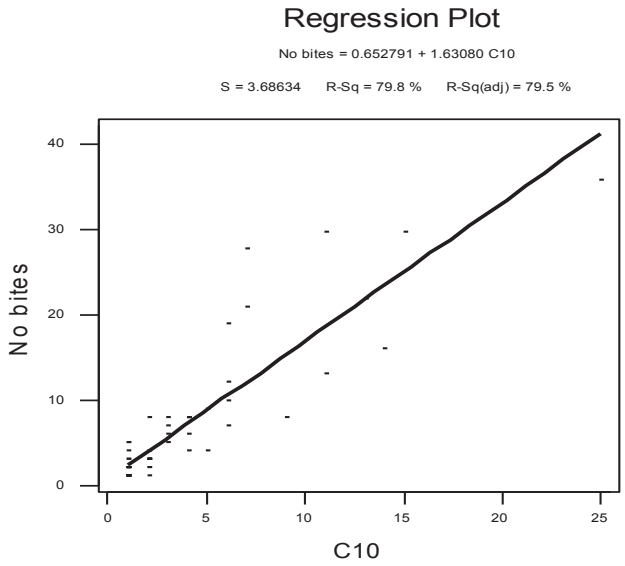

Figure 1. Regression between bites taken and time spent feeding.

No significant correlation was found between the number of bites and feed taken (p=0.851) nor time spent feeding and feed taken (p=0.681).

Age effect

Cows were grouped into four age categories: 30 – 42 months, 43 – 55 months, 56 – 68 months and 68 – 112 months. Since the time spent at the feed bin and the number of bites taken were not normally distributed, the data was log-transformed. The data was then assessed for normality, and differences between the age groups were analysed using ANOVA. No differences were found between any of the age groups and time spent feeding (p=0.564), number of bites taken (p=0.364) or the amount of feed removed from the bin at each visit (p=0.193).

Breed effect

There were no significant breed differences on any of the parameters measured.

Stealing effect

Stealing was observed to have occurred on 55 occasions. To compare instances of stealing between age groups, the Kruskal- Wallis' test was used, and no age effect was found on the frequency of stealing (p=0.726).

In addition to stealing, the behaviours of other herd members towards cows feeding at the feeding bins were observed. Herdmates were observed to show antagonistic behaviours toward feeding cows (poking and pushing away) were observed cows at 48% of the observed feeding visits.

There was a large range in both the amount of time, and the number of bites taken per visit, between different individual cows. The number of bites taken from a feed bin was significantly correlated with the time spent at the bin. This may be an unsurprising finding, but maybe confirms the validity of the set up, and may be a useful confirmation for a different experimental context.

Other findings were mainly negative; there is no evidence that it is possible to predict the intake of dairy cows from observing the number of bites taken at the feeding barrier, or the amount of time spent at the feeding barrier. There was no difference in these behavioural findings with regard to the age of the cows or the breed of the cows. The latter could be the result of not having had equal numbers of cows from each breed, and the tiny number of individuals from the Estonian native breed (n=2).

Stealing from feeding bins by illegitimate cows was frequent. One means by which cows are able to steal from illegitimate bins is the slow speed of closure of the feeding bin gates, and secondly that feeding cows are easily accessible and harassable by other cows. Since each cow does not have its own individually accessible feeding bin, competition at the bin is likely, especially given the slow closure of the gates described above Collings et al. (2011) have described how cows which were lower in the social hierarchy were pushed away from bins more often and consequently fed more frequently during the day, and dry matter intakes and time spent feeding were also lower in these less dominant cows than in more dominant cows.

To eliminate pushing and stealing near the feeding bins, it is important to regulate the gate so that a dominant cow that successfully pushes away a herdmate from a preferred feed bin cannot successfully reward herself with a mouthful of the preferred feed. At the same time the gate should not close every time a cow slightly raises her head as was observed during this trial.

This is important not only to eliminate frustration in the cows, or indeed to upset the delicate balance of nutrition for which the rations are formulated, but casts doubt on intake estimations derived from the automatic recording and evaluation of feed intake data from weighed feed bins.

Conclusions

Variation between individual cows in the time spent at the feed barrier, the number of bites taken, and the feed removed per visit is too high for reliable prediction of intake from these parameters. The number of bites taken per cow and the time spent at the feeding bin were significantly positively correlated. Stealing from feeding bins by herdmates is widespread.

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THICKNESS OF THE SKIN AND ITS LAYERS AT DEGERESS SHEEP OF VARIOUS STRIPES

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Abstract
The article discusses the results of comprehensive studies histomorphological signs of skin and hair of sheep (*Degeress*) of various stripes, especially the thickness of the skin and its layers that are commonly identified indicators. Studies allow to bring the scientific basis for the proper development of activities aimed at increasing production of wool, quantity and quality of which skin microstructure largely depends on. Sheep of different colors have a specific feature on the histological structure of the skin, which is essential for the development of effective methods of selection that enhance productivity of sheep wool.
The researches have established that brown suit sheep has thickened skin as compared to red and gray color ($p<0.01$). In turn, pilar layer of the skin is also much thicker at brown coloration sheep (2029.7×10^{-6} m) than in sheep red (1850.6×10^{-6} m) and sulfur (1773.7×10^{-6} m) suits, the difference is statistically highly significant ($p<0.001$). The thickness of the pilar layer is up to 70% of the dermis, which varies depending on sheep lear ranging from 1773.7×10^{-6} m to 2029.7×10^{-6} m. Reticular layer thickness varies depending on sheep lear ranging from 743.5×10^{-6} m to 826.4×10^{-6} m.
Key words: thickness of skin, sheep, epidermis, pilar layer, reticular layer.

Introduction

Questions to study patterns of development of productive-biological characteristics of sheep (*Degeress*) and identification of the nature of formation of the most important properties of economically useful animals are very closely linked to food production and providing industry with raw materials for the production of high quality products for the consumer.

Sheep (*Degeress*) characterized by a peculiar combination of elements histostructure skin that distinguishes them from other fat-tailed breeds, but at the same time, has significant inbreeding variability indicators such as the thickness of skin and its individual layers, depending on the fineness of the animals wool (Nogaybekov, 1987; Montagna and Parakkal, 1974).

Formation of the structure of the skin and its individual elements causes physical and technical properties of semi-finished products, and ultimately determines the quality of finished products in terms of the strength of the leather, its softness, durability, its presentation, etc. In that case, the study of the histological structure of the sheep's skin is relevant and has important scientific and practical importance in ensuring internal and external markets, consumer goods of high demand.

In this regard, the age-structure formation of the skin and its individual elements, determines physical and technical properties of the half finished products and ultimately determines the quality of finished products in terms of the durability of leather, its softness, its presentation, etc. (Mohammadi et al., 2009).

Skin is an important and very functionally multilateral body. It forms a dense and durable cover that protects the internal tissues and organs from mechanical damage. The skin is an organ involved in thermoregulation. It is an important sensory organ, which has tactile, thermal and painful nerve endings. Also, the skin is involved in metabolism. In the skin, sheep's wool is formed and it plays a physiological role as a component of thermoregulatory mechanisms. Leather - one of the organs, which provides a constant contact with the external environment. Sheep's skin development and its structural elements become particularly important in the view of fact that it is a carrier of valuable products, and it is not only the fur, but also the skin itself. The skin has a significant impact on the growth of different lengths of wool (Ismailov, 2001; Trukhachev, 2007).

Part of the problem of knowledge formation of sheep wool products is studying the structure of the skin and the quality of wool fibers, which has not only general biological, but also has a great practical importance. In this regard, studies allow to bring the scientific basis for the proper development of activities aimed at increasing the production of wool, quantity and quality of which skin microstructure largely depends on.

Skin – is an important protective and adaptive tissue of sheep. The morphology of skin is closely related to the type of animal constitution, which in turn causes productivity, especially fur productivity. According to the results of morphological studies of features of sheep skin, there is a possibility to adjust the selection of pairs to estimate genetic feature to

predict future productivity in the earlier stages of ontogeny animals (Begembekov, 1989).

Improving the quality of the skin, the formation of quantitative and qualitative characteristics in sheep productivity largely depends on the scrutiny of the skin, structure and functions of the relevant structures of the skin. Consequently, the study of sheep skin goes far beyond the interest of the morphology and becomes important in practical sheep breeding.

New breeds and types of sheep created in Kazakhstan are grown taking into account the specific geographical and natural feeding conditions of the regions for which they were created, where paratypic factors had the main influence on the type and productivity of animals.

In connection with above mentioned, the sheep (*Degeress*) bred in different conditions characterized certain adaptive qualities, respectively reflected in their morphological indicators and economically useful productivity.

The purpose of the study was to study the histological structure of the sheep (*Degeress*) skin and its individual elements, which determine physical and technical properties of half finished products and ultimately determine the quality of finished products. **Work task** resulting from the purpose was to set the main features of the sheep's skin (*Degeress*) histostructure: the epidermis, the derma, thickness of pilar layer and reticular layer, the increasing thickness bundles of collagen fibers, the layer thickness of the subcutaneous tissue.

Materials and Methods

Sheep (*Degeress*) have double productivity both meat and wool. They successfully combine high fleece quality, precocity and meat productivity, have a strong constitution, strong, well-developed bones, correct forms of build, hardy and well adapted to the specific conditions of their breeding areas in southern Kazakhstan. Besides wool and meat products, also fur sheepskin from these sheep can be obtained.

The material for research was skin samples obtained from ewes semi-coarse type breed in Limited partnership (LP) 'MKS-Akboz' that is located at Panfilov district of Almaty region (Kazakhstan). The skin samples were taken for histological examination by a skin biopsy on the side of the test animals.

A selection of animals was carried out by random sampling method of pair peers. Experimental animals were similar in age, gender and productivity. There were 5 studied animals in three groups according to the methods of Diomidova N.A., Panfilova E.P., Suslina E.S. (Diomidova et al., 1960; Korostyleva et al., 2009; Plohinskiyi, 1969). Investigated experimental animals were divided by suit into three groups: brown, red, gray.

The study of the development of the sheep skin was performed according to the method of Diamidova N.A. and other scientists:

1. Ablution after fixation;
2. Dehydration in spirits of increasing concentration (50 °C and 60 °C) for 4-6 hours, at 70 °C, 80 °C, and 90 °C for 8-12 hours, and in two portions of 96 °C alcohol for 12-24 hours;
3. Alcohol–chloroform treatment (2-3 hours);
4. Immersion in pure chloroform (2-3 shifts for 1.5 hours);
5. Impregnation in chloroform and paraffin at 37 °C for 3-6 hours. At 56 °C for 1.5 hour;
6. Paraffin impregnation (in three portions for 1-1.5 hours);
7. Paraffin treatment.

The side was a topographical plot of sampling. Histological preparations were prepared in conventional manner. Staining with hematoxylin of Erlich and eosin counterstaining was performed; Veyger's hematoxylin and then van Gieson's picrofucsin.

An isolated piece of skin was placed in the fixative solution in a ratio of not less than 20, and each one was put into a separate bowl. The skin sample covered with hair was attached to the cortical plate flesh side up with plant needles, and it was sunk down in the fixative solution in the same ratio of 1 cm^2 skin to 20-25 cm^3 fixative.

Fixative liquids are used depending on the objectives of the study. Fixative, which is used to detect the general characteristics of the structure of the skin, was 10% formalin. In the above mentioned fixative, the object was kept for 24 hours. After that it was transferred into 5% formalin, where it can be kept safe for a long time.

After fixation, the skin with the hair was oriented to obtain the desired direction of roots and glands. A section was cut out of a length of 2 cm, a width of no thicker than 0.3-0.5 cm on a fixed sample of skin.

For skin preparations, they were covered with celloidin + paraffin.

The skin covered with hair is recommended to take through gelatin to freeze using carbon dioxide and to prepare cuts on a freezing microtome. Speed processing through gelatin, leaving out dehydration through alcohols of increasing content, preserves the true location and integrity of the tissue elements, which are greatly distorted when processing through alcohols and gives incomparably incorrect data when measuring. However, for purposes of studying cytological elements in the skin of animals of all ages, it is absolutely necessary to use a fill of celloidin + paraffin wax and one paraffin wax that provides preparation of thin sections of 6-8×10^{-6} m and

identifying the finest structures inside cells. When cutting on a freezing microtome the thickness of sections is not less than 15-20×10^{-6} m.

The process of getting paraffin and gelatinous units ready for cutting microtomes, passed the preliminary preparation. After fixing in solutions of Zenker and Gelli and the subsequent removal of mercuric chloride in alcohol solution with iodine, the skin was conducted through the spirits of increasing concentration in a mixture of celloidin with ether, and then the paraffin was poured. Time delay in separate mixtures did not exceed 6 hours, except for a mixture of chloroform with paraffin, where it was kept up to 12-24 hours.

The study of morphological changes in the structure and evolution of the skin was produced by describing separate structures and elements and the determination of their quantitative indicators. With this purpose vertical and horizontal sections were used. The measurements were performed with the help of micrometrology line, which was placed inside the eye-glass. Before the measurements the price of one division of the eyepiece micrometer was set, which was achieved by comparison with divisions of object micrometer.

The thickness of the skin and its layers were determined on the vertical sections. On histological preparations diameter bundles of collagen fibers were studied, angle weave between themselves, which gave the opportunity to consider the impact of link types on trademark properties and characteristics of sheepskin. Bundles of collagen fibers, their sizes and link types, which were formed by the interweaving of these fibers, influenced on such trademark properties of fur sheepskin, like the strength of the leather on the gap, elasticity, firmness and others.

Results and Discussion

It is known that the thickness of the skin is closely connected with many of characteristics of the organism, but above all it depends on the overall development of the animal, its productivity, and hence with breeding feature. It varies depending on the age and conditions of animals.

As can be seen from Figure 1, the epidermal layer of sheep consists of two layers: the superficial stratum and lower sprout.

The surface layer is formed by a single row of flat, horizontally - elongated cells with large round and oval nuclei. Cells of the surface layer are clearly expressed keratinization, as the push, approaching the surface, solidification occurs.

Lower germ or Malpighian layer is the deepest layer of the epidermis, consisting of soft living cells of a cylindrical shape. These cells are adjacent to the fibrous layer of the dermis and are powered via the special conical elevation, called the dermal papilla. The thickness of epidermis depends on the age of animals and the time of taking the material for the study.

Presenting the original outer shell, the epidermis first perceives the external environment. Therefore, it is very important that the animal epidermis was well developed, so it should be 1/20 of the thickness of the skin (Avsadzhanov, 1985; Braun, 1983).

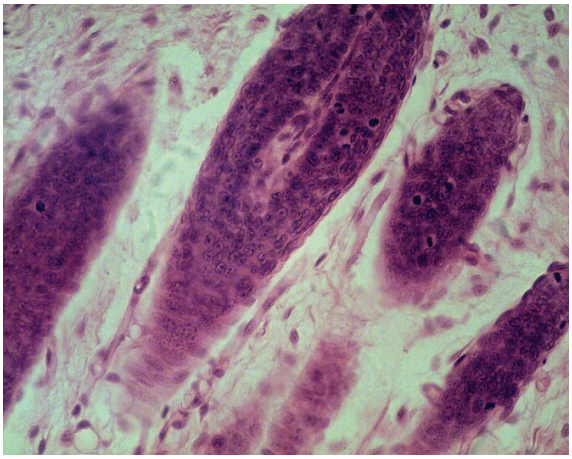

Figure 1. Structure of the epidermal layer of the sheep skin.

Derma (skin actually) is the most powerful layer as for the thickness and the most important for the functional significance. Dermis gives the skin surface strength, provides nutrition and respiration of the epidermis, and participates in all functions of the sheep skin. Derma of sheep is located directly beneath the epidermal layer (Fig.2).

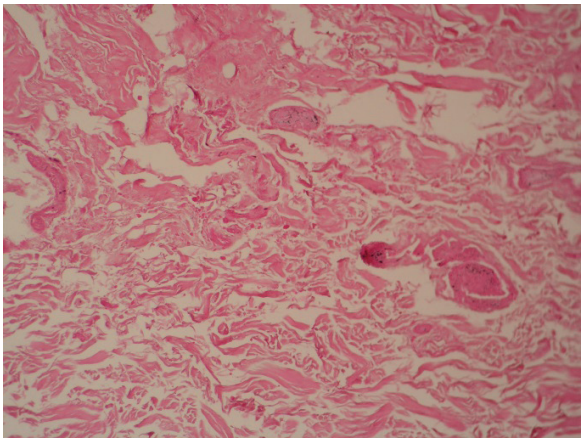

Figure 2. The structure of derma of the sheep skin.

In the dermis, two layers are clearly distinguished: pilar (or papillary) and reticular (or mesh), which occupy 68 – 75% of the total thickness of the skin.

As seen in Figure 3, the thickness of the pilar layer is up to 70% of the dermis, which is the basic structure of the skin, and it is the main reproductive layer. It houses a hair follicle, a dense network of blood vessels and nerve endings, sebaceous and sweat glands, muscles lifting hair, collagen and elastic fibers that provide strong adhesion of all structures. Thickness of pilar layer varies depending on sheep lear ranging from 1773.7×10^{-6} m to 2029.7×10^{-6} m.

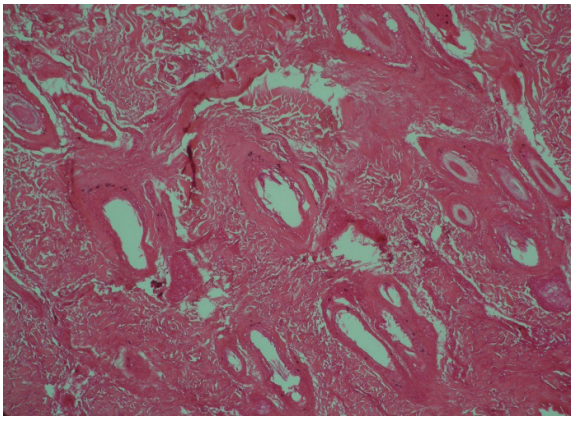

Figure 3. Pilar structure layer of the sheep skin.

Reticular layer (or mesh) is located under the pilar layer (Figure 4). Sheep different directions depending on the age, sex and productivity, reticular layer occupy in the range of 19 to 41% of the dermis. In the test animals with different genotypes, the reticular layer ranges from 30 to 32% of the total thickness of the skin. In this intergroup differences are small and not statistically significant. Reticular layer thickness varies depending on the sheep lear ranging from 743.5×10^{-6} m to 826.4×10^{-6} m.

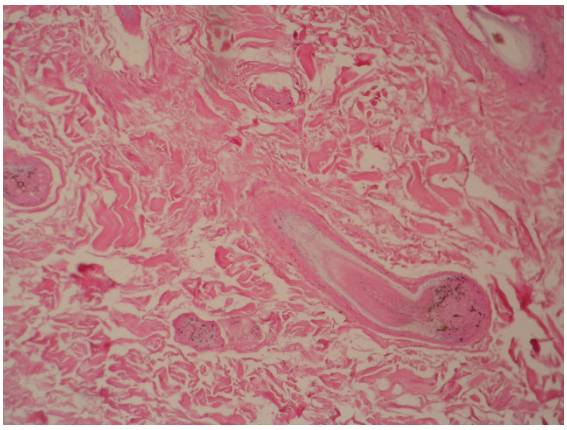

Figure 4. Reticular structure layer
of the sheep skin.

Reticular layer is formed from more densely interwoven bundles of collagen fibers. Collagen is the major protein that makes up the skin of sheep, involved in building tendons, cartilage, bone, dentin, ligaments, fascia, and other connective tissue structures. Skin's derma contains about 98% of the protein.

As seen in Figure 5, our studies have shown that bundles of collagen and elastic fibers of considerable thickness intersect, forming the so-called script. Direction and nature of the tie collagen bundles determines the density and strength of the skin dermis.

This change is reflected in the increasing thickness bundles of collagen fibers and improves the tie by forming more loops, branching and vertically extending beams. In a period of declining growth rate of animals, there is also the simplification and reduction of the thickness of the tie bundles of collagen fibers. Therefore, we can say that at different ages, the skin is not of the same strength.

Collagen fibers are formed from a plurality of thinnest filaments – fibrils, which are interconnected with a particular interfibrillar substance. The thickness is from 1×10^{-6} m to 12×10^{-6} m, wherein the length of the fiber on its thickness varies little. By stretching the skin in different directions, the bundles of collagen fibers are arranged at an angle to each other with a dense weave in certain directions. Character of ligature collagen, thickness of beams and their mutual interlocking depend on the age, sex, and breed of the individual characteristics of sheep.

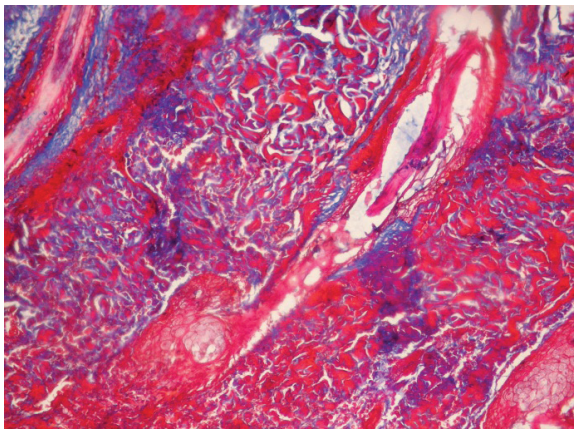

Figure 5. Interlacing bundles of collagen
fibers of the sheep skin.

Subcutaneous tissue of the test animals is composed of loose connective tissue formed of collagen fibers and clay, which is located under reticular layer below bordered by the muscles. Subcutaneous tissue serves as a movable bridge between the dermis and the body of the sheep. It permeates the blood and lymphatic network (Figure 6).

The layer thickness of the subcutaneous tissue varies in different anatomical areas of the sheep skin. This layer is most developed in the blade tip; hence, the bundles of muscle fibers diverge radially along the

back, on the sides and front limbs, gradually thinning. Subcutaneous tissue is strongly developed in the area of breast and under the scalp.

Subcutaneous tissue, being the place of localization of body fat, reduces body heat and softens the blow, thus, protecting the body of sheep from mechanical damage. Subcutaneous tissue forms a loose weave of thin bundles of collagen fibers and elastin fibers network between which fat cells are placed. In the subcutaneous tissue there are many cellular elements, where numerous blood vessels are located.

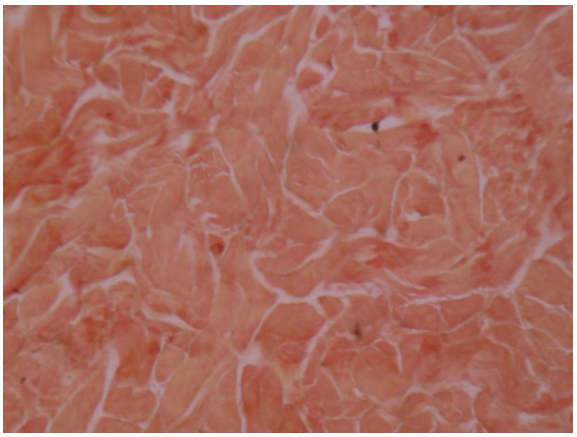

Figure 6. The structure of subcutaneous tissue of the sheep skin.

Thus, the formation of the skin and its derivatives, which begins during fetal development and continues throughout life of sheep, is a complex process.

Diomidova N. (1954), who conducted a detailed description of the formative processes of the skin and wool sheep, notes that the thickness of the skin depends on the breed, sex, age, nutritional status, constitutional and individual characteristics of the animal.

The results of our studies on the histological structure of skin and the total thickness of its individual layers in different sheep lears are presented in Table 1.

As can be seen from these data, in the nature of the structure of the skin, there are significant differences due to the color of the covering hair on the face and legs (suit). Sheep leather of brown suits is thicker than sheep red and gray stripes. The total thickness of the skin on the first 259.0×10^{-6} m or 2.25%, the criterion of reliability of a difference ($t_d = 6.16$) is thicker than that of sheep skin red stripes, which in turn is thicker at 88.1×10^{-6} m or 1.9%, the criterion of reliability of a difference ($t_d = 2.05$) sheep skin gray suit. In turn, the gray skin of sheep is thinner at 202.0×10^{-6} m or 1.14% ($t_d = 8.86$) of the skin brown color sheep.

Thus, thickened skin has brown suit sheep as compared to red and gray color ($p<0.01$).

On development of the epidermis, the brown color sheep also has the advantage, which is accordingly 34.6×10^{-6} m or 1.20% of the total thickness of 28.5×10^{-6} m against the skin, or 1.08% of the total thickness of the skin of the sheep red color. In turn, the epidermis red suit is 28.5×10^{-6} m or 1.08% of the total thickness of 26.4×10^{-6} m against the skin, or 1.04% of the total thickness of the skin of the sheep grays.

Mostly, brown suit sheep compared to red and gray suit ($p<0.01$) have significantly thicker skin. In turn, the pilar layer of the skin is also much thicker at brown coloration sheep (2029.7×10^{-6} m) than in sheep red (1850.6×10^{-6} m) and sulfur (1773.7×10^{-6} m) suits, the difference is statistically highly significant ($p<0.001$).

It should also be noted that the reticular layer is much thicker in sheep brown color (826.4×10^{-6} m) than in sheep skin red (752.6×10^{-6} m) and grey (743.5×10^{-6} m) colors, the difference is statistically

Table 1

Thickness of the skin and its layers at sheep of various stripes, 10^{-6} m

Data	Suit			On average
	brown	red	grey	
Number of animals	5	5	5	
The total thickness of the skin	2890.7±27.06	2631.7±32.18	2543.6±28.31	2688.7±29.18
Including thickness of				
Epidermis	34.6±0.42	28.5±0.27	26.4±0.31	29.8±0.33
%	1.20	1.08	1.04	1.11
Pilar layer	2029.7±16.18	1850.6±29.01	1773.7±19.08	1884.6±21.42
%	70.2	70.3	69.7	70.1
Reticular layer	826.4±7.26	752.6±6.18	743.5±5.32	774.2±6.25
%	28.6	28.6	29.2	28.8

highly significant ($p<0.001$). Differences noted in the reticular layer, did not affect the thickness of the layer of the pilar, where rates fluctuate within $743.5\text{-}826.4\times10^{-6}$ m.

Microscopic study of individual layers of the sheep skin also revealed that histoarchitectonics connective tissue fibers have clearly expressed cellular structure, and it is expressed most clearly in the reticular layer (Figure 4). Spatial density of interposition of connective tissue fibers is higher in the skin samples of sheep that has brown coloration.

Conclusions

1. Sheep (*Degeress*) of different colors have a specific feature on the histological structure of the skin, which is essential for the development of effective methods of selection that enhance productivity of sheep wool.
2. Thickened skin has brown suit sheep as compared to red and gray color ($p<0.01$). Mostly, brown suit sheep compared to red and gray suit ($p<0.01$) have significantly thicker skin. Differences noted in the reticular layer did not affect the thickness of the layer of the pilar, where rates fluctuate within $743.5\text{-}826.4\times10^{-6}$ m.

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CONSUMER ATTITUDES TOWARDS THE INTRODUCTION OF READY-TO-EAT MEALS IN THE LATVIAN MARKET

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Abstract
Nowadays the demand for fast and easy-to-make meals is ever increasing. This type of food is an essential dietary component of a large section of people in developed countries. Given the economic importance of ready-to-eat meals there is a need for a better understanding of the factors that determine their consumption. The aim of this research was to determine consumer attitudes towards the introduction of ready-to-eat meal (MRE) sets in the Latvian market. A survey was performed to determine the Latvian citizens' attitudes towards the introduction of this type of quick-to-make, ready-to-eat products into the market of Latvia. The survey included a total of 800 respondents who answered 14 questions which were related to the interest in MRE sets that can be prepared (warmed up etc.) in less than 10 minutes and taste like home-cooked food, and the types of MRE sets these consumers would like to purchase. The demand forecast for MRE sets in the Latvian market is rather high as 67% of the respondents expressed a desire to purchase these products; the development and implementation of MRE sets is topical. The results show that the highest rated MRE set the respondents, a total of 77.1%, expressed a desire to purchase, was chicken fillet with vegetables. No significant differences in terms of liking were observed between chicken fillet with vegetables and chicken fillet with rice, the second highest rated MRE set by 73.9% of the respondents ($p>0.05$).
Key words: ready-to-eat meal, consumers, market.

Introduction

Over the last few years, the development of innovative food products has been receiving an increasing attention. It is mainly because the demand for safe and easy-to-use food with extended shelf life has been increasing in both consumer and merchant groups (Valceschini, 2006). Nowadays, a significant trend has been observed in Europe: an increasing number of consumers choose for easy-to-cook foods (Olsen et al., 2012). Easy-to-cook foods are a good option for any meal in any group of Latvian consumers, allowing to reduce the daily consumption of junk food, which is becoming increasingly popular. Consumers spend less and less time to prepare meals, and because of long working hours and spending a lot of time on the way, they try to increase their already limited free time. There is an ever increasing demand for services and products that are quick and easy to use. Over the past 10 years, the market for ready-to-eat meals (hereafter referred to as MRE, i.e., meals, ready-to-eat) has increased in Europe and experienced a steady growth (Olsen et al., 2010; Oliver and Salvadori, 2012). MRE include such important features as easy use and short cooking time (Valceschini, 2006; Carini et al., 2013).

The most widely used preparation type for MRE sets is heat treatment. This type of product treatment ensures a long-term storage at room temperatures, maintaining the quality and microbiological safety (Sansone et al., 2012; Ito et al., 2014). This technology provides a number of advantages; it allows you to effectively manage the heat from the steam or water to the product through the packaging, preventing recontamination of the product. It also excludes off-flavour formation and oxidation of the product, preventing moisture and nutrient losses during evaporation and loss of volatile compounds during cooking, as well as preventing development of aerobic microorganisms in the product (Bindu et al., 2007).

With an increase in demand for high quality food, one of the quality assurances includes the possibility of a new, improved and safe packaging use. Advanced packaging technologies offer new and alternative ways to protect the products from the loss of colour, flavour and nutrients, as well as the formation of off-flavours (Chen et al., 2013). One of the most important functions of packaging is a convenient trade and communication with consumers. The communication function serves as a link between consumers and food producers (Marshall et al., 2006). The packaging contains key information about the weight of the product, the origin of the ingredients and their nutritional value (Puligundla et al., 2012).

Food choices are largely dependent on socio-economic and demographic factors, health status and lifestyle factors of the consumers (Geeroms et al., 2008). Based on these factors, consumer attitudes towards the introduction of new food products into the market are not identical. Women show significantly greater distrust of new food products and technologies on the market compared to men (Rollin et al., 2011). Studies related to consumer awareness and shopping motives are extremely important to the food industry, especially in new product development during the initial stages. Understanding how consumers perceive food can cause problems for researchers, because consumers often find it difficult to express or explain the underlying motives for the purchase of the product

(Vidal et al., 2013). One of the principal factors that influence the consumers like or dislike of products is sensory properties of the product. The taste has to be perfect because of its significant impact on food choices (Lyly et al., 2007). Today, there is a high demand for foods processed in packing, because they have long shelf life and are easy to use; these products are popular as daily food (Ito et al., 2014).

It is necessary to evaluate the potential interest of consumers and the attitudes towards this type of product before beginning a new product development. Therefore, the aim of this research was to determine consumer attitudes towards the introduction of MRE sets in the market of Latvia.

Materials and Methods

Food purchase and cooking habits of Latvian adults, as well as the interest in MRE sets that can be prepared in less than 10 minutes and taste like home-cooked food, were analysed in this study.

Eight hundred respondents participated in the 14 question survey, of which 28% were men and 72% were women (Fig 1, description insertion 'Results and Discussion'). The average age of respondents was 31.4 years. Initially, the questions were structured to determine how often respondents cook at home, how long it takes and what products are usually used for meal preparation, as well as the key factors when purchasing food. Since there is a variety of processed (cooked, canned) foods available in the market, the use of which enables to shorten the cooking time, consumer attitudes towards traditionally thermal treated products was determined. One of the most significant questions in the survey was the consumer attitude towards the introduction of MRE sets into the market of Latvia. If the attitude was positive, consumers were asked to choose MRE sets they would like to purchase from 20 different (Table1.) MRE set options.

The obtained data were processed using SPSS software package 16.0; differences among results were considered significant if p-value < 0.05. One way analysis of variance (ANOVA) and Tukey's test were used to interpret the results.

Results and Discussion

The highest activity in completing the survey was observed in age group 26 to 35 years, but the least active respondents were 18 years old or younger. The youngest respondent who participated in the survey was 17 years old, while the oldest was 77 years old (Fig.1).

The level of education was taken into account when analysing the results of the survey. The majority of the respondents (72.8%) had higher education, 16.7% had secondary education, 9.2% had vocational education and only 1.3% of respondents had basic education. Assessing consumer attitudes towards the need of MRE sets in the market of Latvia, the activity of respondents in the labour market was determined. The results showed that 90.8% of the respondents were employed, while 9.2% were unemployed. In order to fully evaluate each of the obtained results the socio-economic and demographic situation of the respondents involved in the survey was an important factor.

Given the fast pace of today, respondents were asked how often they prepared lunch or dinner meals at home (Fig. 2). The results showed that the majority of respondents (69.9%), most of who work, prepare lunch or dinner meals every day. 16.3% of the respondents do not cook at home and prefer to eat out.

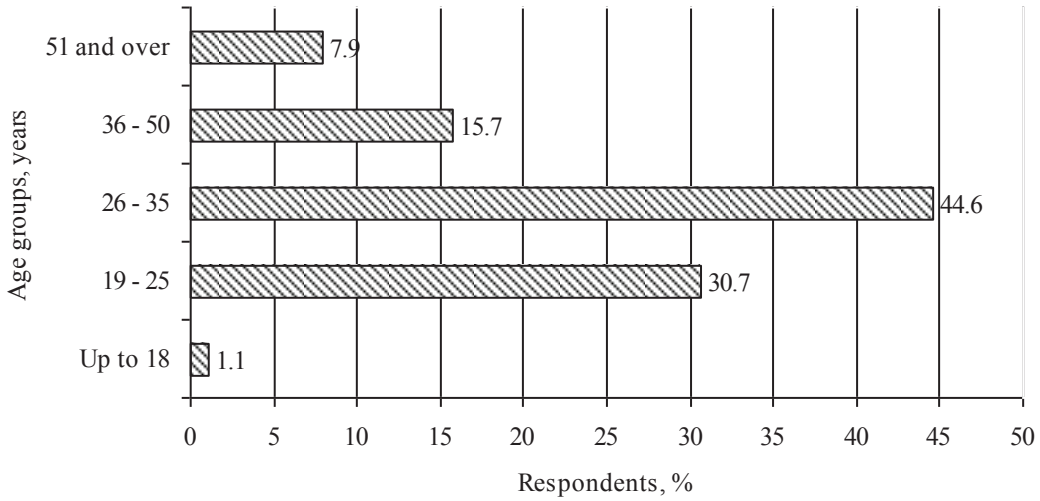

Figure 1. Distribution of respondents by age, %.

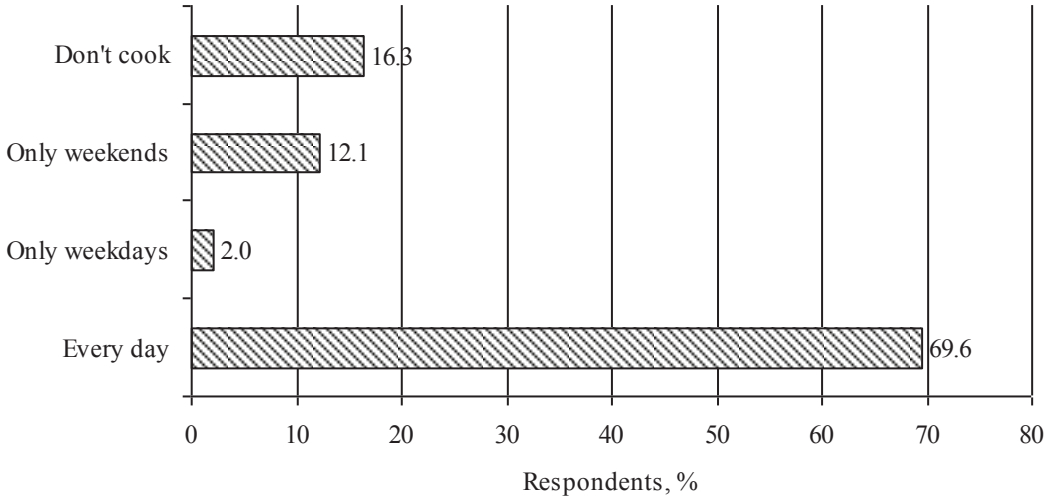

Figure 2. Preparation frequency of lunch or dinner meals at home.

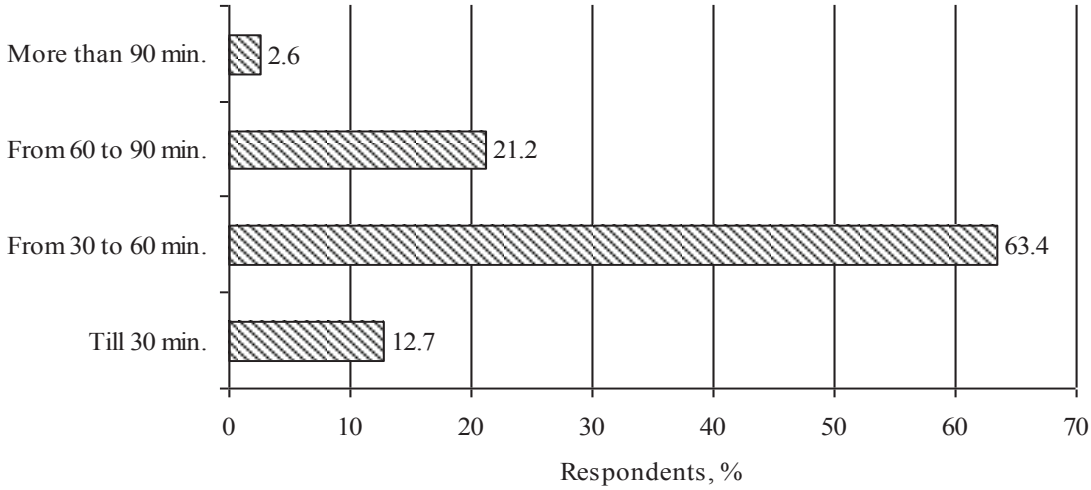

Figure 3. Time spent for preparing one meal a day.

The authors found that 63.4% of the respondents devote 30 to 60 minutes a day for the preparation of one meal (Fig. 3), while 21.2% of respondents spend 60 to 90 minutes a day. Since most of the respondents prepare meals every day, they lose on average 3.5 to 7 hours of their free time on weekly basis. Using MRE sets as lunch or dinner meals would save time for other types of important activities or pursuits.

To be able to understand the main factors of food choice by consumers, and the product characteristics that influence food choices, respondents were asked to assess five key factors: price, quality, producer, packaging and the composition of food.

Food choice and consumption is a part of human behaviour (Mahon et al., 2006). Results show that the quality of the product is the factor consumers evaluate the highest when making a purchase. M. Lyly et al. (2007) noted that the consumer overall preference of foods is greatly affected by the organoleptic properties which is clearly demonstrated in this study as 55.9% of the respondents rated quality as a very important when making a food choice (Fig. 4).

In recent years, more attention has been paid to the ingredients of food products and this study also reflects the fact that Latvian consumers pay more attention to the composition of food. 43.1% of the respondents claimed that the composition of food is an important factor when making a food choice. 45.8% of the respondents said that the price of the product is an important factor when choosing food for daily consumption. However, only 32.7% of the respondents evaluate the producer before making the purchase; significantly more attention is paid to the quality and the price of the product. When asked about the importance of product packaging, 32.4% of participants felt that this factor is of a little importance, although packaging is one of the

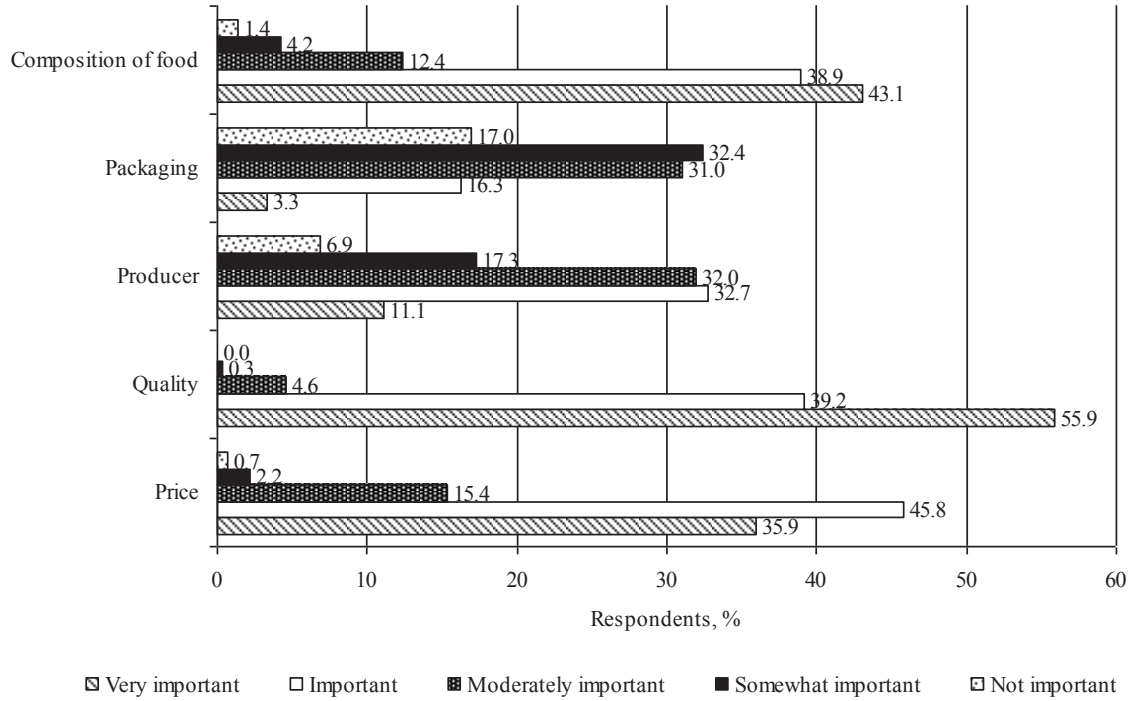

Figure 4. Factors that influence consumer food choices, %.

most important processes in the final stages of food production because it ensures the quality of products during storage and transportation.

Food packaging is designed to contain and protect food, as well as to provide all the necessary information about the product. The primary function of food packaging is to ensure that the food is safe for consumption while brought from the producer to the consumer; properly selected food packaging can significantly extend the shelf life of food products. The secondary function of food packaging is marketing (Han, 2014).

One of the important tasks of the survey was to determine the consumer interest in the introduction of Latvian produced MRE sets into the market of Latvia. These MRE sets could be prepared in less than 10 minutes and would taste like home-cooked food. Consumer view and attitude are the key factors in the development of new products, because the negative attitude may hinder the integration of the product in the market (Haugaard et al., 2014). Survey results show that 67.2% of the respondents would be interested in the introduction of MRE sets into the market of Latvia, while only 32.8% of the respondents claimed that such products are not necessary in the market of Latvia and they have no interest in purchasing MRE sets. These results show that the number of the respondents which are interested in MRE sets is significantly higher than the number of disinterested respondents ($p<0.05$). Thus, the development and implementation of MRE in the market is relevant.

With the introduction of new food products to the market, it is necessary to ascertain the requests of consumers. Food choice is a complex phenomenon with multiple interaction factors that determine which foods are consumed at a particular place and time. Food selection and consumption is a natural and integral part of human behaviour (Mahon et al., 2006).

MRE sets vary in composition of ingredients and in the way of preparation, therefore to find out the product pairs which could be chosen for daily consumption, consumers were asked to choose the MRE sets from 20 different MRE set options that they would be keen to purchase or would not purchase as lunch or dinner meals (Table 1). This question was asked only to the respondents who were interested in the introduction of MRE sets into the market of Latvia, a total of 67.2% of the surveyed consumers. The MRE set which, as approved by most, consisted of 'vegetables with chicken fillet'; 77.1% of the respondents stated that they would like very much to purchase this MRE set for lunch or dinner meals. In terms of preference, there were no significant differences ($p>0.05$) between the MRE sets 'vegetables with chicken fillet' and 'rice with chicken fillet'. The 'rice with chicken fillet' set was approved by 73.9% of respondents. The last set in top three was 'potatoes with chicken fillet' that 67.4% of respondents would like to purchase and use as the key element for everyday meals. It can be concluded that consumers prefer MRE sets containing chicken instead of pork or beef. On the other hand, the MRE sets, which the consumer would not like to

Table 1

The opinion of the respondents on MRE sets

Groups of meals, ready-to-eat	Types of meal ready-to-eat sets	Respondents, %
		Would Buy
Meals, ready-to-eat with chicken fillet	Vegetables with chicken fillet	77.1
	Potatoes with chicken fillet	67.4
	Rice with chicken fillet	73.9
	Pasta with chicken fillet	56.4
	Buckwheat with chicken fillet	56.0
Meals, ready-to-eat with beef	Vegetables with beef	63.3
	Potatoes with beef	51.8
	Rice with beef	48.2
	Buckwheat with beef	45.4
	Pasta with beef	40.8
Meals, ready-to-eat with pork	Vegetables with pork	65.6
	Potatoes with pork	62.8
	Rice with pork	58.3
	Buckwheat with pork	56.9
	Pasta with pork	54.6
Meals, ready-to-eat with sauce	Vegetables with sauce	45.4
	Potatoes with sauce	31.7
	Rice with sauce	27.5
	Pasta with sauce	26.6
	Buckwheat with sauce	24.3

see as lunch or dinner meals, were buckwheat with sauce (75.5%), pasta with sauce (73.4%) and rice with sauce (72.5%) with no significant differences between the rating of disliking ($p>0.05$). Respondents would not purchase these MRE sets for daily consumption.

The survey data show that consumers would choose the MRE sets containing chicken, beef and pork more often than the MRE sets with sauce.

For a MRE set (they would like to purchase) 62.2% of the respondents replied that the optimal weight should be 500 g. (Fig.5). About one third of the respondents (29%) informed that the optimal weight should be 250 g, and 8.8% of the respondents claimed they would like to purchase a MRE set that weighs 1000 g. The weight of MRE sets highly depends on family situation (marital status) and the number of people in families.

In addition, a question about an appropriate price for a MRE set with meat was asked (Fig.5). Respondents were offered to choose from four

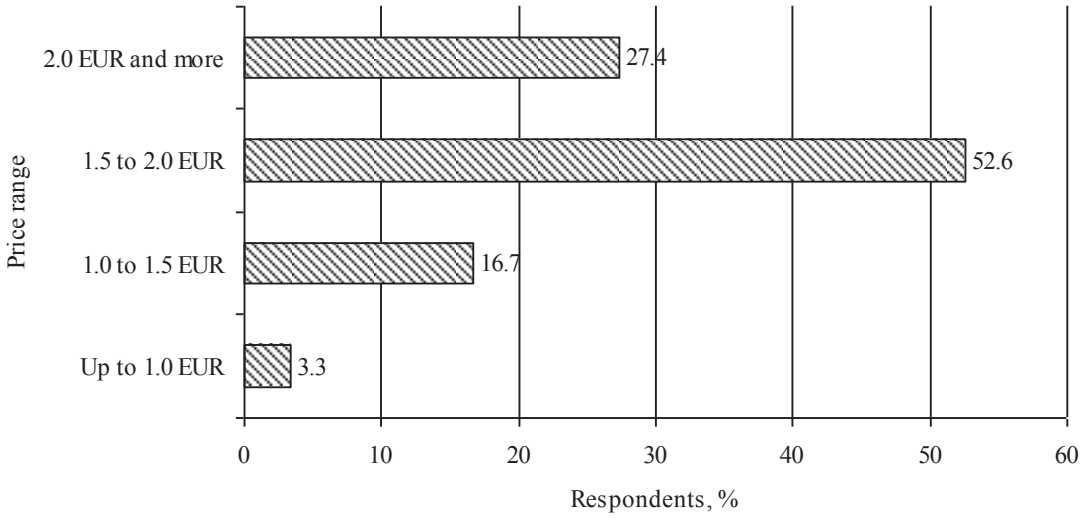

Figure 5. What should be the optimal price for one MRE set with meat that you would be willing to pay?

different MRE set prices. Majority of respondents (52.6%) chose 1.5 to 2.0 EUR as an optimal price. In turn, 27.4% of respondents reported that they would be willing to pay more than 2.0 EUR for a set of MRE. The results of such a response are due to the economic factors which can significantly influence the food choice.

Conclusions

1. The results about the demand forecast for MRE sets in the market of Latvia lead to the conclusion that the development and implementation of MRE is relevant, as 67.2% of the respondents expressed a desire to purchase such products.
2. The target audience for MRE sets is young adults and people in their 30s (age group 19-35 years) with active daily rhythm who work or study and choose to spend more of their free time in other types of activities than preparing meals.
3. When making a food choice, a lot of attention is paid to such factors as product quality, composition of food ingredients and price, while the packaging and producer are less important. The demand for new products in the market of Latvia depends on the food purchase habits of consumers and the factors characterising the product quality.

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ATTITUDES OF LATVIAN ADULTS TO THE CONSUMPTION OF PULSES

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Abstract
Pulses (*Fabaceae*) are an excellent source of nutrients with protein content equal to the protein of meats. Regularly choosing such meat alternatives as pulses can help minimize the amount of saturated fat and increase the amount of fibre in the diet. New pulse products could benefit vegetarians and people struggling with new diet changes. The aim of this study was to determine pulse consumption patterns of Latvian adults. An 11 question survey was developed on an online survey website www.visidati.lv to analyse consumer attitudes towards pulse consumption in Latvia. The questionnaire was completed by 780 respondents from September to December 2013. During the Baltics food industry fair 'Riga Food 2013' five question survey was carried out after vegetarian bean spread tasting. The questions were related to pulse consumption and preference; five question survey consisted of overall preference and quality determination of the product. The results show that the majority of Latvian omnivore adults consume pulses about once a week or less, while pulse intake in vegetarian adults is significantly higher ($p<0.05$). Latvian adults prefer green peas (*Pisum sativum* L.), navy and broad beans (*Phaseolus vulgaris* L.), lentils (*Lens culinaris* Medik.), chickpeas (*Cicer arietinum* L.) and maple peas (*Pisum sativum* L. var. *arvense*). Overall preference of vegetarian bean spread with sun-dried tomatoes (*Solanum lycopersicum* L.) is 'like very much' (4.5 – 4.6) and most of the respondents would purchase this product if it was available in a store.
Key words: pulse consumption, survey, overall preference.

Introduction

Legumes are plants in the family *Fabaceae*, or the fruits or seeds of such plants. Legumes are grown agriculturally, primarily for their food grain seed, for livestock forage and silage, and as soil-enhancing green manure. Pulses (grain legumes) are dry seeds of leguminous plants which are distinguished from leguminous oil seeds by their low fat content. Pulses yield from one to twelve seeds of variable size, shape, and colour within a pod and are mainly cultivated for human consumption (Codex Alimentarius Standart 171-1989, Rev. 1, 1995).

Pulses are staple foods for millions of poor people in developing countries, and have always been a part of Latvian diet. Traditional pulses in Latvian cuisine are maple peas (*Pisum sativum* L. var. *arvense*), garden peas (*Pisum sativum* L.), broad beans (*Vicia faba* L.), lima beans (*Phaseolus lunatus* L.), scarlet runner beans (*Phaseolus coccineus* L.), as well as navy (white), pinto and kidney beans (*Phaseolus vulgaris* L.). Lentil (*Lens culinaris* Medik.), chickpea (*Cicer arietinum* L.), mung bean (*Vigna radiata* (L.) R. Wilczek), urad bean (*Vigna mungo* (L.) Hepper), and adzuki bean (*Vigna angularis* (Willd.) Ohwi and Ohashi) are also widely used in the world. Pulses are available dried, canned, pre-cooked and also frozen. They are used in chilli con carne, burritos, stews, salads, side dishes, casseroles, soups, vegetarian versions of meat dishes and as baked or refried beans (Elliot, 2000).

Pulses are nutritionally important since they usually provide the bulk of the diet and are an excellent source of nutrients with a low glycaemic index. They are a relatively cheap source of energy, protein, slowly digestible carbohydrates and fibre, and important vitamins (niacin, folic acid, vitamin B_1, B_2, B_6) and minerals (iron, zinc, calcium, magnesium, phosphorus and copper) (McCrory et al., 2010).

Pulses constitute an important source of dietary protein for large segments of the world's population particularly in those countries in which the consumption of animal protein is limited by non-availability or is self-imposed because of religious or cultural habits (Boye et al., 2010). Protein content in pulses ranges from 17% to 40%, contrasting with 7–13% of cereals, and being equal to the protein content of meats (18–25%) (De Almeida Costa et al., 2006). It should be noted that pulse proteins are incomplete because of relatively low quantities of the essential amino acid methionine (McCarty et al., 2009). Nevertheless, most plant proteins are incomplete and by combining complementary foods from two or more incomplete protein sources, a complete protein can be created. Grains (which are deficient in lysine) are commonly consumed along with pulses to form a complete diet of protein. Pulses are among the best protein sources in the plant kingdom and unlike conventional animal food sources of protein such as beef or milk, pulses are packed with hormone-free, steroid-free and antibiotic-free plant protein (Papanikolaou and Fulgoni, 2008).

Pulses have shown numerous health benefits, e.g. lower glycaemic index for people with diabetes, increased satiation, cancer prevention, reduction in cholesterol levels, prevention or alleviation of constipation, and protection against cardiovascular diseases due to their dietary fibre content (Wang et al., 2010). Total dietary fibre content in pulses range from 11.8% to 23.3% (Khan et al., 2007), and insoluble dietary fibre constitutes 80 to 85% of total

dietary fibre in whole pulses. Besides these nutritional benefits, pulses are also gluten-free, so products made from pulse flours provide alternatives to wheat flour based products (Siddiq et al., 2013).

Among European countries, higher pulse consumption is observed around the Mediterranean, with per capita daily consumption between 8 and 23 g, while in Northern Europe, the daily consumption is less than 5 g per capita (Bouchenak and Lamri-Senhadji, 2013). In 2009 pulse (including soy and nuts) consumption represented only 0.7% of total regularly consumed foods in Latvian food basket, with daily consumption 32 ± 2 g per capita (Joffe et al., 2009). According to the Latvian Central Statistical Bureau data, the average pulse consumption was 9 g per capita per day and 63 g per capita per week in 2013 (Consumption of..., 2013). Pulses are included in two (out of the five) food groups that are the building blocks for a healthy diet. A serving of pulses as a part of meat/meat alternative or vegetable group is 75-80 g (½ cup) cooked beans, lentils, chickpeas, split peas or canned beans. The U.S. Dietary Guidelines (2005) recommend eating three cups (450 – 480 g) of pulses per week. Regularly choosing such meat alternatives as pulses can help minimize the amount of saturated fat in the diet but there are no guidelines for pulse consumption in Latvia. According to European Guidelines on cardiovascular disease prevention (Perk et al., 2012), recommended fibre intake is 30-45 g per day. One serving (½ cup) of pulses covers about 25% of daily fibre needs and contains more healthy fibre than any other food group (Agurs-Collins et al., 2006).

Pulses have always been an important source of protein for vegetarians and since vegetarianism is a growing trend in Latvia, pulse consumption among vegetarians could be higher compared to omnivores. However, one does not need to abandon their dietary practice in order to replace animal protein with plant protein on a weekly basis or choose a healthier dietary option. New pulse products, e.g. various pulse spreads, could benefit vegetarians and people struggling with new dietary changes but before beginning new product development it is necessary to evaluate the potential interest of consumers and the attitude towards this type of products (Lyly et al., 2007). Therefore, in order to ascertain the attitudes of Latvian adults to the consumption of pulses the aim of this study was to determine pulse consumption patterns of Latvian adults.

Materials and Methods

A questionnaire was developed to analyse consumer attitudes towards pulse consumption in Latvia. The questionnaire consisted of 11 questions; of which 7 questions were related to pulse consumption and preference (e.g., intake and preference of pulses, the most common method of preparation and serving), and the rest of the questions were aimed at obtaining basic information about respondents. The survey was developed with both multiple-choice and open-type questions; it was carried out during the Baltics food industry fair 'Riga Food 2013' (90 respondents; 67% women and 33% men) and posted on an online survey website 'www.visidati.lv' (690 respondents; 56% women and 44% men) from September to December 2013. Complete information about the respondents is given in Results and Discussion.

An additional 5 question survey was carried out during 'Riga Food 2013' after vegetarian bean spread tasting. Respondents (90 respondents; 67% women and 33% men) were asked to evaluate vegetarian bean spread with sun-dried tomatoes (*Solanum lycopersicum* L.) with 5-point hedonic scale (5 – like very much and 1 – dislike very much) in order to determine the overall preference of the sample (ISO 4121:2003). The rest of the questions were related to the quality of the product and the likeliness to buy it in a store. The vegetarian bean spread with sun-dried tomatoes was prepared at the laboratory of Faculty of Food Technology (Latvia University of Agriculture) according to the vegetarian spread preparation technology (Ķirse and Kārkliņa, 2014).

The obtained data processing was performed using mathematical and statistical methods with statistical software *R 3.0.2*; differences among results were considered significant if p-value < 0.05. One way analysis of variance (ANOVA), Tukey's test and independent samples *t*-test were used (Næs et al., 2011).

Results and Discussion

The questionnaire was completed by 780 respondents; of which 360 respondents followed vegetarian diet (46.2%) and 420 respondents were omnivores (53.8%). The mean distribution of respondents by gender was 64.5% women and 35.5% men. Respondents were from all regions of Latvia: Kurzeme (16.6%), Zemgale (26.3%), Vidzeme (14.8%), Latgale (13.3%) and Riga (29.0%). There were two noticeably larger respondent groups: one from age 35 to 44 and the other from age 25 to 34; while the smallest group of respondents was aged 65 and older (Fig. 1). During their late 20s to early 40s people use internet quite often compared to seniors who go online seldom. The level of education of the majority of respondents (62.3%) was higher education, followed by vocational (23.8%), secondary (11.7%) and basic (2.2%) education.

Vegetarians, by definition, abstain from the consumption of red meat, poultry, and seafood, therefore their main protein source is plant protein products (Fig. 2). According to the survey data, some

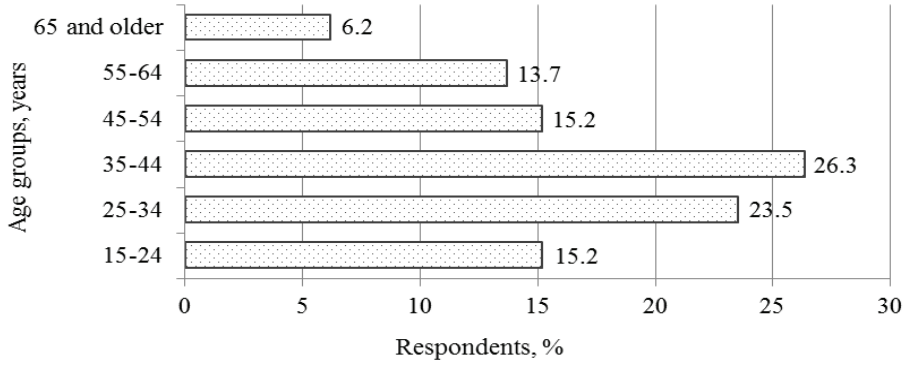

Figure 1. Distribution of respondents by age.

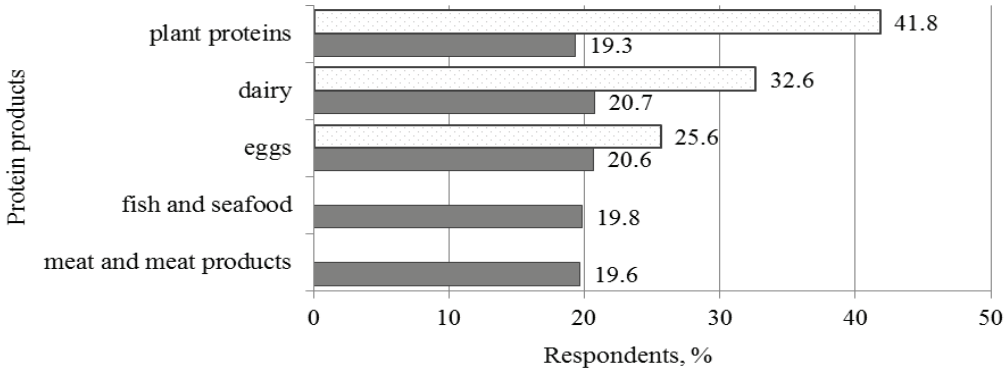

Figure 2. Preference of protein sources in the daily diet of the respondents:
□ vegetarian, ■ non-vegetarian.

of the vegetarian respondents (56 women and 24 men) replied that they only use plant protein products; they could therefore represent vegans. The data show that non-vegetarian respondents consume products from all five protein sources with no significant preferences to one or more protein sources in their daily diet ($p=0.072$). A little over 58% of the protein in Latvian vegetarian diet comes from animal products (dairy and eggs) and it corresponds to the data of other studies which state that about 40-60% of the protein in non-strict vegetarian diets is derived from animal products (Mangels et al., 2011).

The majority of respondents consume pulses about once a week, while only vegetarian respondents eat pulses every day (Fig. 3). Pulses are consumed 3 to 5 times a week by one fifth of non-vegetarian respondents and almost one third of vegetarian respondents. Pulse intake in Latvian vegetarian adults is significantly higher ($p<0.05$) comparing with Latvian omnivore adults. Belgian researchers found a trend towards a higher intake ($p<0.1$) of pulses when comparing vegetarian with omnivorous subjects, nevertheless reaching only borderline significance (Clarys et al., 2013). The differences between Latvian and Belgian dietary patterns could be the main reason for diverge in pulse intake as well as the product offer at grocery stores in Belgium. A variety of plant-derived protein products are available in Belgium such as vegetarian sausages, fillets, meat balls, burger patties and chops (Clarys et al., 2013) while in Latvia these products are not available or very expensive and not affordable on a daily basis. In Belgium vegetarian products in stores are equally available to both omnivores and vegetarians while the majority of Latvian omnivores are not aware of the small amount of vegetarian alternatives available. As pulses have always been a part of Latvian diet, it is possible to incorporate pulses in everyday meals for a reasonable price or prepare such meat substitutes as burger patties and meat balls from pulses at home.

More than one third of non-vegetarian respondents and a little over 20% of vegetarian respondents consume pulses less than once a week, and this less frequent consumption of pulses is the result of various reasons. The main reason for low pulse intake in both groups of respondents is meteorism (21.4%) (also known as flatulence). Pulses contain oligosaccharides (raffinose, stachyose, and verbascose) which are resistant to human enzymes but are digestible by gastrointestinal methane-producing microflora

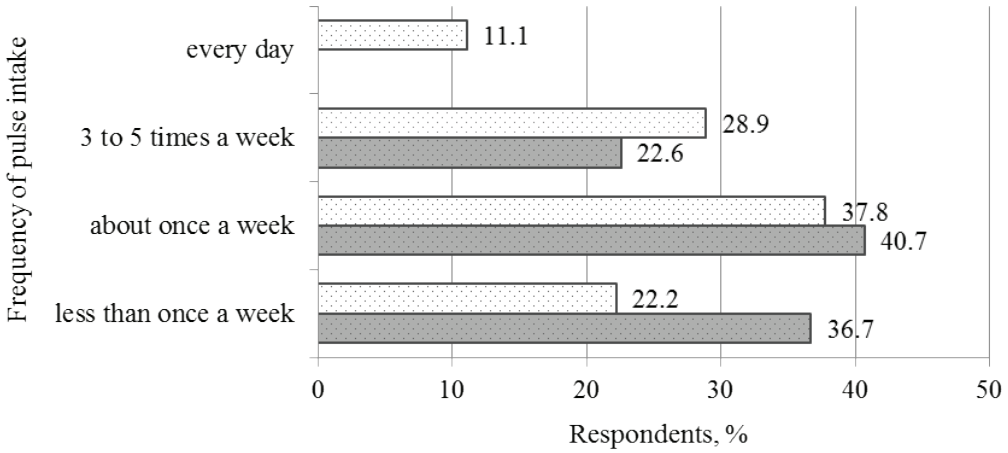

Figure 3. Frequency of pulse intake by their dietary practice:
□ vegetarian, ▩ non-vegetarian.

(*Methanobrevibacter smithii*) that causes flatulance (Ohge et al., 2005). Meteorism can be avoided by cooking pulses with anise, cumin, coriander, caraway or asafoetida which have the ability to prevent the formation of gas in the gastrointestinal tract (McGee, 1984). Other reasons for eating pulses less than one a week include long cooking time (20.4%), pulses not being in favour in respondents' families (15.3%) and problems with making delicious dishes (13.5%). Non-vegetarian respondents also find the taste or aroma unappealing and are not ready to plan ahead because dry pulses need soaking; 10.3% of non-vegetarian respondents follow blood type or paleo diet that excludes pulses. Vegetarian respondents often find pulses hard-to-cook and it prevents them from preparing home-cooked pulses more frequently. Seldom pulse consumption is also linked with limited pulse availability and living arrangements (e.g. easier to make other foods in dormitories).

Non-vegetarian respondents prefer traditional pulses (Fig. 4): green peas (18.1%), navy beans (15.6%), broad beans (13.4%), maple peas (12.6%), kidney beans (12.0%) and split peas (11.0%). Vegetarian respondents prefer lentils (14.4%) and chickpeas (12.8%) instead of split and maple peas. Pulses are the perfect meat alternative for vegetarians thus they are more open to trying different pulses. Mung beans which are fairly common in Indian cuisine are consumed three times less by non-vegetarians and soy is consumed twice as much by vegetarians. Soy (*Glycine max* (L.) Merr.), green peas (*Pisum sativum* L.) and snap beans (*Phaseolus vulgaris* L.) were included in this question because people consider them pulses, however they are, in fact, not grain

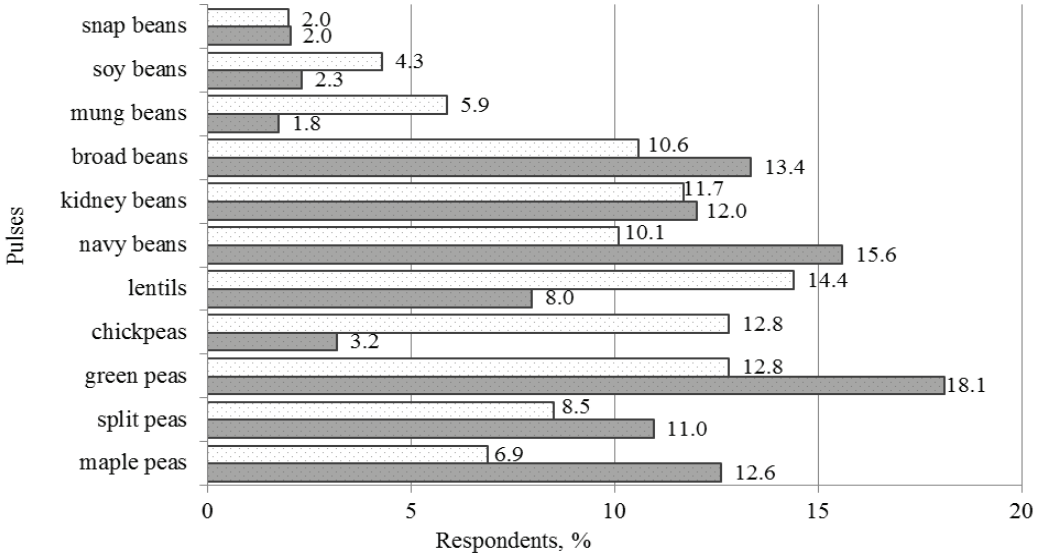

Figure 4. Preference of different pulses: □ vegetarian, ▩ non-vegetarian.

legumes and belong to oilseed crops and vegetable crops, respectively.

The majority of vegetarian respondents choose to buy dry pulses at a grocery store and cook them at home (Fig. 5) instead of buying pulse preserves which is the preferred use of pulses by non-vegetarian respondents. This could be the reason why some vegetarian respondents consume pulses less than once a week – they have experienced hard-to-cook phenomenon in home-cooked pulses. Although more expensive than dried pulses, canned pulses tend to be softer and sometimes more convenient or practical than soaking and boiling dried ones. Both groups of respondents use pulses grown in their gardens but hardly anyone consumes pre-cooked pulses. Non-vegetarian respondents also favour frozen pulses (mainly green peas) but consume little pulse spreads. Vegetarians consume store bought and home-made pulse spreads mainly from chickpeas (hummus) and beans.

The most common way of eating pulses is boiled pulses (with or without such additives as onions, bacon, lard) as a main dish and in stews or casseroles (Fig. 6). Non-vegetarian respondents also enjoy eating pulses in salads and soups as well as eating them raw (e.g. green peas). More than 10% of vegetarian respondents consume sprouted pulses which have a higher nutritional value than their unsprouted counterparts (Wang et al., 2005). Germination helps to reduce anti-nutritional factors that interfere with absorption of iron, zinc and calcium; sprouting increases the content of vitamin C and B vitamins (thiamine, niacin and riboflavin), also flatulence causing oligosaccharides are broken down thus making sprouted pulses more digestible (Limón et al., 2013). Vegetarian patties, which are only consumed by vegetarian respondents, are mostly made of beans, lentils and chickpeas for vegetarian burgers.

The majority of respondents would like various pulse spreads to be commercially available;

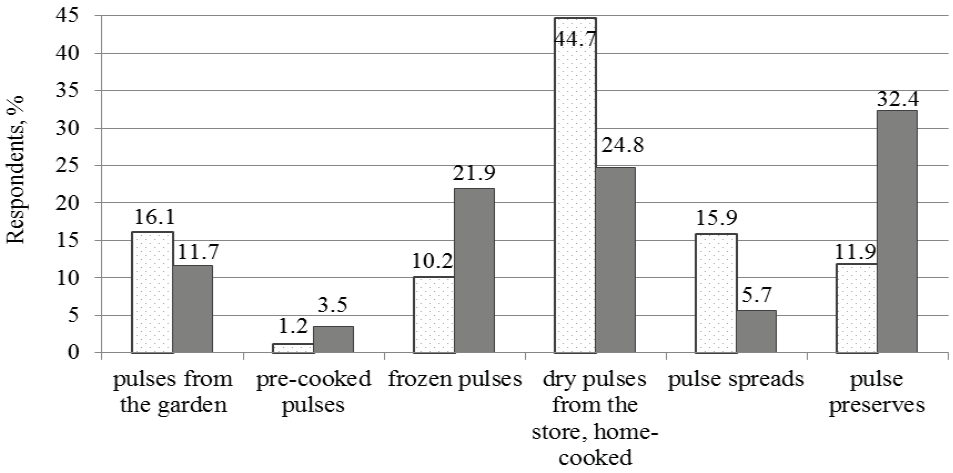

Figure 5. Most often chosen types of processed pulses: □ vegetarian, ■ non-vegetarian.

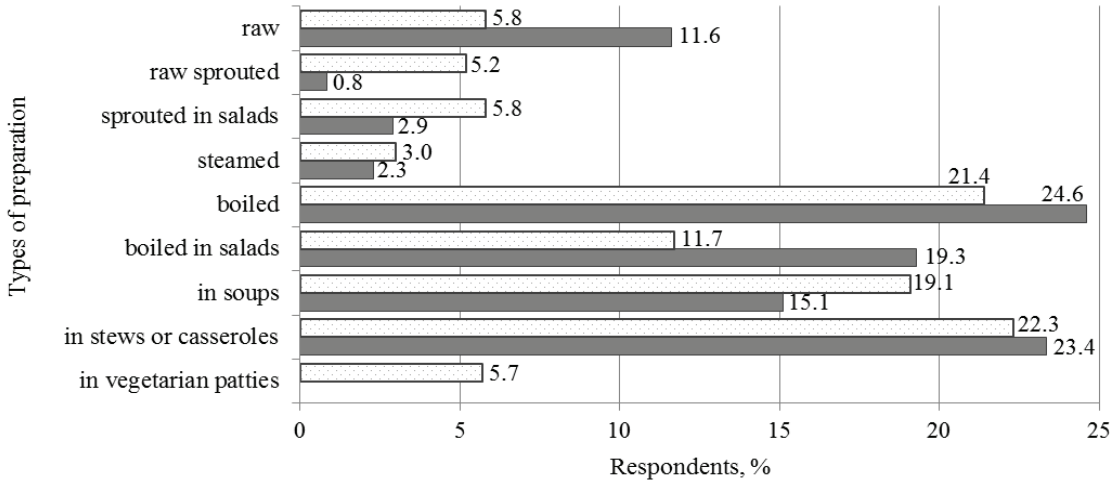

Figure 6. Consumption of prefered pulses: □ vegetarian, ■ non-vegetarian.

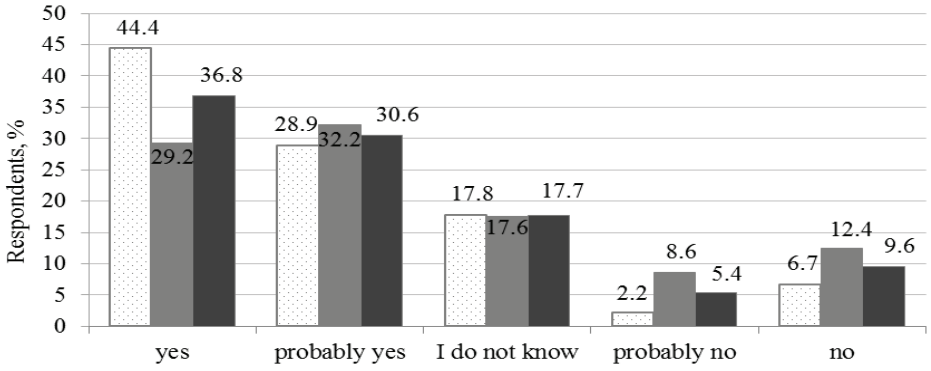

Figure 7. Would you like for more pulse products to be commercially available, e.g., various pulse spreads?
□ vegetarian, ■ non-vegetarian, ■ overall.

vegetarians are more interested in having pulse spreads commercially available than non-vegetarian respondents (Fig. 7). About ⅓ of respondents are not interested in more pulse spreads being available at grocery stores. A few of vegetarian respondents would like to have greater dry pulse selection at common grocery stores instead of new pulse products.

To better understand consumer demand on new bean products, visitors of the Baltics food industry fair 'Riga Food 2013' were offered to evaluate a new vegetarian bean spread with sun-dried tomatoes prepared at the Faculty of Food Technology. After tasting the product visitors were asked to complete a five question survey. They were requested to evaluate the product on a scale from 1 to 5 (5 – like very much and 1 – dislike very much) and the results show that the overall preference of vegetarian bean spread with sun-dried tomatoes is 'like very much' (4.5 – 4.6) with no significant differences between men and women ($p=0.168$).

Most of the respondents (60%) rated the quality of vegetarian bean spread with sun-dried tomatoes as 'good', followed by 'excellent' (35%) and 'average' (5%). Respondents were also questioned about the sensory attributes they liked and disliked about the product. Commonly the sensory attributes that influence acceptance of bean products are general visual appearance, texture and flavour (Ghasemlou et al., 2013). Sensory properties that male respondents liked most about the vegetarian bean spread with sun-dried tomatoes were flavour, freshness and similar taste to meat or hummus. Women, on the other hand, liked flavour and texture the best, followed by the ingredients used, as well as the bean flavour and aftertaste. Some of the male respondents disliked the texture, while female respondents noted the unappealing colour and wanted the product spicier. As consumer appetite for food is stimulated or dampened by its colour because the colour of food indicates the flavour of food, improving the colour should be taken into consideration. Respondents of both genders disliked the fact that the product was not on the market yet.

The last question on the additional survey was related to the likeliness to purchase vegetarian bean spread with sun-dried tomatoes at a grocery store. Majority of the respondents (75% men and 58% women) said they would buy this product as soon as it was available in a store, the rest of the respondents were undecided. Men were more likely to purchase vegetarian bean spread than women.

Conclusions

1. The majority of omnivore adults in Latvia consume pulses about once a week or less, while pulse intake in vegetarian adult group is significantly higher ($p<0.05$).
2. Latvian adults prefer green peas, navy and broad beans, lentils, chickpeas and maple peas, therefore they should be considered for vegetarian pulse spread preparation. These pulses are mainly purchased as preserves or dry, and then home-cooked and consumed boiled as a main dish and in stews or casseroles. The majority of the respondents would like for various pulse spreads to be commercially available.
3. Overall preference of vegetarian bean spread with sun-dried tomatoes is 'like very much' (4.5 – 4.6) and its quality is rated as 'good'.
4. Most of the respondents would purchase vegetarian bean spread with sun-dried tomatoes if it was available in a store.

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MIRCOBIOLOGICAL COMPOSITION ASSESSMENT OF BREAD KVASS

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Abstract
Kvass is a non-alcoholic beverage produced by fermenting kvass mash with yeast; alcohol content in kvass must be less than 1.2% alcohol by volume. Microbiological safety of kvass is an important issue because European Regulation No 2073/2005 on microbiological criteria for foodstuffs does not provide microbiological criteria for kvass production. Microbiological safety of kvass depends on raw materials, personal hygiene, environment, kvass blending and filtration. Experiments were carried out at the Latvia University of Agriculture Department of Food Technology from November 2013 to January 2014. The aim of this work was to assess the microbiological environment changes during kvass production process and shelf-life. Understanding the development of dynamic of microbiological environment provides a better management for kvass production processes. Samples of bread kvass were analysed during production (12 and 13 h) and storage (36, 60, 84, 132, 136 h) at 3 ± 1 °C to determine kvass quality. Yeasts (LVS EN ISO 21527 - 2: 2008), lactic acid bacteria (ISO 9332:2003) and total plate count (LVS EN ISO 4833:2003) were determined in kvass samples. Microorganisms in kvass were identified using API identification system; the dominating microflora in kvass was *Saccharomyces cerevisiae* and *Leuconostoc mesentericus*. Changes of total plate count during fermentation and maturation were not significant ($p>0.05$).
Key words: kvass, yeast, microbiological composition.

Introduction

Production of non-alcoholic beverages has an important part in the Latvian food industry. One of the best known non-alcoholic beverages in Latvia is naturally fermented kvass. Kvass is a non-alcoholic beverage produced by fermenting kvass mash with yeast. The alcohol content in kvass must be less than 1.2% alcohol by volume. Nowadays most of the commercially available beverages sold as kvass are kvass drinks and malt extract drinks. They are made by diluting grain extract concentrates with water and adding colourings and different flavours (Klosse, 2013) and artificial sweeteners.

The rapid segment production of naturally fermented kvass can be explained by high quality taste, tonic effect and beneficial effects on digestion and on pancreas (Feik et al., 2007). Many consumers choose naturally fermented kvass over kvass drinks. It is technologically more complicated to produce naturally fermented kvass with good quality than kvass drinks from grain extract concentrates.

Naturally fermented kvass is made from dried rye bread by soaking it in hot water for a few hours. After separating water-bread extract from soaked bread, it is fermented by adding bread yeast. Fermented kvass production process must be monitored carefully in order not to exceed the permitted amount of alcohol, as well as hygiene standards must be strictly abided to avoid the product contamination with undesirable microflora, which may result in an adverse effect on product quality. An increased count of microorganisms indicates the general norms of hygiene have been disregarded in a food company, leading to food contamination during processing and manufacturing. It can also lead to high contamination levels during improper storage (Wong et al., 2005). Therefore, it is important to investigate and to determine microbiological environment and possible risk factors during kvass production; bread rusks are one of the possible risk factors. It is essential for any manufacturer to obtain high quality product with a longer shelf life, retaining its characteristic properties during storage.

Microbiological criteria for kvass are not defined by European Regulation No 2073/2005 on microbiological criteria for foodstuffs. Therefore, it is important to study microbiological environment of kvass and existing microorganisms that can cause product spoilage. Moulds, yeasts and bacteria cause food spoilage (Da Silva et al., 2013). There are various microorganisms that can cause kvass spoilage: *Pseudomonas* spp. (42 ± 1 °C, pH 6.6-7.0), *Acinotobacter* spp. (35 ± 1 °C, pH 6.5-7.5) and *Moraxella* spp. (36 ± 1 °C, pH 7.0-9.0) spoil food products by producing substances that give undesired taste and odour (Wilson, 2005; Jeyalakshmi and Kanmani, 2008; UK Standards..., 2011). *Clostridium perfringens* and *Clostridium botulinum* produce toxins dangerous to humans. Facultative anaerobic bacteria which can grow in kvass environments and spoil it are *E. coli*, *Staphylococus aureus* and *Salmonella* species. Using spoiled foodstuff in human nutrition can cause various toxicosis and intoxications (Krämer, 1992). Optimal environment for mould growth is pH 4.0-6.0 at 27 ± 3 °C, and wild yeasts proliferate at 25 ± 1 °C (Baumgart, 1993).

Research on microbiological environment of bread kvass made from rye bread rusks has not been done in Latvia yet. The aim of this work was to assess

the microbiological changes during kvass production process and shelf-life.

Materials and Methods

Experiments were carried out at the Latvia University of Agriculture, Faculty of Food Technology Microbiology research laboratory from November 2013 to January 2014.

Preparation of kvass

For bread kvass production the following materials were used: 'Liepkalni Ltd' rye bread rusks, baker's yeast *Saccharomyces cerevisiae*, lactic acid bacteria *Leuconostoc mesentericus*, 'Ltd Dansukker' beet sugar and 'Liepkalni Ltd' dark rye-barley malt. For preparing of 1 L kvass mash, 200 g of rye bread rusks and 2 g dark malt are soaked in 2 L of hot water (78 ± 2 °C). Bread rusks are left soaking for 3 h, then the water-bread rusk suspension is filtered (300 microns) and the liquid fraction is cooled down and used in further kvass production stages. 1 g baker's yeast, 2 units of lactic acid bacteria starter and 1/3 of the estimated quantity of sugar are added to 1 L of kvass mash. The total quantity of sugar for kvass production is 30 g; 10 g of sugar are added prior to fermentation. The fermentation of kvass mash takes 9 h at 27 ± 1 °C. After fermentation kvass is placed in a refrigeration chamber to cool down at 3 ± 1 °C. After cooling, the yeasts are filtered (5 microns) and the remaining sugar is added. Kvass is maturated for 12 h at 6 ± 1 °C and then it is ready for drinking (total production process of 25 h). Kvass was filled in 0.5 L PET bottles and stored for 156 h in total in order to complete the microbiological analysis. The stages of kvass production process are shown in Table 1.

Table 1

Stages of kvass production process

Stage	Materials and technological process	Time, h
S_0	Rye bread rusks, before soaking	0
S_1	Kvass after fermentation	12
S_2	Kvass after blending and the start of maturation	13
	End of kvass production process	*25*
S_3	Kvass during storage	36
S_4	Kvass during storage	60
S_5	Kvass during storage	84
S_6	Kvass during storage	132
S_7	Kvass during storage	156

Microbiological analyses

Preparation of test samples, initial suspensions and decimal dilutions for microbiological examination was completed according to LVS EN ISO 6887-5:2011. The total plate count (TPC) was determined according to the standard LVS EN ISO 4833-1:2014, moulds and yeasts were determined according to the standard ISO 21527-2: 2008 and lactic acid bacteria were determined according to the standard ISO 9332:2003.

Microorganisms in kvass were identified using API (analytical profile index) identification system: lactic acid bacteria – ID 50 CHL and yeasts – ID 32C. API test was completed with ready-to-use kvass samples after storage for 10 h and 20 h. Photos of identified microorganisms were taken with Axioscop 40.

Data analyses

The obtained data processing was performed with Microsoft Excel 13 for Windows; mean values and standard deviations were calculated. For data cross-comparison ANOVA and correlation were used. Both t-test and F-test were used in order to assess the significance of changes and inter-comparison of the obtained data. For the interpretation of the results it is assumed that α=0.05 with 95% confidence.

Results and Discussion

Microbiological analyses were carried out during all production stages of laboratory produced kvass. The initial total plate count (TPC) in bread rusks was 4.16 log cfu g^{-1} (Figure 1). The first stage (0 hours) of naturally fermented kvass production was TPC determination in the main raw material – rye bread rusks. TPC during kvass mash fermentation was unstable. During the first 13 hours the increase in TPC was low, compared to other dominant organisms in the environment.

Yeasts and lactic acid bacteria are the main microorganisms in kvass fermentation process (Salovaara and Gänzle, 2011), both of which form substances that give kvass its specific taste and aroma. During fermentation the alcohol and lactic acid are produced which are considered as natural preservatives that partially protect kvass from undesirable development of microorganisms.

During the first 12 h of naturally fermented kvass production (9 of which was fermentation), yeast concentration increased from 4.55 log cfu g^{-1} (dry bread rusks at 0 hours) to 6.06 log cfu g^{-1} which is between the growth rate of TPC and lactic acid bacteria. This indicated that the raw material already contained a certain amount of yeast cells. No moulds were detected during kvass production process.

A slightly lower amount of lactic acid bacteria was found in bread rusks - 3.34 log cfu g^{-1}. The initial line segment for the growth of lactic acid bacteria is steeper than the other two groups, suggesting a higher growth rate. During the first 12 h of naturally

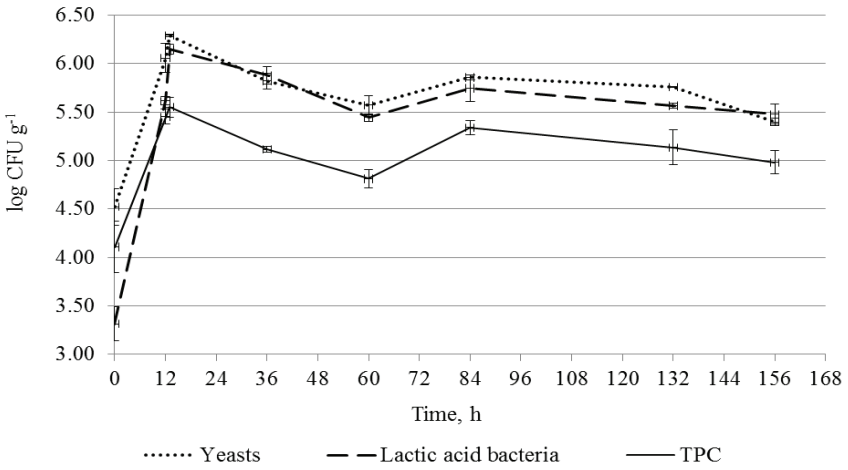

Figure 1. Changes of total plate count, lactic acid bacteria and yeast count during kvass production and storage.

fermented kvass production, the changes in growth of lactic acid bacteria were greater compared to TPC and yeasts ($p<0.05$).

The literature indicates that yeasts and lactic acid bacteria are symbiotic microorganisms (Ramos et al., 2011). Lactic acid bacteria create an acidic environment that is optimal for yeasts, while yeasts produce amino acids and vitamins that are vital for microorganisms. Lactic acid bacteria and yeasts compete for nutrients at the same time. Reduction of dry matter and increase in acidity create more favourable conditions for lactic acid bacteria growth. Excessive environment acidity suppresses both yeasts and lactic acid bacteria; therefore it can promote the growth of undesirable microorganisms (Помозова, 2006). During kvass fermentation, the count of lactic acid bacteria and yeasts was slightly superior, but the growth of aerobic colonies decreased more rapidly than the changes in other two groups of microorganisms.

There are some similarities in the development of growth dynamics in all tested groups of microorganisms, which indicatesthat kvass is a beneficial and suitable environment for a variety of microorganisms. The added yeast and lactic acid bacteria starterhave certain growth advantages because they are dominant due to the high microorganism count in this particular environment. The changes of pH in kvass, due to the increase of lactic acid concentration, promote the growth of yeasts and lactic acid bacteria while preventing the growth of saprophytic bacteria (Lidums, 2011).

The changes in the determined microorganisms during the technological process of kvass production can be explained partially by the changes in temperature. The temperature during fermentation (27 ± 1 °C for 9 h) is optimal for yeast and lactic acid bacteria growth; however, during kvass maturation (6 ± 1 °C for 12 h) it is significantly below the optimum levels.

During the fourth stage of kvass production process active bacteria and yeast count decreased; the next stage showed a repeated bacteria and yeast cell growth. The increase in cell count can be explained by the end of cellular adaptation to the reduced temperature conditions and the added sugar prior to fermentation. At this stage a trend was observed, - a higher growth rate was found in TPC group ($p<0.05$), while the increase was equally slower for lactic acid bacteria and yeasts.

The total kvass storage time was 130 h (the total production time 168 h); there were practically no nutrients available for microorganisms in kvass environment; ethanol and lactic acid were created, and a gradual decrease in the number of microorganisms began with a similar negative rate in all groups.

Correlation of changes in detected microorganism count in kvass

Based on correlation coefficients of microorganisms, the growth of yeasts, TPC and lactic acid bacteria proceeded similarly (Table 2). The changes in microorganism count during kvass maturation had a strong positive correlation.

There were significant differences in the initial count of microorganisms between groups ($p<0.01$), and yeast count was greater than the count of lactic acid bacteria. During the first 12 h of kvass fermentation, yeasts dominated over other groups ($p<0.001$), the lactic acid bacteria and TPC developed equally, with a small but significant superiority of TPC ($p<0.05$). After adding the additional dose of sugar, a significant increase in the number of yeasts was observed.

In the next stages of kvass production process (S_2 to S_4), the number of microorganisms decreased; however, lactic acid bacteria and yeasts

Table 2

The values of correlation coefficients (r) between TPC, lactic acid bacteria and yeasts during kvass maturation (n=30)

Group	Yeasts	Lactic acid bacteria	TPC
Yeasts	1	0.94	0.97
Lactic acid bacteria	0.94	1	0.92
TPC	0.97	0.92	1

continued to dominate over TPC group ($p<0.001$). Microbiologically detectable decrease in the count of lactic acid bacteria and yeasts did not differ significantly ($p>0.05$). During storage (60 to 84 h), a repeated increase in microorganism growth was observed; this period is characterized by the temperature raise of approximately 1.5 °C.

Identification of microorganisms

API identification system was used to isolate yeasts and lactic acid bacteria. Two types of microorganisms were isolated and identified in naturally fermented bread kvass – *Saccharomyces cerevisiae* and *Leuconostoc mesentericus spp. cremoris*.

Conclusions

1. Lactic acid bacteria and yeast count increase during production process of bread kvass.
2. The dominating microflora in kvass was *Saccharomyces cerevisiae* and *Leuconostoc mesentericus*.

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PURIFICATION OF EGG YOLK OIL OBTAINED BY SOLVENT EXTRACTION FROM LIQUID EGG YOLK

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Abstract
There are different methods of egg yolk oil extraction, but still solvent extraction is commonly used. Due to the high cost of egg yolk powder production, extraction of lipids from liquid egg yolk remains very topical. Crude egg yolk oil obtained by solvent extraction from liquid egg yolk contains high amount of water which can decrease shelf life of egg oil promoting lipid oxidation. High concentration of residual solvents limits the usage of egg oil in food or in cosmetics due to the health risks. The aim of this study is to purify egg yolk oil obtained by solvent extraction from liquid egg yolk. Results show that it is possible to decrease the water content in egg oil from 14.26 ± 1.29% to 0.88 ± 0.13% by eliminating lecithin from egg oil. Solvent evaporation in the rotary film evaporator under the vacuum cannot remove solvents completely from the extract, but nitrogen streaming through the extract as a last step in evaporation process removes solvent residues, leaving behind high quality egg yolk oil suitable for food application.
Key words*:* egg yolk oil, solvent extraction, purification.

Introduction

Egg yolk oil is a good source of unsaturated fatty acids, oil soluble vitamins, phospholipids and cholesterol, and it can be widely used as an ingredient in food and cosmetics, but extraction of lipids from egg yolks is a complicated process. First of all, the structure of egg yolk does not allow using standard lipid extraction methods like pressing. Egg yolk lipids are associated with proteins, which makes lipid extraction process even more complicated (Ahn et al., 2006). There is a method which requires protein degradation by the heat, but egg yolk proteins are a very valuable product to be discarded such way. That is why solvent extraction of egg yolk lipids is the most justified method. Solvent extraction of egg lipids can be applied on egg yolk powder and liquid egg yolk. Production of egg powder is an expensive process which requires high amount of energy. For that reason, the extraction of liquid egg yolks is studied as a most economically reasonable method. Usage of organic solvents has some benefits in egg oil production such as antimicrobial effect that allows using liquid egg yolk for extraction without heat treatment reducing production costs. But the usage of organic solvents is related to the health risks; therefore, solvent presence in final product must be prohibited.

In previous studies 2-propanol/hexane solvent mixture was chosen as the best solution for extraction of egg lipids from liquid egg yolk. Lipids in egg yolk are presented as polar phospholipids and non-polar or neutral lipids. 2-propanol denatures egg yolk proteins and extracts polar lipids. Hexane, as a non-polar solvent, extracts neutral lipids (Christie, 1993). But egg yolk oil extracted that way contains high amount of water which can decrease oil quality during the storage promoting lipid oxidation.

The aim of this study was to purify egg yolk oil obtained by the solvent extraction and determine possible changes of its highly valuable compound content.

2-propanol is a polar solvent and it is miscible with water forming a hydrogen-bonded structure (Tabata et al., 1994). But it is also quite non-polar, because of its hydrophobic alkyl group, what makes it soluble in non-polar solvents such as hexane. This can be one of the reasons for high water content in oil micelle before solvent evaporation. 2-propanol high solubility in water makes extraction of this compound from aqueous phase into a non-polar solvent very difficult (Tabata et al., 1994). 2-propanol extraction to the hexane can be promoted by process called 'salting out' where inorganic salt, usually sodium chloride, is dissolved in water to reduce solubility of the 2-propanol in water phase (Hasseine et al., 2009).

2-propanol, due to its polar nature, will extract polar phospholipids (lecithin) from the egg yolk. Lecithin is a good emulsifier and it can form very stable emulsions (Maximiano et al., 2008). Lecithin binds water presented in liquid egg yolk by the swelling. In vegetable oil refining this process is used for wet degumming. Wet lecithin can contain up to 50% of water and its drying is a big challenge. Wet lecithin in egg yolk oil can be another reason for high water content in crude egg yolk oil because lecithin drying in the rotary evaporator is not so efficient (Palacios and Wang, 2005a). So, one of the possibilities for decreasing water content in egg yolk oil is lecithin elimination during the extraction process.

For the separate phospholipid extraction more polar solvent is needed. In this case ethanol can be the best choice. Ethanol is more polar than 2-propanol and it will also denature yolk proteins. But in this time extraction must be done in two stages. First, liquid egg yolk needs to be extracted with ethanol to eliminate polar phospholipids and then neutral lipids will be extracted from precipitates with hexane. The

big difference between ethanol and hexane polarities causes phase separation in two layers when they are mixed together. Increasing of reaction surface can improve extraction between two phases, but it needs to be aware of vigorous mixing to avoid emulsion formation. After phase separation all polar compounds, like phospholipids, salts, sugars and soluble proteins will take place in polar ethanol-water phase, but neutral lipids will be diluted in non-polar hexane phase which must be free from water (Palacios and Wang, 2005a). Egg yolk oil can be obtained from this non-polar phase by the evaporation of the hexane.

Volatile organic solvents can be removed from lipid extracts in rotary evaporators. For decreasing the evaporation temperature this process usually occurs under the vacuum. But this process is not sufficient for total removal of solvents from the extracts. Increasing the evaporation temperature can cause unwanted changes in lipids. Pure and dry nitrogen is commonly used in laboratories for solvent evaporation from the extracts. Nitrogen is inert gas and in comparison with air it does not contain oxygen, which can react with lipids.

The combination of rotary vacuum evaporation and nitrogen blowing through the extract may have a good result for the total solvent removal. Nitrogen bubbles going through egg oil will sufficiently increase evaporation surface and pick up solvent vapors from the product. The same process conditions of rotary evaporation, such as temperature and vacuum, will intensify solvent and nitrogen removal from egg oil. Nitrogen will also remove air from the oil that will decrease oxidation of lipids by the air oxygen.

This method can also be used for large scale solvent removal using industrial nitrogen generators, which produce pure and dry nitrogen.

Materials and Methods

Materials

Hen eggs from Lohman Brown Classic breed were provided by JSC Balticovo (Iecava area, Latvia). Egg yolks were separated from egg whites and homogenized. Moisture content of homogenized egg yolks were determined using Moisture balance MOC-120H (Shimadzu, Japan) at 120 °C until weight difference from the previous weighting was less than 0.05%.

All solvents (ethanol, hexane, 2-propanol) used in egg yolk oil extraction were analytical grade from Sigma Aldrich (Germany). Compressed nitrogen gas with purity 99.999% HiQ Nitrogen 5.0 was from Linde AG (Germany).

Egg yolk oil extraction

Extraction of egg yolk lipids with 2-propanol/ hexane was done as follows - solvents were mixed by volume at ratio 30:70 and poured into a beaker. Liquid homogenized egg yolk was added to solvent mixture with a thin squirt vigorously mixing. The ratio 2:1 between solvent mixture and egg yolk was used. Extraction was done at temperature of 20 °C vigorously mixing for 30 minutes. Extracts were filtered using vacuum filtration, collected in to a clean container and then transferred to a separatory funnel.

Salting out 2-propanol from the egg yolk lipid extract

For salting out of 2-propanol saturated sodium chloride water solution (26 g 100 ml^{-1}) was added to the extract at dosage 10 ml for 100 ml of extract and well mixed. After mixing, extract was left for 1 hour for phase separation. Then bottom aqueous layer was drained by the opening in a stopcock of separatory funnel.

The oil was recovered by evaporation of the solvent mixture using rotary evaporator IKA RV 10 Control (IKA-Werke GmbH & Co. KG) at 80 °C and 400 m bar pressure.

Two stage lipid extraction with ethanol and hexane

Lipid extraction with ethanol and hexane from liquid egg yolk was made by following steps. First, lecithin was extracted with ethanol from liquid egg yolk and then neutral lipids were extracted from precipitate with hexane (Schreiner, 2006). For lecithin extraction, 200 g of homogenized liquid egg yolk was added to 400 ml of ethanol and stirred until egg yolk proteins denatured and completely dispersed. Extraction was done at 20 °C for 30 minutes. Then, the mixture was filtered by vacuum filtration, and the supernatant was collected and transferred to a separatory funnel. The precipitate was extracted with 400 ml hexane vigorously mixing for a 30 minutes at 20 °C using a magnet stirrer. Extract was filtered by vacuum filtration and supernatant was collected and added to the same separatory funnel. Both ethanol and hexane extracts were thoroughly but gently, to avoid emulsion formation, mixed to extract polar lipids and impurities to a polar ethanol-water phase and neutral lipids to a non-polar hexane phase. Then the mixed extracts were left for 1 hour for phase separation. Bottom ethanol/ water layer, containing polar lipids and water soluble compounds such as salts, sugars, soluble proteins, was drained from separatory funnel through the open stopcock and collected in a clean container. After evaporation in the rotary evaporator, crude lecithin was an object for water content determination. The yield of crude lecithin (polar lipids) was expressed in percent from liquid egg yolk.

Egg yolk oil was obtained from the extract by evaporation of the hexane in the rotary evaporator IKA RV 10 Control V (IKA®-Werke GmbH and Co. KG) at the temperature of 70 °C and 400 mbar pressure.

Solvent residue removal using nitrogen gas

After solvent evaporation in the rotary evaporator, as the last step of the solvent removal, the pure nitrogen gas was laid through the egg oil for a 10 minutes in the same rotary evaporator with the same evaporation conditions by means of a plastic tube immersed in the oil.

Analysis

Water content in egg yolk oil and crude lecithin was measured using evaporation scale 'Moisture balance MOC-120H' from Shimadzu (Shimadzu Corporation, Japan) at 120°C until the difference in sample weight was less than 0.05 percent from previous weight measurement.

Hexane residue in egg yolk oil was determined in accordance with the standard method ISO 9832:2002, HS-GS-FID (headspace gas chromatography with flame ionization detector).

Ethanol residue in egg yolk oil was determined by HS-GS-FID (headspace gas chromatography with flame ionization detector) (Stenerson and Verma, 2011; Tiscione et al., 2011; Restek, 2000).

Fatty acid profile was determined in accordance with the standard methods ISO 12966-2 and ISO 5508, GC-FID (gas chromatography with flame ionization detector).

For GC analysis, Shimadzu GC 2010 Plus gas chromatograph with flame ionization detector (Shimadzu Corporation, Japan) was used.

β-carotene content was determined in accordance with the standard method ISO 17932:2011 using UV spectrophotometer UV-1800 (Shimadzu Corporation, Japan).

The results were presented as the means and standard deviation for three replicates. Means were compared by t-test and analysis of variance (ANOVA). Significance was defined at $p<0.05$. Statistical analysis, tables and figures were carried out by Microsoft Excel 2010 version software.

Results and Discussion

Water content in egg yolk oil

Figure 1 illustrates the water content in egg yolk, crude lecithin and egg yolk oil before and after purification.

Water content in fresh liquid egg yolk used for extraction of egg yolk oil was 49.52 ± 1.26 g 100 g^{-1}. After extraction of egg yolk oil some part of yolk containing water together with some solvents will stay in denatured yolk proteins, but the other part will be extracted together with lipids to the oil micelle. In case of using 2-propanol as a polar solvent in 2-propanol/hexane oil extraction, it binds water and brings it to

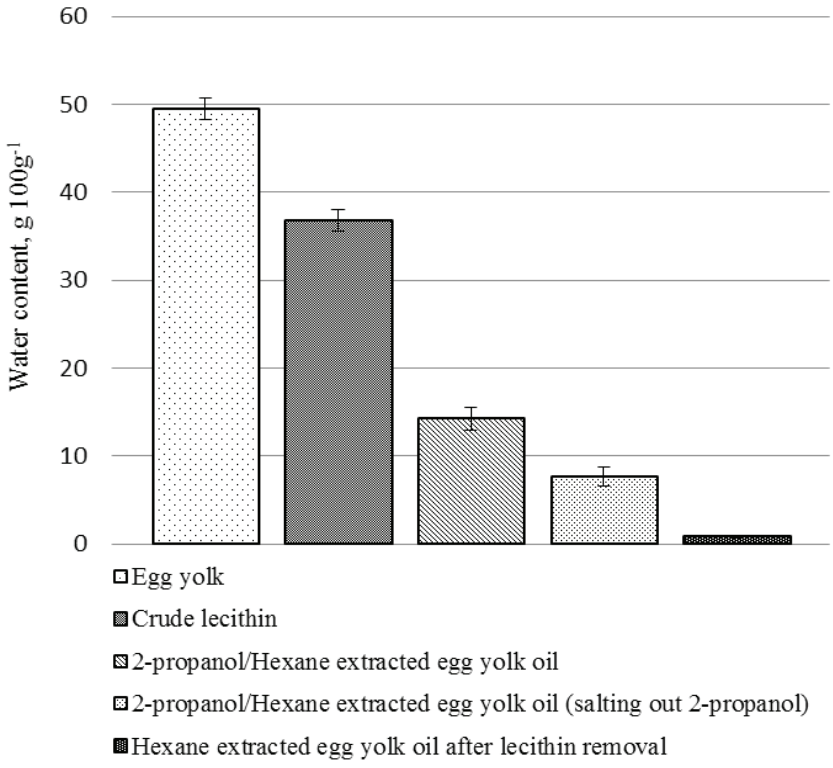

Figure 1. Water content in egg yolk, crude lecithin and egg yolk oils.

the non-polar phase. It is confirmed by the forming of quite homogeneous extract solution. A very negligible phase separation was observed. After evaporation of the 2-propanol/hexane solvent mixture egg yolk oil was an object for water content determination. The result shows that egg yolk oil extracted this way contains high amount of water (14.26 ± 1.29 g 100 g^{-1}). Water content in food oils is one of the main quality parameters because it has a very big influence on lipid oxidation during the storage (Nelson and Labuza, 1992). That is why decreasing of water content in edible oils is required.

To confirm that high water content in egg yolk oil is related to the lecithin which absorbs water contained in liquid egg yolk, the water content in crude lecithin, extracted from the liquid egg yolk with ethanol, was determined. In different lecithin extraction methods water content in crude lecithin varies from 30 till 50 percent (Palacios and Wang, 2005b). Water content in crude lecithin affects the yield of crude lecithin. In our study the yield of extracted crude lecithin was 11.43 ± 2.65% from the liquid egg yolk. Water content in our crude lecithin sample was 36.79 ± 1.17 g 100 g^{-1}, which is an average parameter.

2-propanol salting out decreases its solubility in water, causing the separation of water in a clearly visible aqueous phase. After solvent evaporation, the water content in this lipid extract was significantly ($p<0.05$) lower (7.66 ± 1.03 g 100 g^{-1}) than in the previous sample without 2-propanol salting out. But the best result of the water content decreasing in egg yolk oil was obtained by elimination of phospholipids by extraction of liquid egg yolk oil with ethanol. The big difference between ethanol and hexane polarities allows to get neutral lipid extract in hexane almost without the water presence and low water content (0.88 ± 0.13 g 100 g^{-1}) in egg yolk oil obtained by this method proves it.

Solvent residue removal

Solvent residues in egg yolk oils presented in Table 1.

High solvent residue in egg yolk oil not only affects oil sensory parameters, but is also dangerous for human health. Egg yolk oil, obtained in previous studies contained high level of 2-propanol and hexane residue. The content of these solvents in food product is strictly regulated by the EU legislation (Directive 2009/32/EC, 2009). Changing 2-propanols to more volatile ethanol has little decrease of total solvent residue in egg oil. But the usage of nitrogen in oil treatment allows removing totally any solvent residues. The amount of used nitrogen and treatment time need to be studied to decrease nitrogen consumption for this process.

Fatty acid content

Fatty acid profile of purified egg yolk oils obtained by solvent extraction presented in Table 2 is quite similar to the fatty acid profile of crude egg yolk oil. Egg yolk oil is rich in palmitic, stearic, oleic and linoleic acids. Docosahexaenoic and α-linolenic acid content in purified, ethanol/hexane extracted egg yolk oil is lower than in crude egg yolk oil obtained by 2-propanol/hexane extraction. It can be related to the elimination of lecithin, by the extraction process from egg yolk oil during purification.

β-carotene content

The determined β-carotene content in purified egg yolk oil was 79.16 ± 1.08 mg kg^{-1}, which is less than 81.02 ± 0.37 mg kg^{-1} in oil obtained by extraction of liquid egg yolk with 2-propanol/hexane solvent mixture.

As lecithin was removed from the egg yolk before extraction with hexane, β-carotene content in purified egg yolk oil must be higher due to its concentration in a smaller amount of the oil. β-carotene is particularly non-polar compound and cannot be extracted with polar ethanol in an aqueous phase; it must be located in a non-polar hexane phase. Decrease in β-carotene content in purified egg oil can be explained, probably, by the content of carotenoids in hen feed till it has direct influence on the β-carotene content in eggs (Jiang et al., 1994).

Table 1

Solvent residues in egg yolk oils after extraction

Solvent residue	Extraction solvent		
	2-propanol/hexane	Ethanol/hexane	Ethanol/hexane + Nitrogen solvent removal
2-propanol, mg kg^{-1}	264.14 ± 7.18	-	-
Hexane, mg kg^{-1}	2.03 ± 0.02	1.16 ± 0.06	<0.01
Ethanol, mg kg^{-1}	-	50.30 ± 6.38	<0.01

Table 2

Fatty acid content in non-purified and purified egg yolk oils

Fatty Acids	Extraction and purification methods		
	2-propanol/Hexane (crude oil)	2-propanol/Hexane salting out 2-propanol	Ethanol/Hexane+N_2
	g $100g^{-1}$	g $100g^{-1}$	g $100g^{-1}$
Myristic acid (C14:0)	0.14±0.02	0.14±0.03	0.16±0.02
Myristoleic acid (C14:1)	0.04±0.01	0.02±0.01	0.02±0.01
Pentadecylic acid (C15:0)	0.08±0.01	0.09±0.02	<0.01
Palmitic acid (C16:0)	22.72±0.04	23.48±0.16	23.22±0.08
Palmitoleic acid (C16:1)	0.28±0.02	0.44±0.08	0.39±0.03
Margaric acid (C17:0)	0.19±0.02	0.13±0.01	0.21±0.02
Heptadecenoic acid (C17:1)	0.12±0.01	0.11±0.01	<0.01
Stearic acid (C18:0)	6.20±0.21	6.00±0.52	6.79±0.30
Oleic acid (C18:1)	52.61±0.06	51.43±2.18	51.83±1.20
Linoleic acid (C18:2)	13.67±0.03	14.02±0.03	14.16±0.03
α-linolenic acid (C18:3)	1.72±0.01	1.64±0.01	0.05±0.01
Paullinic acid (C20:1)	0.23±0.01	0.08±0.01	0.04±0.01
Eicosadienoic acid (C20:2)	0.01	<0.01	1.03±0.01
Behenic acid (C22:0)	0.04±0.01	0.05±0.01	0.11±0.01
Dihomo-γ-linolenic acid (C20:3)	0.19±0.02	0.11±0.01	0.23±0.02
Erucic acid (C22:1)	0.03±0.01	0.02±0.01	0.02±0.01
Arachidonic acid (20:4)	0.07±0.01	0.11±0.05	0.02±0.01
Eicosapentaenoic acid (C20:5)	0.03±0.01	0.02±0.01	<0.01
Nervonic acid (C24:1)	0.02	0.03±0.01	<0.01
Docosatetraenoic acid (C22:4)	0.08±0.02	0.06±0.02	<0.01
Docosapentaenoic acid (C22:5)	0.05±0.01	0.02±0.01	<0.01
Docosahexaenoic acid (C22:6)	1.02±0.02	1.23±0.14	0.04±0.01
Other	0.46±0.01	0.77±0.64	1.68±0.55
Sum of saturated fatty acids	29.37	29.89	30.49
Sum of monounsaturated fatty acids	53.33	52.13	52.30
Sum of polyunsaturated fatty acids	16.84	17,21	15.49

Conclusions

The high water content in crude egg yolk oil is related to lecithin solubility in water contained in the egg yolk. Eliminating the lecithin from lipid extract by the two stage extraction with polar and non-polar solvents significantly reduces the water content in egg yolk oil. Solvent residues from egg yolk oil can be totally removed by streaming nitrogen through the oil as the final stage of rotary evaporation under the vacuum. Purified egg yolk oil is a good source of unsaturated fatty acids and β-carotene.

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EFFECT OF ENZYMATIC HYDROLYSIS ON BRAN MICROFLORA

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Abstract
The present study was undertaken to estimate enzymatically hydrolysed and non–hydrolysed wheat (*Triticum aestivum*) and rye *(Secale cereale)* bran microflora. Enzymatic hydrolysis was accomplished by α – amylase from *Bacillus amyloliquefaciens* and by Viscozyme L which contain a wide range of enzymes responsible for the breakdown of carbohydrates into simple sugars. Wheat and rye bran samples were collected from native mills, namely Stock Company (SC) 'Rigas dzirnavnieks' wheat bran with large particle size (WLSR), SC 'Jelgavas dzirnavas' rye bran with small particle size (RSSJ), SC 'Dobeles dzirnavnieks' wheat bran with small particle size (WSSD) and wheat bran with large particle size (WLSD). Gained results indicate that before enzymatic hydrolysis all of the bran samples showed similar microbiological contamination with total plate count (TPC), yeasts and lactic acid bacteria. Enzymatic hydrolysis of bran give the possibility to partially eliminate the microbiological contamination with TPC, yeasts and lactic acid bacteria. The amount of microorganisms after enzymatic hydrolysis (before storage) were decreased and ranged from 5.26 ± 0.04 to 5.45 ± 0.01 log CFU g^{-1}, from 4.81 ± 0.01 to 5.60 ± 0.05 log CFU g^{-1}, and from 4.09 ± 0.01 to 5.10 ± 0.05 log CFU g^{-1}, respectively. After eight weeks of storage (temperature – 20 ± 1 °C, relative humidity – 40 ± 1%) enumeration of microorganisms showed significant decrease of colony–forming units in all bran samples. The amount of TPC, yeasts and lactic acid bacteria in the control bran samples fluctuated in a range from 4.84 ± 0.04 to 5.49 ± 0.05 log CFU g^{-1}, from 4.86 ± 0.03 to 5.25 ± 0.03 log CFU g^{-1}, 3.53 ± 0.03 to 4.21 ± 0.02 log CFU g^{-1} respectively.
Key words: microbiological contamination, enzymatic hydrolysis, Viscozyme L, depolymerisation.

Introduction

Wheat (*Triticum aestivum*) bran is the coarse outer layer of the wheat kernel that is separated from the cleaned and scoured kernel. It consists mainly of the large pieces of bran remaining after the flour has been extracted from the wheat (Radenkovs and Klava, 2012). Wheat bran is a composite material formed from different histological layers, and three different strips can be obtained from the soaked outer layers. The outer strip corresponds to outer pericarp (epidermis and hypodermis), the inner one corresponds to the aleurone layers, and the intermediate one remains a composite of several tissues (inner pericarp, testa, and nuclear tissue (Hemery et al., 2010).

Rye *(Secale cereale)* bran like wheat bran is by-product of the rye milling. Likewise wheat bran rye bran composed from different histological layers such as: fruit coat (pericarp), seed coat (testa), aleurona layer.

Different types of bran have different chemical compositions, it depends on grain genetics, agricultural background and milling process (Harris et al., 2005).

The chemical composition of the wheat as well as rye bran depends on certain factors associated with the grain chemical composition or with milling processes. Wheat and rye bran has a significant amount of carbohydrates, proteins, minerals (magnesium, potassium, phosphorus, iron, manganese, and zinc), bioactive compounds (tocopherols and tocotrienols, phenolic compounds, alkylresorcinols), and other growth factors, which support growth of microorganisms, including the fastidious lactic acid bacteria (Hemery et al., 2010).

Due to its nutritional value, low cost, and potential use in human nutrition, many studies have been conducted to evaluate its use in food.

Microbiological conditions during harvesting together with outer part residues and starch contamination during dehulling and polishing processes limit its direct use as food.

The residues may carry a high level of microbiological impurities, such as yeast, fungi, the spores of which are resistant to heat and are able to produce mycotoxins. The most common mycotoxins contamination in cereals is *Aspergillus spp.*, *Penicillium spp.*, *Fusarium* spp. and *Claviceps* spp. (Finnegan, 2010). Mycotoxins are formed during cereal growth or in post–harvest storage during the wet season; sun drying practised by most farmers may not adequately reduce the moisture content in grains. As a result, grains with moisture content higher than permissible level enter the storage system.

Fermentation may be a useful strategy for reducing microbiological contamination. Enzymatic or microbial fermentation is a process of bioconversion of organic substances by microorganisms and/or enzymes of microbial, plant or animal origin. It is one of the oldest forms of food preservation which is applied globally. Indigenous fermented foods such as bread, cheese and wine, have been prepared and consumed for thousands of years and are strongly linked to culture and tradition, especially in rural households and village communities. It is estimated that fermented foods contribute to about one–third of the diet worldwide (Food and Agriculture Organization, 2004).

Microbial fermentation leads to a decrease in the level of carbohydrates, as well as some non–digestible poly and oligosaccharides. Certain amino acids may be synthesised and the availability of B group vitamins may be improved (Nout and Ngoddy, 1997). During the fermentation of cereals by lactic acid bacteria the content of free amino acids was increased. Several studies imply that the fermentation gained the positive effect on cereal nutritional value, on the content of essential amino acids, particularly lysine, methionine and tryptophan (Adams, 1990). During the microbial fermentation the optimal pH is necessary for enzymatic degradation of cereal substances e.g. cell walls, protein/starch matrix. It was reported in the literature that the more appropriate condition of enzymatic pre–treatment with Viscozyme L is pH 4.6 (Guan and Yao, 2007), while for most of microorganisms this pH is critical for growing and developing. Temperature, pH, the control of water activity, and use of antimicrobial agents are the available methods to prevent the growth of organisms or production of microbial toxins in food. Reducing pH below 4.0 – 4.5 by fermentation of acidification with acid foods can similarly inhibit proliferation and the availability of water to microorganisms by adding salt or sugar, or by freezing (Brown et al., 1998).

The aim of this work was to estimate enzymatically hydrolysed and non–hydrolysed wheat and rye bran microflora.

Materials and Methods

The experiments were performed at the Faculty of Food Technology of Latvia University of Agriculture in collaboration with the Latvia State Institute of Fruit–Growing. All analyses were conducted with threefold repetition.

Bran samples

Summer wheat (*Triticum aestivum*) and rye *(Secale cereale)* bran samples were collected from industrial mills of Latvia:

1) SC ‘Dobeles dzirnavnieks’– wheat bran with large particle size (~441 μm) (**WLSD);**
2) SC ‘Dobeles dzirnavnieks’– wheat bran with small particle size (~215.8 μm) (**WSSD);**
3) SC ‘Rigas dzirnavnieks’– wheat bran with large particle size (~600.0 μm) (**WLSR);**
4) SC ‘Jelgavas dzirnavas’– rye bran small particle size (~276.0 μm) **(RSSJ).**

Two different methods were used for pre–treatment of bran samples: enzymatic hydrolysis (by using enzymes, heating, pH adjustment);

the control treated bran samples (by using heating, excluding adding of enzyme and citric acid). This type of pre–treatment is needed to decide whether the temperature (100 ± 1 °C) has any influence on the colony–forming units or not;

the control samples – samples that were not treated but used like raw material.

Enzymes

Industrial enzyme preparations were produced by ‘Novozyme Corporation’ (Bagsvaerd, Denmark) and purchased from Sigma – Aldrich. Two commercial preparations of enzymes: α – amylase from *Bacillus amyloliquefaciens* (EC 232 – 560 – 9) and Viscozyme L from *Aspergillus spp*., (EC 263 – 462 – 4) were used to hydrolyse carbohydrates. The α – amylase has a declared activity ≥250 units g^{-1}, optimum conditions of enzymatic pre–treatment is pH 5.0 – 8.0, temperature 55 ± 1 °C and incubation time 0.5h (Demirkan et al., 2004) form Viscozyme L declared activity is 100 fungal beta glucanase (FBG) g^{-1}, optimum conditions are pH 4.6, temperature 44 ± 1 °C and incubation time 3.2 h (Guan and Yao, 2007).

Enzymatic Hydrolysis

For starch hydrolysis, wheat bran (10g) was mixed with 90 mL of distilled water in 1000 – mL Reagent bottle with screw cap with dilutions 1:9, and then 500μL of α – amylase was added. Hydrolysis was carried out in a water bath at temperature 55 ± 1 °C, incubation time 0.5h and shaking intensity 60 rpm. After starch hydrolysis bran mash was homogenized for 3 minutes, the pH of the suspension was adjusted to pH 4.6 with 0.2 mL of 50% citric acid in each dilutes and Viscozyme L 400μL was added. Incubation time is 3.2h, temperature 44 ± 1 °C, and shaking intensity 60rpm. After enzymatic hydrolysis and enzymes inactivation (10 min at temperature 100 ± 1 °C), bran mash was cooled to room temperature (20 ± 1 °C) and then freeze–dried (temperature (– 50 °C, vacuum – 0.5 mbar, drying time – 72 ± 5h), and then stored at room temperature (20 ± 1 °C).

Microbiological analysis

Microbiological evaluation of bran was performed according to the standard ‘Microbiology of food and animal feeding stuffs’ LVS EN ISO 7218:2007. All microbiological evaluations were conducted with threefold repetition.

Enumeration of aerobic colony count (ACC) was performed according to the standard LVS EN 4833:2003, colony–count technique at 30 °C (Fig. 1).

Microbiology of food and animal feeding stuffs – Horizontal method was used for the enumeration of yeasts and moulds – LVS ISO 21527 – 2:2008, and colony count technique in products with water activity lower than or equal to 0.95.

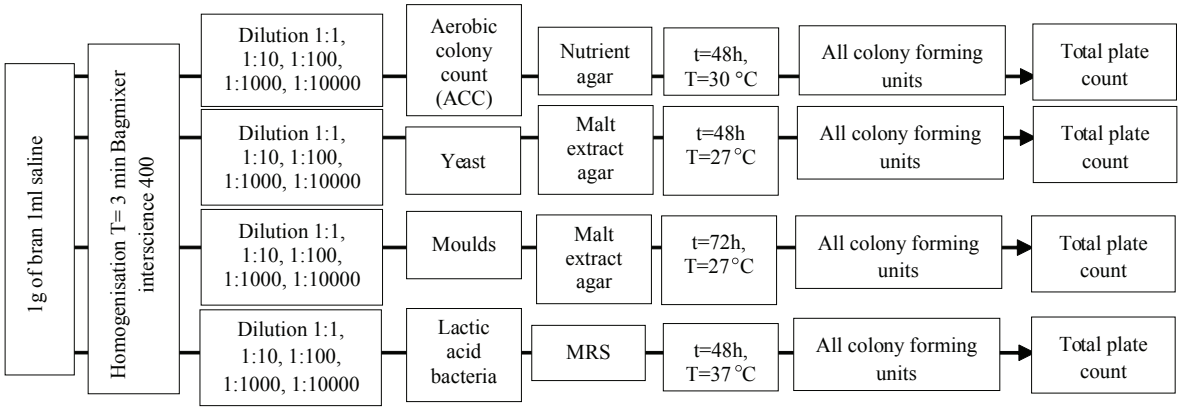

Figure 1. Scheme of microbiological testing of enzymatically treated and non–treated wheat and rye bran.

Enumeration of mesophilic lactic acid bacteria was performed according to standard LVS ISO 15214:1998.

Bran samples were packaged in plastic bags and stored at room temperature (temperature – 20 ± 1 °C, relative humidity – 40 ± 1%). Microbiological evaluation was conducted before storage (initial microflora), after the third, the fifth (the data are not included) and eighth week of storage. Particularly the eighth week of storage showed significant (p=0.001) decreasing of microorganisms comparing with initial microflora.

Moisture, pH value and water activity (a_w)

Moisture content was analysed using 'Determination of the Moisture Content of Cereals and Cereal Products method'– ICC Standard No, 110/1, by drying for 2 h at 150 °C. Procedure was carried out in triplicates.

pH was measured using 'Hydrogen–Ion Activity (pH)Electrometric method' – AACC 02 – 52.01, using JENWAY 3520 (Barloworld Scientific Ltd., ESSEX, UK) pH–meter. The pH electrode was dipped into a mixture of homogenized sample and distilled water. For a more precise measurement the calibration of pH meter has been done using 'Two–Point Calibration Procedure'. Measuring procedure was carried out in triplicates.

Water activity was measured using 'LABSwift a_w measurement device'. Before the measurement of water activity in the samples, calibration was done by using re–usable saturated salt calibration standard. Measuring procedure was carried out in triplicates.

Statistical analysis

Data was processed by SPSS software version 17.0. Data was analysed using descriptive statistics and processed by one–way analysis of variance ANOVA (one way ANOVA), as well as for comparing all bran samples depending on pre – treatment ways two-way ANOVA were used. Microsoft office software version 2007 was used to determine significant differences between the samples.

Results and Discussion

Moisture content, pH value and water activity of analysed bran.

Wheat and rye bran is a by–product of the milling process of flour, and is a composite material formed from different histological layers and three different strips. The outer strip corresponds to outer pericarp (epidermis and hypodermis), the inner one corresponds to the aleurone layers, and the intermediate one remains a composite of several tissues (inner pericarp, testa, and nuclear tissue) (Hemery et al., 2010).

One of the most important factors for microbial contamination, as well as for microorganism development is grain harvesting and storage conditions. Influence of temperature is closely associated with grain moisture (Dimic et al., 2009). Fungal infection, as well as insect invasion is of particular concern with wheat and rye stored at moisture content, time or length of storage and storage temperature (Karunakaran et al., 2001). It was reported that fungi produce mycotoxins, such as ochratoxin A, which was produced by *Aspergillus* and *Penicillium* spp., as a result it presents a health risk. Several studies imply that synthesis is highest when the product humidity is above 13% and temperature is between 24 ± 1 and 37 ± 1 °C. That is why, warm and wet geographic regions are the most favourable environments for mycotoxins (Dimic et al., 2009). For elimination of fungal contamination it is strongly recommended to control the moisture content of grain before harvesting, as well as during storage.

One of the factors which can affect bran moisture content is grain milling and drying technology. In the previous experiments it was ascertained that the

increase of moisture content might be caused by the increase of particle sizes in bran that leads to intensive growth and reproduction of fungi spore (Radenkovs et al., 2013).

In total, twelve samples including four controls were treated without enzymatic modification and four enzymatically treated bran samples were analysed. The results obtained from the determination of moisture content are summarized in Figure 2. The obtained results give a possibility to conclude that the highest moisture content was detected in the control bran samples without enzymatic modification. The content of moisture fluctuated in a range from 7.66 ± 0.07 to 15.38 ± 0.05%, while the lowest content was recorded in the control samples which were treated similarly to enzymatically hydrolysed bran, provided they are not enzymatically modified. The amount of moisture content in the tested bran samples ranged from 4.60 ± 0.09 to 6.1 ± 0.04%. Analysing data of moisture obtained after enzymatic modification suggest that the moisture content was significantly ($p<0.05$) decreased comparing with the control bran samples, for WSSD from 13.69 ± 0.05 to 10.21 ± 0.05% ($p=0.0001$) and for WLSD from 15.38 ± 0.05 to 11.73 ± 015% ($p=0.0001$). An exception was detected for WLSR, as well as for RSSJ bran samples, the content of moisture of which has increased from 7.66 ± 0.07 to 7.92 ± 0.14% ($p=0.009$) and from 12.05 ± 0.08 to 13.20 ± 0.11% ($p=0.002$), respectively. Differences between the samples can be explained with the fact that during the freeze-drying the uneven moisture elimination has occurred. It is because the water holding capacity in brans with small particle size is weaker, compared to the brans with large particles.

It is important to know not only the moisture content of bran, - another important marker is water activity (a_w) which can influence the development of microorganisms. Bacteria need higher water activity than yeasts and moulds, consequently foods with low water ability may be contaminated mainly with yeasts and moulds (King, 2009). Our obtained results indicate that the highest water activity of bran was recorded in the control samples (Figure 3). Water activity fluctuated in a range from 0.30 ± 0.01 to 0.71 ± 0.01. After enzymatic hydrolysis as well as bran treatment without enzymes gave positive results in decreasing the water activity. Water activity was decreased particularly 2 times and ranged from 0.38 ± 0.01 to 0.39 ± 0.01 for control samples treated without enzymes and from 0.35 ± 0.01 to 0.37 ± 0.01 for enzymatically hydrolysed bran samples.

The results obtained from the determination of pH value of the samples are summarized in Figure 4.

The highest pH value was found in the control bran samples, as well as in bran samples which

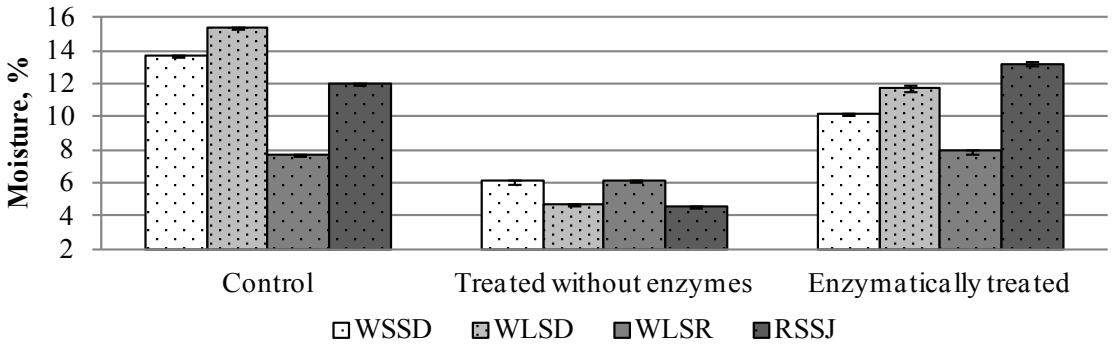

Figure 2. Moisture content of different bran samples, %.

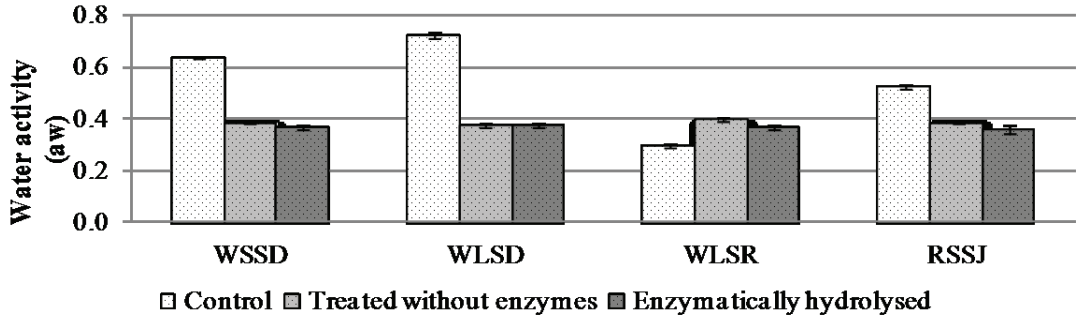

Figure 3. Water activity of different bran samples, %.

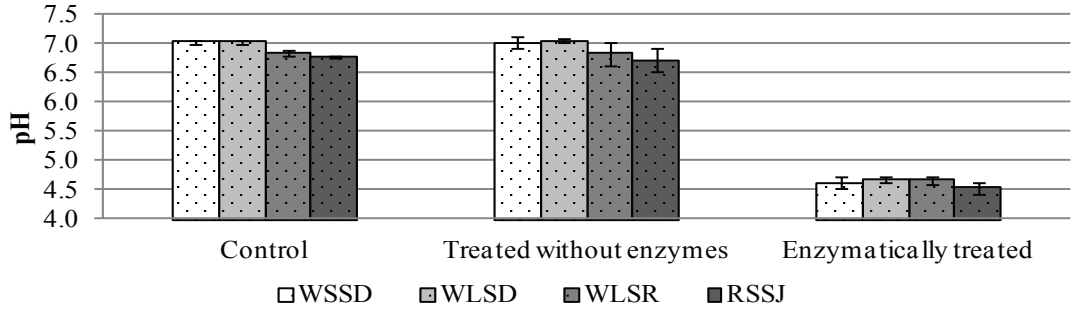

Figure 4. Comparison of pH value in wheat bran samples.

were treated without enzyme addition. The pH value for these bran samples ranged from 6.79 ± 0.02 to 7.04 ± 0.01 and from 6.72 ± 0.20 to 7.06 ± 0.04, respectively. Our previous studies show similar results in pH value among bran samples (Radenkovs and Klava, 2012). Analysing data of pH value obtained after enzymatic modification suggest that pH was significantly ($p<0.05$) decreased, pH value fluctuated in a range from 4.54 ± 0.10 to 4.69 ± 0.05. The decrease of pH may be explained with the fact that during enzymatic hydrolysis pH was adjusted to 4.6 with addition of citric acid. The pH 4.6 is necessary for enzymatic degradation of cell walls with Viscozyme L.

Microbiological contamination

The microbiological contamination of cereals as well as cereal products is diverse and includes a wide range of microorganisms, basically moulds, bacteria, yeasts, lactic acid bacteria, rope–forming bacteria (*Bacillus spp.*), pathogens, enterococci and coliforms. The microbiological contamination occurs mostly during growth, harvest and storage time, and is dominated by the moulds. The most important genera of the storage fungi are *Penicillium* and *Aspergillus*, although species of *Fusarium* may also be involved in spoilage when grain is stored under moist conditions (Adams and Moss, 2000). In our results obtained from four bran samples and by two different pre–treatments of bran we did not find any mould impurities, while our previous study indicated that moulds were detected in all bran samples with the genus *Penicillium* being the most frequent. Fungal counts ranged from 5 to 8 log CFU g^{-1} (Radenkovs et al., 2013). Differences between the samples may be explained by the fact that obviously the microbiological contamination has occurred during transporting, milling or storage of cereals.

Contamination with lactic acid bacteria

The results obtained from four bran samples and by two different methods of pre–treatment of bran suggest that the highest contamination with lactic acid bacteria was in the control bran samples, particularly in WLSD bran samples (5.75 ± 0.04 log CFU g^{-1}) (Figure 5). It was detected that during the storage of wheat, as well as rye bran samples, the colony–forming units have significantly decreased ($p<0.05$) comparing with starting point (1 week). The obtained results suggest that after eight weeks of storage in treated without enzymes bran samples lactic acid bacteria were completely inactivated in the WLSR bran sample, but in other bran samples significantly ($p<0.05$) decreased. In enzymatically hydrolysed bran samples the concentration of colonies significantly ($p<0.05$) decreased, and this amount was equal to 4.19 ± 0.07 log CFU g^{-1} in WSSD, 3.04 ± 0.03 log CFU g^{-1} in WLSD, 3.68 ± 0.06 log CFU g^{-1} in WLSR and 3.28 ± 0.05 log CFU g^{-1} in RSSJ.

During the research of literature about enzymatic hydrolysis and their impact on cereal microflora no explanation was found for colony count decrease; the limitation of literature review does not allow the possibility to completely describe the obtained

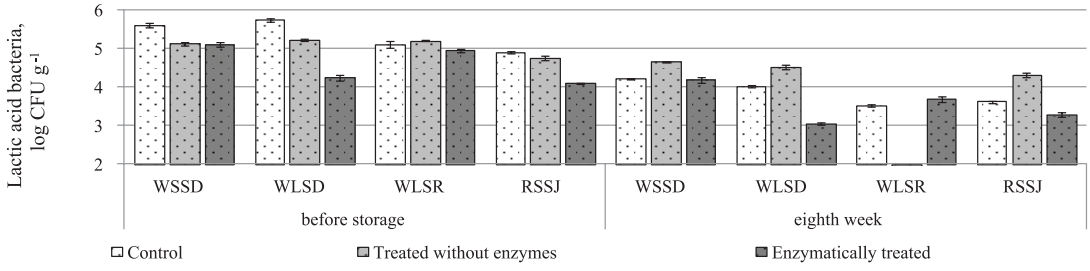

Figure 5. Colony – forming units of particular microbiological contamination with lactic acid bacteria.

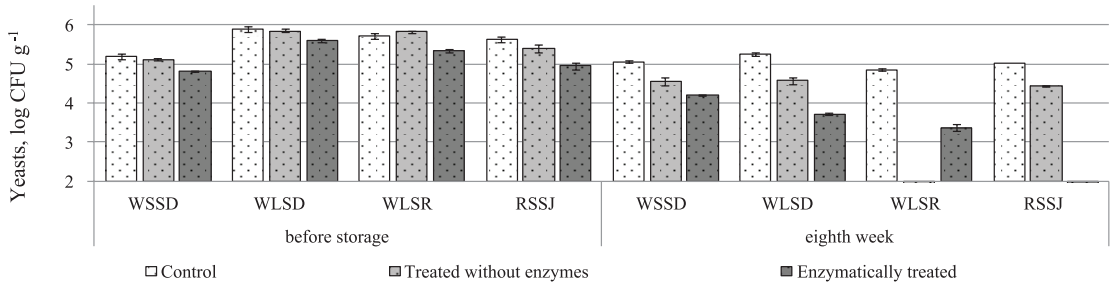

Figure 6. Colony – forming units of particular microbiological contamination with yeasts.

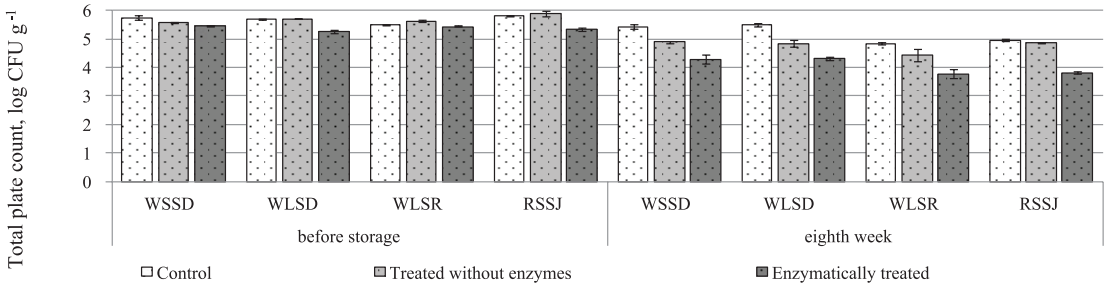

Figure 7. Colony–forming units of particular microbiological contamination with total plate count.

results. But we can assume that during the enzymatic modification of bran, water activity (a_w), moisture, nutrient accessibility (carbohydrate hydrolysis), as well as the pH value were changed, which can significantly alter the implementation of the development of microorganisms (Rushing et al., 2004).

Contamination with yeasts

Similar results were obtained for presence of yeasts in bran (Figure 6), - their development has occurred most intensively in the control bran samples. Among four bran samples the highest amount was in WLSR (5.89 ± 0.08 log CFU g^{-1}), while the lowest amount was found in WSSD bran samples (5.19 ± 0.07 log CFU g^{-1}). Our previous studies suggest that the control wheat and rye bran samples had the highest contamination with yeasts. The highest colony forming units were recorded in WLSD, and corresponding log 10 CFU g^{-1}, while the lowest contamination was in RSSJ, which corresponds to log 7 CFU g^{-1} (Radenkovs et al., 2013).

A positive fact was it that after eight weeks of storage enzymatically hydrolysed bran the presence of yeasts in RSSJ bran sample was not detected. This can be explained by the fact, that rye has a higher tolerance to disease, because the grain–filling assimilates are photosynthesized mainly in the stalk and head, comparing with wheat. It is probable that during the enzymatic hydrolysis the liberating of bound bioactive compound has occurred, and it has influenced the colony counts of yeasts. In the other bran samples after eight weeks of storage the colony forming units had significantly ($p<0.05$) decreased. The amount of colony forming units in WSDD comparing with initial microflora decreased from 4.81 ± 0.01 to 4.21 ± 0.00, from 5.60 ± 0.05 to 3.72 ± 0.07 log CFU g^{-1} (WLSD), from 5.35 ± 0.04 to 3.37 ± 0.09 log CFU g^{-1} (WLSR).

The results (Figure 7) showed contamination with total plate count. The initial study showed that the highest contamination with microorganisms was in the control bran samples. Among four control samples the amount of colony ranged from 5.50 ± 0.02 log CFU g^{-1} to 5.82 ± 0.04 log CFU g^{-1}.

Similar to the previous case, the lowest colony count was found after eight weeks of storage in enzymatically hydrolysed bran samples, the amount of TPC ranging from 3.79 ± 0.16 to 4.31 ± 0.05 log CFU g^{-1}.

Conclusions

1. This study asserts that no mould presence was detected in any of the bran samples, which allows to assume, that the moisture content and water activity of samples during storage, as well as after enzymatic hydrolysis were not appropriate for fungal growing and developing.
2. Analysing data of bran initial contamination with TPC, yeasts and lactic acid bacteria, suggest that all bran samples contain contamination of these microorganisms. Partial reduction of microbiological impurities was gained after enzymatic hydrolysis.

3. In all bran samples TPC, yeasts and lactic acid bacteria were detected in a range from 5.50 ± 0.02 to 5.82 ± 0.04 log CFU g^{-1}, from 5.19 ± 0.07 to 5.89 ± 0.08 log CFU g^{-1}, and from 5.11 ± 0.09 to 5.75 ± 0.04 log CFU g^{-1}, respectively.
4. After enzymatic hydrolysis the concentration of colony forming units decreased significantly ($p<0.05$) with yeasts ($p=0.002$) and lactic acid bacteria ($p=0.001$) which corresponds 4.81 ± 0.01 to 5.60 ± 0.05 log CFU g^{-1} and from 4.09 ± 0.01 to 5.10 ± 0.05 log CFU g^{-1}, respectively.
5. Analysing the data obtained after eight weeks of storage indicate significant decrease of TPC, yeasts and lactic acid bacteria. The amount of TPC fluctuated in a range from 3.79 ± 0.16 to 4.310.05 log CFU g^{-1}, yeasts from 3.37 ± 0.09 to 4.21 ± 0.08 log CFU g^{-1} and lactic acid bacteria 3.04 ± 0.03 to 4.19 ± 0.04 log CFU g^{-1}.

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INVESTIGATION OF TOTAL PROTEIN CONTENT AND AMINO ACID COMPOSITION OF WHOLE GRAIN FLOUR BLEND FOR PASTA PRODUCTION

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Abstract
The purpose of the current research was to investigate the total protein content and amino acid composition of flour blend made from several types of whole grain flour for pasta production. Conventional rye, hull-less barley, triticale and wheat grain was used in the experiments. For the flour blend obtaining white wheat flour type 550 was used. Using standard methods the following quality parameters were analysed: protein content in grain and flour samples by using InfratecTM model 1241 Grain Analyzer, in flour blend – by AACC 46–20, amino acid content by LVS ISO 13903:2005. In the present research it was determined that it is possible to increase the total protein content in wheat flour type 550 by 11% if adding whole wheat or whole triticale flour, and by 7% if adding whole grain flour of hull-less barley. Higher total amino acid content was obtained for whole wheat flour sample; lower – for whole rye flour sample. Significantly lower total amino acid content was obtained in whole rye, hull-less barley and triticale flour comparing with whole grain wheat flour. No significant differences (p=0.779) were found in the analysed essential amino acid content made of different flour blend – the content of essential amino acids in the analysed flour blend samples was very similar.
Key words: grain, protein, amino acids, pasta, blend.

Introduction

Whole grain consists of the intact, ground, cracked or flaked kernel after the removal of inedible parts such as the hull and husk. The principal anatomical components - the starchy endosperm, germ and bran - are present in the same relative proportions as they exist in the intact kernel (Healtgrain forum…, 2013). Currently, no common internationally accepted definition exists. Therefore, exact definitions of whole grain and whole grain foods processed in various ways, and knowledge about the components providing health effects in food as consumed, are crucial issues for whole grain research and recommendations (Andersson et al., 2014). It has been shown in epidemiological and certain intervention studies that whole grain cereals, containing a multitude of bioactive components, may protect against obesity, diabetes, cardiovascular diseases (CVD), hypertension and certain cancers (Fardet, 2010; Okarter and Liu, 2010; Lillioja et al., 2013). It was suggested that aleurone in bran, which is rich in minerals (magnesium and zinc), protein and certain bioactive components such as ferulic acid, is certainly more than just indigestible dietary fibre and was thus concluded to be a critical grain constituent for health effects (Lillioja et al., 2013).

Whole wheat flour (WWF), one of the most common and important whole grain, retains wheat bran and germ, and acts as a rich source of dietary fibre, vitamins, minerals and antioxidants. From epidemiological evidence and clinical trials, many studies have shown that the long-term intake of whole grain can provide tangible benefits to sufferers of chronic diseases such as diabetes mellitus, obesity, and cancer leading to a greater focus on whole grain foods. Corresponding dietary recommendations have also recommended consumers to eat more whole grain. Meanwhile, many specialty foods have been produced: whole wheat bread, whole wheat pasta, whole wheat noodles and some local traditional snacks (Wang et al., 2014). Wheat (*Triticum spp.*) is the main cereal crop used for human consumption in many areas worldwide. Traditionally, pasta is manufactured solely from durum wheat (*Triticum durum* Desf.), which results in a product considered to be of superior quality, to pasta made from cheaper common wheat (*Triticum aestivum* L.) or a blend of the two species. Nevertheless, price differentials between both wheat species could provide some manufacturers with the incentive to benefit economically from undeclared addition of common wheat. The manufacture of pasta from mixtures of durum and common wheat without adequate labelling is usually considered as adulteration. Durum wheat pasta for export outside the European Union may contain a maximum of 3% common wheat from unavoidable adventitious contamination during agricultural processing. Pasta is consumed in large quantities throughout the world. Scientific research has been undertaken to understand the parameters influencing pasta processing and the final product quality. It is firm and resilient with no surface stickiness and little if any cooking losses (Sissons et al., 2005; Troccoli et al., 2000). The quality of dietary protein is also important in determining changes in protein metabolism. The requirement for dietary protein consists of two components:

1. Nutritionally essential amino acids (isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val)) under all conditions, and for conditionally essential amino acids (arginine (Arg),

cysteine (Cys), glutamine (Gln), glycine (Gly), histidine (His), proline (Pro), taurine, and tyrosine (Tyr)) under specific physiological and pathological conditions.

2. Nonspecific nitrogen for the synthesis of the nutritionally dispensable amino acids (aspartic acid, asparagine, glutamic acid, alanine, and serine) and other physiologically important nitrogen-containing compounds such as nucleic acids, creatine, and porphyrins (Mauro, 2007).

Gluten is the main structure forming protein in the flour and responsible for the elastic characteristics of the dough (Gallaghe et al., 2004). These properties derive largely from the gluten proteins, which form a continuous viscoelastic network within the dough. Gluten contains hundreds of protein components, which are present either as monomers or, linked by interchain disulphide bonds, as oligo- and polymers (Wrigley et al., 1998). They are unique in terms of their amino acid composition, which are characterized by high contents of glutamine and proline and by low contents of amino acids with charged side groups. Traditionally, gluten proteins have been divided into gliadins and glutenins, according to their polymerisation properties: gliadins are monomeric proteins that form only intra-molecular disulphide bonds, if present, whereas glutenins are polymeric proteins whose subunits are held together by inter-molecular disulphide bonds, although intra-chain bonds are also present. Among these storage proteins, glutenins (polymeric proteins) have been shown to be extremely important in determining rheological properties (Jia et al., 1996). Gluten proteins are susceptible to heat treatment and their behaviours subjected to relatively high temperatures have been studied by a number. It was shown that molecular size of the glutenin aggregates increases and, hence, their extractability decreases (Weegels et al., 1994b). At the macromolecular level, pasta is essentially a large protein network formed by irreversible protein–protein crosslinks through thermal dehydration, which encapsulates starch granules (Drawe, 2001). Bran from whole grain flour can interfere with water migration during this step, increasing water retention within the pasta (Villeneuve et al., 2007). Bran and germ particles also disrupt the continuity of the protein network, resulting in weaker, less firm pasta (Manthey et al., 2002). The structure of cooked pasta is generally described as a compact matrix, with the starch granules trapped in the network formed by the gluten proteins (Cunin et al., 1995). The resulting structure is responsible for the peculiar sensorial and nutritional properties of pasta, and its formation relates to the characteristics of the starting material and to various steps of the technological process (Scanlon et al., 2005). Rye (*Secale cereale*) could be exploited more efficiently in new types of cereal products due to its positive health effects. Nowadays, its use is limited mainly as a result of the problems arising from its flavour; not all European consumers are familiar with the somewhat foreign, rye-like flavour, perceived as bitter and intense. However, rye consumption in Europe might increase if ingredients were produced with the specific rye-like flavour modified to a slightly milder one, without significantly decreasing the contents of fibre and bioactive compounds in the rye (Heiniö et al., 2003). Triticale (*Triticosecale wittmack*) is a type of small grain created by genetically combining wheat and rye. Triticale grain, flours, and prepared products are available through both health food and commercial outlets on a limited basis. The data indicate that while the nutritional quality of triticale is considered superior to wheat, the higher ash content, lower milling yields of flour, and inferior loaf volume and texture distract from commercial baking use of triticale. In comparison with bread wheat, triticale has low gluten content, efficient gluten viscoelasticity and, therefore, inferior bread-making quality (Doxastakis et al., 2002). Hull-less barley (*Hordeum vulgare L.*) has been intensively investigated in respect to its food, feed and industrial applications. The advantage of hull-less barley compared to hulled barley in food uses is that pearling is not needed, so that the outer part of the endosperm, the aleurone, which contains proteins with essential amino acids and vitamins, is retained, as well as other bioactive compounds. Another advantage of hull-less barley is that there is no hull hampering the milling process. It can be milled using conventional equipment available for wheat milling, with extraction yields of 73%. Hull-less barley flour has been successfully used in chemically leavened products such as muffins, pancakes, biscuits and cookies (Andersson et al., 2004). Conventional farming has played an important role in improving food and fibre productivity to meet human demands but has been largely dependent on intensive inputs of synthetic fertilizers, pesticides, and herbicides. Certain conventional farming practices and associated chemical inputs have raised many environmental and public health concerns. Prominent among these are the reduction in biodiversity, environmental contamination, and soil erosion. Public concerns over environmental health and food quality and safety have led to an increasing interest in alternative farming practices with both lower inputs of synthetic chemicals and greater dependence on natural biological processes (Tu et al., 2006).

In the scientific literature practically cannot be found data about whole grain flour made form hull-less barley, rye, triticale and wheat application in pasta production, as well as protein content and amino acid composition of possible whole flour blend for pasta

making. Therefore, the purpose of the current research was developed as follows – to investigate the total protein content and amino acid composition in dry blends made from several types of whole grain flour for pasta production.

Materials and Methods

The study was carried out at the Agronomy Research Laboratory of Latvia University of Agriculture (LUA), at the scientific laboratories of Faculty of Food Technology at Latvia University of Agriculture and at the laboratory of the JSC "Jelgavas dzirnavas" (Latvia), at the laboratories of Biotechnology and Veterinary Medicine Scientific Institute "Sigra" (Latvia).

Conventional rye (′Kaupo′) harvested from State Priekuli Plant Breeding Institute (Latvia) in 2012, hull-less barley (line ′PR 5099′) and triticale (line ′9405-23′), as well as wheat (′Zentos′) grain form LUA research station „Peterlauki" (Latvia) were used in the experiments. To obtain the flour blend, white wheat flour type 550 (Latvia) was used.

Before experiments grain was grounded in laboratory mill Hawos (Hawos Kornmuhle GmbH, Germany) obtaining fine whole grain flour.

At the previous experiments the following optimal wheat flour type 550 and whole grain proportion was obtained: 70% type 550 wheat flour and 30% whole wheat flour (W/W); 80% type 550 wheat flour and 20% whole rye flour (W/R); 70% type 550 wheat flour and 30% whole triticale flour (W/T); 80% type 550 wheat flour and 20% whole hull-less barley flour (W/H).

For measurement of protein content in analysed grain samples the InfratecTM model 1241 Grain Analyzer from Foss Tecator AB has been used for the spectroscopic investigation of the grain samples according to the express method ISO 12099. The instrument had an extended wavelength range of 570 – 1100 nm (Petterssona et al., 2003).

The total protein content of flour blend was determined under the standard method AACC 46–20 by means of a Kjeldal method.

The total and essential amino acid content in flour and flour blend was measured by HPLC Waters Alliance 2695/3100MS/2998FD (Waters Corporation, USA) using LVS ISO 13903:2005 standard method.

Microsoft Excel software was used for the research purpose to calculate the mean arithmetic values and standard deviations of the mathematical data used in the research. SPSS 20.0 software was used to determine the significance of research results, which were analysed using the two-factor ANOVA analyses to explore the impact of factors and their interaction, and the significance effect (p-value).

Results and Discussion

Protein is the second most common constituent of cereal grain, following starch. Depending on cereal species, variety, and agronomic conditions, the protein content in cereals can range from 5 to 20%. The type and amount of protein in cereal grain are important in terms of nutritional values as well as impacts on functional properties of food or feed containing the protein (Shewry, 2007).

Significant differences (p=0.004) in total protein content were found in the analysed whole grain flour samples. Lower protein content was obtained in rye flour 108 ± 3 g kg^{-1}, higher in wheat as 146 ± 1 g kg^{-1}, respectively (Figure 1). However, it is necessary to mention that there were not found any significant differences (p=0.774) between wheat and triticale whole grain flour protein content – results were very similar, as 146 ± 1g kg^{-1} and 143 ± 2 g kg^{-1}, respectively. The differences in total protein in the analysed flour samples could be explained mainly with grain individuality.

For the comparison, wheat flour type 550 protein content is 105 ± 2 g kg^{-1}, which is very similar to rye whole grain flour protein content and significantly

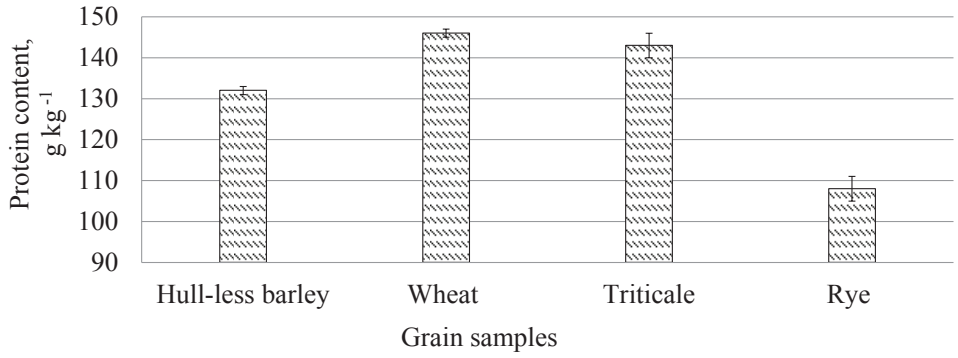

Figure 1. Total protein content in grain.

lower (p=0.001) comparing with whole grain hull-less barley, wheat and triticale flour.

Higher protein content in whole grain flour mainly could be explained with all grain (including cover) grinding. Because of their different production systems and levels of fertiliser use, it is difficult to obtain comparative values for the protein contents of different cereals. However, consideration of values reported indicates that relatively small differences exist within and between species and that these are amplified by environmental factors. Thus, ranges of 58 – 77 g kg^{-1} of protein on a dry weight basis have been quoted for rice; 80 – 150 g kg^{-1} for barley and 90 – 110 g kg^{-1} for maize. However, these ranges almost certainly reflect the impact of environment as well as genotype. For example, if grain nitrogen varied from 1.27 to 2.01% (about 72 – 115 g kg^{-1} protein) in a single variety of barley in which N fertilisation (Shewry, 2007).

Within the present experiment it was ascertained that it is possible to increase the total protein content in wheat flour type 550 by adding several types of whole grain flour in several proportions (Figure 2). As the results of our research demonstrate, it is possible to increase the total protein content by 11% in wheat flour type 550 by adding whole wheat or whole triticale flour. As a result, the flour blend for pasta production could be with higher nutritive value. No significant differences (p=0.417) were found in protein content between the analysed flour blends by adding rye flour (Figure 2). However, it is possible to increase the protein content in flour blend by 7% (which is not significant - p=0.068) if adding the hull-less barley whole grain flour. Therefore, it is possible to foresee higher protein content in pasta made from wheat flour type 550 and whole grain wheat flour, as well as from wheat flour type 550 and whole grain triticale flour.

Cereals and cereal products contain variable amounts of free amino acids depending largely on the species, cultivar and growing conditions. Free amino acids in raw materials of heat-treated foods take part in the Maillard reaction, which is important for cereal food quality (Mustafa et al., 2007).

During the present experiment significant differences (p=0.004) in the total amino acid content were obtained for analysed whole grain flour samples in dry matter (Table 1). Higher total amino acid content

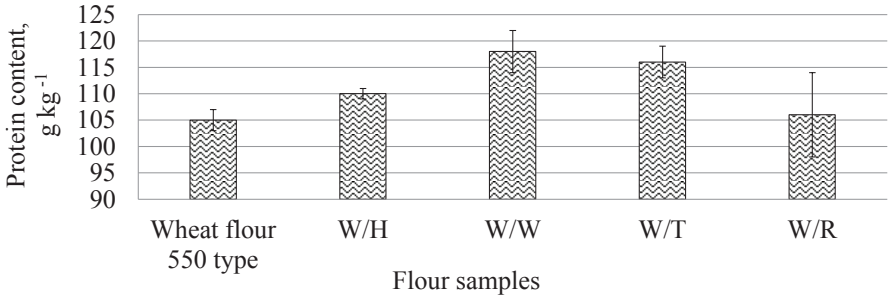

Figure 2. Total protein content of flour blend.

W/H – 80% type 550 wheat flour and 20% whole hull-less barley flour; W/W – 70% type 550 wheat flour and 30% whole wheat flour; W/T – 70% type 550 wheat flour and 30% whole triticale flour; W/R – 80% type 550 wheat flour and 20% whole rye flour

Table 1

Amino acid composition of analysed flour samples

Samples	Total amino acid content, g kg^{-1}	Essential amino acids total content g kg^{-1}
Whole rye grain	13.525±0.004	5.101±0.001
Whole hull-less barley grain	14.764±0.001	5.354±0.011
Whole triticale grain	15.115±0.012	5.361±0.007
Whole wheat grain	21.148±0.001	9.617±0.004
Wheat flour type 550	16.170±0.008	6.458±0.001
70% type 550 wheat and 30% whole wheat (W/W)	17.626±0.018	7.406±0.012
70% type 550 wheat and 30% whole triticale (W/T)	15.817±0.008	6.129±0.008
80% type 550 wheat and 20% whole rye (W/R)	15.641±0.004	6.186±0.004
80% type 550 wheat and 20% whole hull-less barley (W/H)	15.889±0.048	6.237±0.008

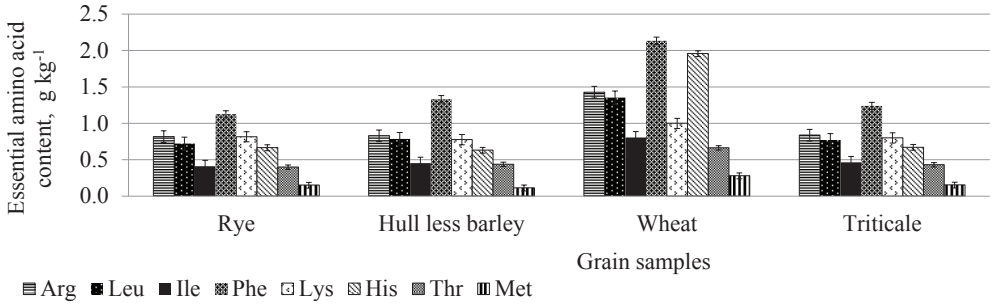

Figure 3. Essential amino acid content in whole grain flour samples.

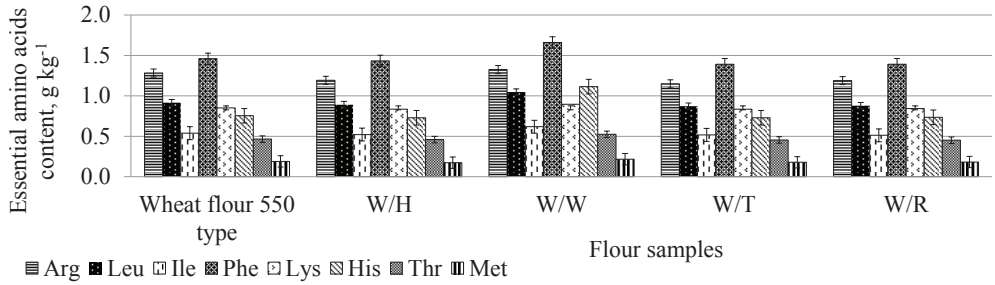

Figure 4. Essential amino acid content in flour blends.

W/H – 80% type 550 wheat flour and 20% whole hull-less barley flour; W/W – 70% type 550 wheat flour and 30% whole wheat flour; W/T – 70% type 550 wheat flour and 30% whole triticale flour; W/R – 80% type 550 wheat flour and 20% whole rye flour

was obtained for whole wheat flour sample, lower – for whole rye flour sample. Differences could mainly be explained with grain individuality. Mathematical data analyses show, that there are no found significant differences ($p=0.068$) in the total amino acid content between: whole rye and whole hull-less barley flour ($p>0.05$), whole hull-less barley and whole triticale flour ($p>0.05$). However, significantly lower ($p<0.05$) total amino acid content was obtained in whole rye, hull-less barley and triticale flour comparing with whole grain wheat flour (Table 1). Consideration of values reported indicates that relatively small differences exist within and between varieties and that these are amplified by environmental factors (Shewry, 2007).

In the present research it was found that wheat flour type 550 has a significantly higher ($p=0.05$) total amino acid content comparing with made flour blends (Table 1), with whole triticale, and rye and hull-less barley flour. The obtained results could mainly be explained with lower total amino acid content in same analysed whole grain flour samples. However, it is necessary to indicate that by mixing wheat flour type 550 with whole grain wheat flour, it is possible to increase the total content of amino acids by 8%, comparing with wheat flour type 550 amino acid results. It could be explained with the fact of all grain milling for whole grain flour production, and in the scientific literature (Mustafa et al., 2007) it is mentioned that an essential part of amino acid concentrates in the aleurone layer of the grain. Very similar results were obtained during essential amino acid total content analyses (Table 1).

It is necessary to mention that amino acids are not stored in the body like fats or carbohydrates; there are no specialized cells in the body to maintain a reservoir. Of course, amino acids are ubiquitous, being present in structural proteins, enzymes, transport proteins, etc. Some of these proteins (notably serum albumin) can be degraded under conditions of fasting or starvation, to release free amino acids. Dietary intake of amino acids is typically not balanced to exactly match the body's demands for various amino acids. Amino acids taken via the diet must be chemically modified and rearranged to provide adequate levels of all the amino acids needed. There is a large number of pathways in the body for balancing the pool of amino acids, both for synthesis and for degradation. The number of enzymes involved creates a great potential for genetic diseases. Furthermore, disruption (by mutation of just one enzyme) in the metabolism of only one amino acid can have profound consequences for growth and development; some of the genetic diseases are fatal. Adult humans are unable to synthesize all twenty amino acids needed for protein synthesis; those which cannot be synthesized and which must then be acquired via the diet are referred to as essential.

The ten which the body can synthesize on its own are nonessential (Essential and nonessential…, 2014). Therefore, it was very necessary to analyse essential amino acid composition in whole grain flour samples and in flour blend.

It was found that phenylalanine presence in the analysed whole grain flour samples is more pronounced, comparing to others amino acids (Figure 3). However, in smaller amount methionine was obtained in all analysed flour samples. In the scientific literature it is mentioned that lysine is the limiting amino acid for all cereals. Whole wheat flour, as aleurone and embryo tissues of grain, do contain higher contents of essential amino acids (Shewry, 2007). Such results differ from those obtained during the present research: the amount of lysine in all analysed whole flour samples was higher than histidine, or threonine, or methionine (Figure 3).

Lysine is one of the most limiting amino acids in plants consumed by humans and livestock. Recent genetic, molecular, and biochemical evidence suggests that lysine synthesis and catabolism are regulated by complex mechanisms (Ferreira et al., 2005). However, within experiments no significant differences (p=0.447) were found in lysine content between analysed whole grain samples.

In our study we have found significant differences (p=0.067) composed of phenylalanine. Lower phenylalanine content was obtained in the flour blend with whole triticale grain flour as 1.39 ± 0.07 g kg^{-1}, however, higher analysed amino acids content was found in flour blend with whole wheat grain flour as 1.66 ± 0.06 g kg^{-1}, respectively. The found differences could be explained with the fact that aleurone and embryo tissues of grain contain higher contents of essential amino acids (Shewry, 2007). No significant difference (p=0.799) was found among the other essential amino acids in analysed whole grain flour samples (Figure 4).

Conclusions

1. Lower protein content was obtained in whole grain rye flour 108 ± 3 g kg^{-1}, higher in wheat – 146 ± 1 g kg^{-1}, respectively. There are not found significant differences (p=0.774) between wheat and triticale whole grain flour protein content – the obtained results were very similar, as 146±1 g kg^{-1} and 143 ± 2 g kg^{-1}, respectively.
2. It is possible to increase the total protein content in wheat flour type 550 by adding several types of whole grain flour. It is possible to increase the total protein content in wheat flour type 550 by 11% if adding whole wheat or whole triticale flour and by 7% if adding hull-less barley whole grain flour.
3. Higher total amino acid content was obtained for whole wheat flour sample (21.148 g kg^{-1}); lower – for whole rye flour sample (13.525 g kg^{-1}).
4. No significant differences were found in the total amino acid content between whole rye and whole hull-less barley flour (p>0.05), whole hull-less barley and whole triticale flour (p>0.05). However, significantly lower (p<0.05) total amino acid content was obtained in whole rye, hull-less barley and triticale flour comparing with whole grain wheat flour.
5. In this study significant differences (p=0.067) were found composed of phenylalanine. Lower phenylalanine content was obtained in the flour blend with whole triticale grain flour as 1.39 ± 0.07 g kg^{-1}, however, higher analysed amino acid content was found in flour blend with whole wheat grain flour - 1.66 ± 0.06 g kg^{-1}, respectively.

Acknowledgement

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MICROBIOLOGICAL CONTENT OF COW MILK DEPENDING ON SEASON AND HERD SIZE IN LATVIAN ORGANIC FARMS

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Abstract
The objective of the study was to investigate the microbiological content of cow (*Bos primigenius*) milk in Latvian organic farms according to season and herd size with a purpose to detect their impact on the distribution of mastitis causing pathogens in milk. Samples were collected in 14 organic dairy farms of Latvia, 4 times through 2012: in winter, autumn, spring and summer period. Raw milk samples (n=564) obtained from cow composite milk were studied. The samples were divided into three groups in accordance with the number of cows in the cow-shed: A (3-30), B (31-60) and C (61-124). The total colony count (TTC) and the isolation of mastitis causing bacteria were analysed using standard methods. Bacterial growth occurred in 90.4% of samples. Isolated microorganisms belonged to 35 species, and the following bacteria were the most prevalent agent, including Coagulase negative staphylococci in 29.4%, *Staphylococcus aureus* in 24.1%, *Kocuria kristinae* in 12.9%, and *Enterobacteriaceae spp.* in 10.3% out of 564 milk samples. Depending on the season, the average number of TCC was the lowest in summer (4.66 ± 4.01 $\log_{10}$ CFU mL^{-1}), moderately higher in spring and winter (4.72 ± 4.18 and 4.82 ± 3.54 $\log_{10}$ CFU mL^{-1}, respectively), but significantly higher in autumn (5.43 ± 4.80 $\log_{10}$ CFU mL^{-1}). Coliforms were not isolated from group A herds instead of B (3.1%) and C (4.6%). Occurrence of *Staphylococcus aureus* was noticeably higher in B (19.1%) and C (20.8%) than in A (12.6%) herds. Most of group A herds met the milk quality requirements, while group C herds produced more contaminated milk.
Key words: raw milk, microbiological quality, organic farming.

Introduction

Over the past years, in the European Union, including Latvia, and also worldwide, the demand for organic agricultural products has noticeably increased. The organic sector in the EU has been rapidly developing during the past years. During the last decade, organic area in the EU enlarged by about 500,000 hectares per year. The whole organic area represents 5.4% of the total utilised agricultural area in Europe. In Latvia the organic dairy cows (*Bos primigenius*) make up 9.6% of the total dairy cow herd and their produced milk comprises 7% of the total produced milk amount in Latvia (Facts and figures on organic agriculture…, 2013). Based on the current growth rate of organic food consumption, it is predicted that the demand for organically produced dairy products will continue to increase (Batte et al., 2007).

Due to the high nutritional value of milk, together with the neutral pH and high water activity, the raw milk serves as an excellent growth medium for different microorganisms including many spoilage and pathogenic bacteria (Frank, 2007). Researchers have observed that numerous factors contribute to the bacterial variation, such as geographical location, season, farm size, number of animals on the farm, hygiene and farm management practices. However, in spite of the variation, all of these surveys demonstrated quite clearly that milk can be a significant source of foodborne pathogens of human health significance (Oliver et al., 2005).

The objective of the study was to investigate the microbiological content of cow milk in Latvian organic farms depending on season and herd size with a purpose to detect their impact on the distribution of mastitis causing pathogens in milk.

Materials and Methods

Sampling

Five hundred and sixty four raw milk samples from 14 organic dairy farms located in various parts of Latvia - regions of Kurzeme (3 herds), Latgale (3 herds), Vidzeme (2 herds) and Zemgale (6 herds) - were collected in winter (December, 2011), when the dairy cows were housed indoors, in autumn (October, November, 2012), in spring (April, May, 2012) and in summer period (August, 2012) when the cows were kept outdoors grazing on pastures. The organic dairy farms have been registered by the state control institutions. Dairy cows in these farms were housed either in tie stall or free stall cow-sheds. 148 samples were collected in winter, 142 in spring, 168 in summer and 106 raw milk samples in autumn. Herd size varied from 3 to 124 animals in a cow-shed including 4 herds with 3-30 cows (group A herds, n=86 samples), five - with 31-60 cows (group B herds, n=262 samples) and five herds with 61-124 cows (group C herds, n=216 samples) in a cow-shed. Herds of A, B and C group have milk production 4461 ± 257, 4372 ± 517 and 5428 ± 575 kg per cow per year, respectively. Fifteen lactating cows from each herd were chosen for sampling. Milk samples from herds of fewer than

15 cows were collected from all lactating animals. The study included various breeds (Latvian Brown, Holstein and Danish Red), as well as different varieties of cross-breeds from the first to ninth lactation.

Milk samples were collected by trained farm personnel from a cow level (cow composite milk) during sampling procedure of monitoring milk quality according to the standard LVS 175:1999 'Sampling of raw milk'. Samples were collected in sterile, 7 mL vacutainers (Vacutest Kima, Italy) and immediately transported to the Laboratory of Microbiology of the Research Institute of Biotechnology and Veterinary Medicine 'Sigra' (Sigulda, Latvia) maintaining cold chain under the temperature of 10 °C, then frozen at -20 °C for 2-6 weeks until the examination was done.

Microbiological examination

The samples were defrosted at a room temperature and serially decimally diluted with Maximum Recovery Diluent (Oxoid, England) according to the standard LVS EN ISO 6887-5:2011 'Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 5: Specific rules for the preparation of milk and milk products (ISO 6887-5:2010)' and appropriate dilutions were plated on to agars.

Microbiological examination has been described in detail previously (Gulbe and Valdovska, 2012). Samples presenting more than three sizes of microbial pathogens were considered contaminated (Laboratory and field handbook on bovine mastitis, 1999).

Statistical analysis

For statistical analysis, the IBM SPSS Statistics version 21 was used. Descriptive statistics for the total colony count and occurrence of several microorganisms including average standard error and frequencies was done. To determine whether the effect of season and herd size was significant in explaining the variations in the total colony count and isolated bacteria, the data were analysed by bivariate correlation. Data are presented as mean ± standard error, and a probability value $p<0.05$ was considered statistically significant.

Results and Discussion

Milk is a complex biological fluid and by its nature, a good growth medium for many microorganisms. Because of the specific production, it is impossible to avoid contamination of milk with microorganisms, therefore, the microbiological content of milk is a major feature in determining its quality (Torkar and Teger, 2008).

In our results, bacterial growth had occurred in 90.4% of the examined milk samples. Isolated microorganisms belonged to 35 species, - we divided them into 16 groups and included one group of mixed culture (this means that the sample contains 2 and more varieties of bacteria) (Fig. 1a, b).

Coagulase negative staphylococci (CoNS), *Staphylococcus aureus, Kocuria kristinae* and *Enterobacteriaceae spp.* were the most prevalent agents and were isolated in 166 (29.4%), 136 (24.1%), 73 (12.9%) and 58 (10.3%) out of 564 raw milk samples, respectively. Above mentioned bacteria, except for *Enterobacteriaceae spp.,* were distributed in milk samples almost of each herd throughout the year. Bacteria from genus *Enterobacteriaceae* were present mostly in samples from group C herds all year round, more rarely in samples from group B herds (only in spring and autumn), but these microorganisms were not present in samples of group A herds. Isolated *Enterobacteriaceae spp.* includes *E. coli, Klebsiella oxytoca, Serratia marcescens, Kluyvera ascorbata, Pantoea agglomerans* and other undifferentiated species. CoNS group includes *S. equorum, S. kloosii, S. saprophyticus, S. simulans, S. haemolyticus, S. vitulus* and other undifferentiated CoNS species.

More rarely distributed bacteria belongs to genus of *Micrococcus spp.* in 35 (6.2%), *Corynebacterium spp.* in 30 (5.3%), *Bacillus spp.* in 23 (4.1%), a group of other Gram-positive bacteria in 20 (3.5%), and *Streptococcus spp.* in 17 (3.0%) out of 564 raw milk samples. *Micrococcus spp.* includes *M. sedentarius* and other undifferentiated species. *Corynebacterium spp.* includes *C. freundii, C. aquaticum* and *C. kutscheri*. Other Gram-positive bacteria consist of *Lactococcus lactis ssp. lactis, Lactococcus lactis ssp. cremoris, Pediococcus pentosaceus, Gemella haemolysans* and other undifferentiated species. *Streptococcus spp.* includes *S. uberis* and other undifferentiated streptococci except for *S. agalactiae*.

We isolated *Aerococcus spp.* with occurrence 3.7% of examined raw milk samples. Since the *Aerococcus spp.* was isolated only from two herds during one season, this occurrence cannot be applied to all investigated herds.

The frequency of other isolated bacteria does not exceed 3% of all examined samples, and this category includes other Gram-negative bacteria in 14 (2.5%), coagulase positive staphylococci (CoPS) in 10 (1.8%), *Enterococcus spp.* in 10 (1.8%) and *S. agalactiae* in 7 (1.2%) out of 564 milk samples. Other Gram-negative bacteria contains *Acinetobacter baumanii, Arcanobacterium pyogenes* (formerly *Actinomyces pyogenes*), *Pseudomonas fluorescens* and other undifferentiated species. *Bacillus spp.* includes *B. brevis, B. cereus, B. licheniformis, B. subtilis* and other undifferentiated species. *Enterococcus spp.* includes *E. faecalis* and other undifferentiated species, but CoPS contains *S. intermedius* and other

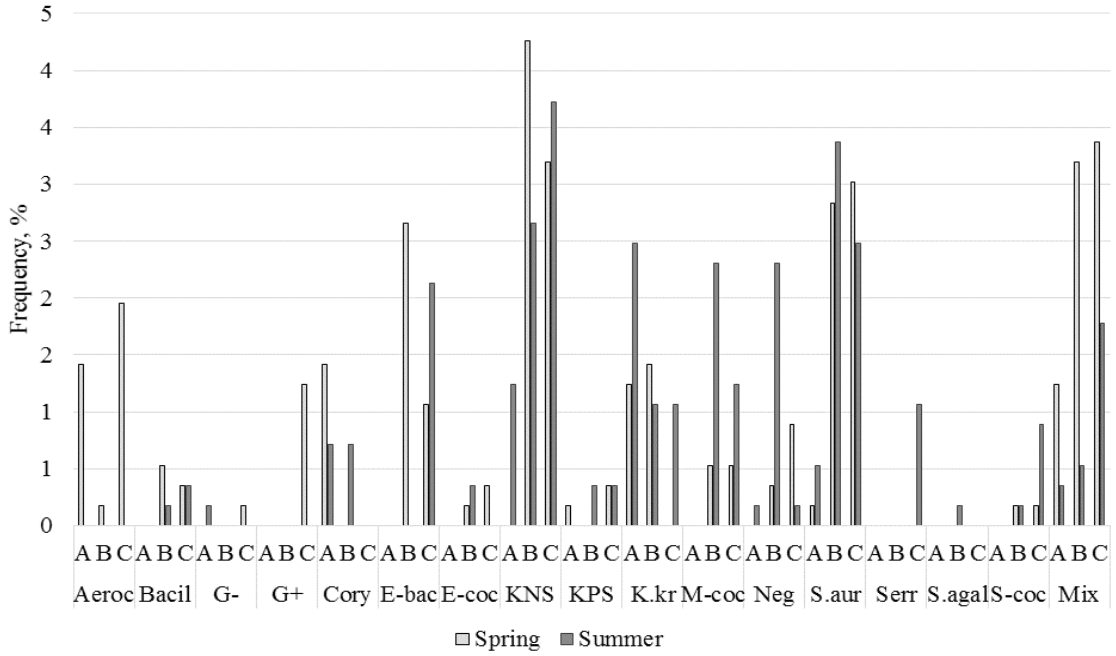

Figure 1a. The occurrence of isolated bacteria in raw milk samples depending on season and herd size, % of samples in each group.

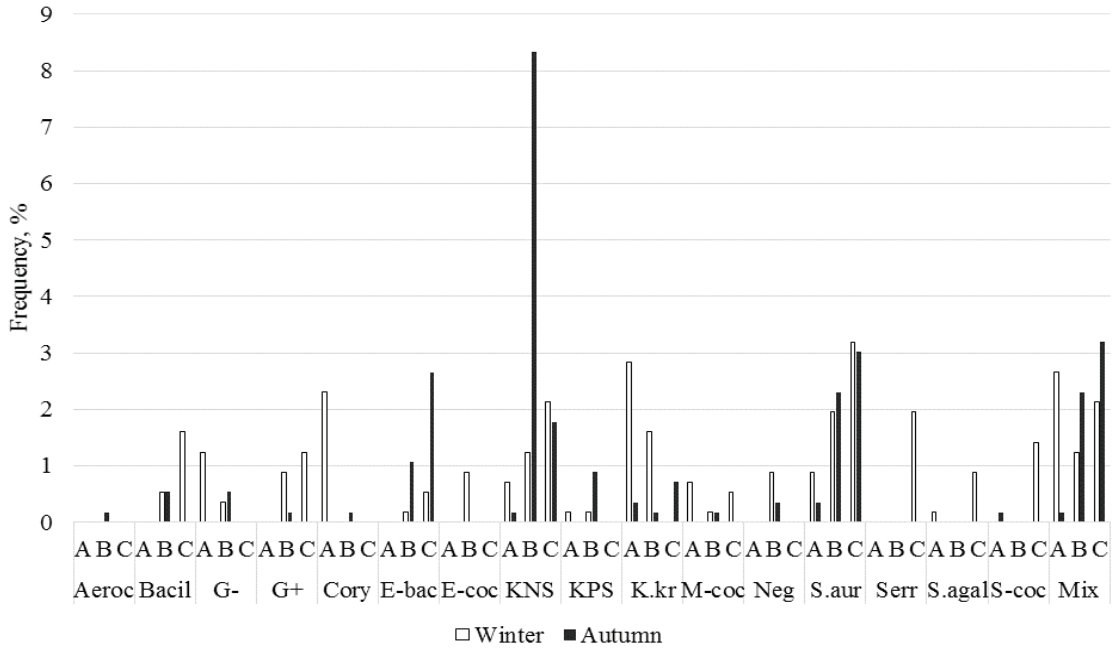

Figure 1b. The occurrence of isolated bacteria in raw milk samples depending on season and herd size, % of samples in each group: ▫ Winter; ■ Spring; ■ Summer; ■ Autumn; Aeroc – *Aerococcus spp.*; Bacil – *Bacillus spp*.; G- - other Gram-negative microorganisms; G+ - other Gram-positive microorganisms; Cory – *Corynebacterium spp*.; E-bac – *Enterobacteriaceae spp*.; E-coc – *Enterococcus spp.*; KNS – coagulase negative staphylococci; KPS – coagulase positive staphylococci; K.kr – *Kocuria kristinae*; M-coc – *Micrococcus spp.;* Neg – negative culture; S.aur – *Staphylococcus aureus*; Serr – *Serratia spp.*; S.agal - *S. agalactiae*; S-coc – *Streptococcus spp*.; Mix – mixed culture.

undifferentiated coagulase-positive staphylococci except for *S. aureus*.

SPSS Bivariate correlation analysis revealed that the effect of herd size, season and isolated bacteria on TTC was significant ($p<0.01$); the impact of herd size and season on isolated bacteria was significant too ($p<0.05$) while the seasonal effect was not significant on the isolated bacteria groups.

All isolated bacteria groups depending on season and herd size are presented in Figure 1a, b.

According to the Directives of European Union (Regulation 853/2004), the geometric average of the total number of microorganisms should not exceed 100,000 CFU per mL of raw cow milk from primary production. We determined the TCC higher than 100,000 CFU mL^{-1} in 14.4% and 85.6% of samples with TCC lower than 100,000 CFU mL^{-1} with mean TCC 5.7 ± 4.9 and 4.1 ± 3.0 $\log_{10}$ mL^{-1} out of all tested samples, respectively. There are quite large variations by TCC in different milk worldwide. K.G. Torkar and S.G. Teger (2008) and D.N. Prabhavathy and D. Sowmya (2012), reported on TCC in 24% raw milk samples in quantity of 4.46 $\log_{10}$ CFU mL^{-1} and 4.5 $\log_{10}$ CFU mL^{-1}, of the total number of microorganisms in raw milk, respectively, and this is similar to our experiment (4.9 ± 4.1 $\log_{10}$ CFU mL^{-1}). Other researchers report significantly higher TCC, for example, E.N. Aaku et al. (2004) and R. Arenas et al. (2004) - 6.7 $\log_{10}$ CFU mL^{-1} and 7-8 $\log_{10}$ CFU mL^{-1}, respectively.

In the literature an opinion prevails that the seasonal climate variations have an effect on microbial content of raw milk (Osteras et al., 2006; Riekerink et al., 2007; Nobrega and Langoni, 2011; Zucali et al., 2011).

K.G. Torkar and S.G. Teger report a decrease of TCC from summer to winter (Torkar and Teger, 2008). In our study the average number of the total colony count (TCC) was the lowest in summer (4.66 ± 4.01 $\log_{10}$ CFU mL^{-1}), moderately higher - in spring and winter (4.72 ± 4.18 and 4.82 ± 3.54 $\log_{10}$ CFU mL^{-1}, respectively), but significantly higher - in milk samples taken in autumn (5.43 ± 4.80 $\log_{10}$ CFU mL^{-1}). The average increase of TCC in autumn samples largely is because of samples from group C herds, as we can see it in Figure 2 that displays distribution of TCC in accordance with season and herd size.

K.G. Torkar and S.G. Teger (2008) explains that there exists a correlation between TCC and certain individual groups of microorganisms because they represent a part of the total bacterial amount in milk. There is an especially positive correlation between the number of coliform and psychrotrophic microorganisms because a lot of coliform bacteria are capable to grow at low temperatures (Torkar and Teger, 2008). A.J. Bramley and C.H. McKinnon (1990) reported that some species of the genera making up the coliform group of bacteria are psychrotrophic and constitute 10 – 30% of the whole group of microorganisms, the majority of these are *Aerobacter spp.*

Figure 3 displays distribution of TCC in accordance with five most frequent, isolated group of microorganisms. It also shows the average TCC

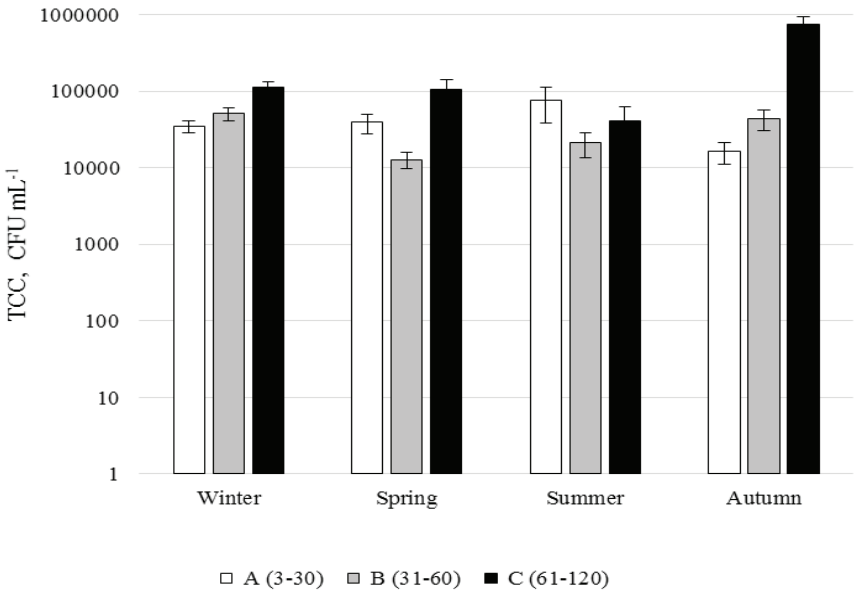

Figure 2. The mean number of total colony count in raw milk samples depending on season and herd size: cows in shed □ 3-30 ▒ 31-60 ■ 61-124.

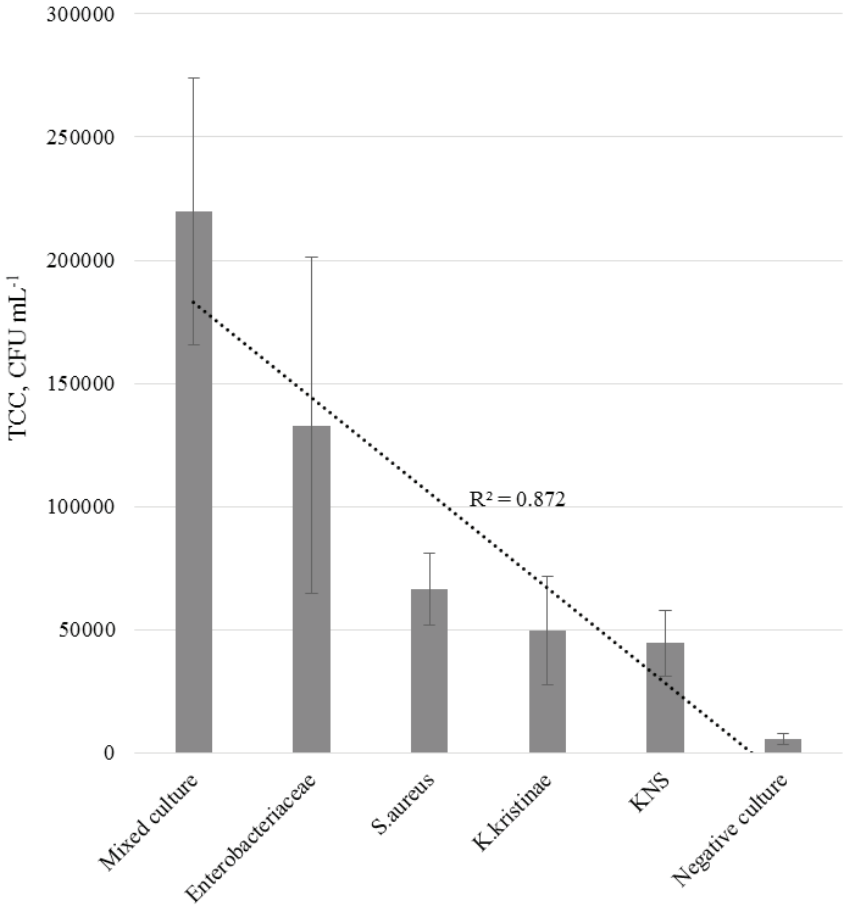

Figure 3. The mean number of total colony count in raw milk samples depending on bacterial agent.

in milk samples with negative bacterial culture when culturing was performed on blood agar and selective mediums.

The highest TCC is associated with mixed culture, substantially lower - with *Enterobacteriaceae spp*., but the major mastitis pathogens - *S. aureus* and *K. kristinae* together with CoNS - creates further reduced increase of TCC in raw milk.

In literature there is also data on the seasonal impact on the prevalence of certain bacteria species in cow milk. O. Osteras with colleagues refer to the highest prevalence of *Streptococcus dysgalactiae* and CoNS during April and May (late indoor season), and the highest prevalence of *S. aureus* and *Streptococcus uberis* - during June and July (the outdoor season) (Osteras et al., 2006). M. Zucali et al. (2011) report that occurrence of CoPS is higher in cold season in comparison with the other staphylococcus. Occurrence of *E. coli* is higher in summer than in winter (Olde Riekerink, 2007).

In this study, analysing the most widely spread bacterial groups we found that *Aerococcus spp.* occurrence is high in spring (n=20), but it was almost absent in samples of other seasons (n=1 in autumn). *Bacillus spp.* is most distributed in winter (n=12) milk samples, less so in other seasons (n=5 in spring, n=3 in summer and winter) and this observation is confirmed by other investigations – A.D. Sutherland and R. Murdoch, who report that the occurrence of mesophilic *Bacillus spp.* was the highest in the winter and lowest in the summer/autumn while psychrotrophic baccili occurrence was conversely lowest in the winter and highest in the late summer/ autumn (Sutherland and Murdoch, 1994). The most of our isolated *Bacillus spp.* belong to mesophilic species (*B. brevis, B. licheniformis, B. subtilis)* while *B. cereus* is psychrotrophic one. Psychrotrophic microorganisms, from a food spoilage perspective, are the most important organisms present in dairy products because they cause unpasteurized milk spoilage during its storage in the refrigerator (Champagne et al., 1994). *Enterobacteriaceae spp.* prevalence is the highest in spring and autumn (n=21 and n=12), less frequent in summer (n=12) and winter (n=4). CoNS are less distributed in raw milk in winter (n=23) than following seasons (n=42 in spring, n=43 in summer,

n=58 in autumn), but prevalence of CoPS is higher in cold season (n=9 in cold season toward n=5 in hot season) – these observations are in line with the investigations of the O. Osteras group (Osteras et al., 2006). *Serratia spp.* are detected only in winter (n=11) and summer (n=6). Prevalence of *K. kristinae* is lower in autumn (n=7) than in the following seasons (n=25 in winter, n=15 in spring, n=26 in summer).

The quality of milk can be estimated by the count of somatic cells (SCC), the total colony count of mesophilic aerobic and facultative anaerobic microorganisms, coliforms and *S. aureus* (Nikolajeva, 2011). In literature there is some data about the impact of the herd size on SCC, but it is hard to find out any findings how the herd size affects the bacterial diversity in milk. Therefore, in our study we made a short comparison of milk quality with other published investigations based on the total colony count toward SCC: several researchers have found out that the herd size has a significant impact on the quality of milk (Allore et al., 1997; Khaitsa et al., 1999; Ely et al., 2003). H.G. Allore et al. observed twice as frequent, elevated (SCC > 500,000 mL^{-1}) somatic cell count in herds with fewer than 27 lactating cows, compared to herds with > 62 cows (Allore et al., 1997), whereas L.O. Ely et al. report that herds with more than 449 cows had lower SCC than other smaller herds (Ely et al., 2003).

In this study, the average number of TTC was the lowest in B (4.50 ± 3.94 $\log_{10}$ CFU mL^{-1}), moderately higher in A (4.62 ± 4.17 $\log_{10}$ CFU mL^{-1}) and the highest in group C herds (5.40 ± 4.84 $\log_{10}$ CFU mL^{-1}). Significantly higher ($p<0.05$) TCC was in the milk samples taken in autumn (5.43 ± 4.80 $\log_{10}$ CFU mL^{-1}).

Coliforms from *Enterobacteriaceae spp*. were not isolated from group A herds instead of B (3.1%) and C (4.6%). Prevalence of *S.aureus* was noticeably higher in B (19.1%) and C (20.8%) than in group A (12.6%) herds. Percentage is calculated for occurrence of bacteria in each herd group during the year.

Conclusions

1. Bacterial growth occurred in 90.4% of examined milk samples. Isolated microorganisms belong to 35 species. Coagulase negative staphylococci, *Staphylococcus aureus, Kocuria kristinae* and *Enterobacteriaceae spp.* were the most prevalent agents and were isolated in 166 (29.4%), 136 (24.1%), 73 (12.9%), and 58 (10.3%) out of 564 raw milk samples, respectively.
2. Depending on season, the average number of total colony count was the lowest in summer (4.66 ± 4.01 $\log_{10}$ CFU mL^{-1}), moderately higher in spring and winter (4.72 ± 4.18 and 4.82 ± 3.54 $\log_{10}$ CFU mL^{-1}, respectively). Significantly higher the total colony count was in milk samples taken in autumn (5.43 ± 4.80 $\log_{10}$ CFU mL^{-1}) from large (C) herds.
3. Depending on the herd size, the average number of total colony count was the lowest in medium (B) (4.50 ± 3.94 $\log_{10}$ CFU mL^{-1}), moderately higher in small (A) (4.62 ± 4.17 $\log_{10}$ CFU mL^{-1}) and the highest in large (C) herds (5.40 ± 4.84 $\log_{10}$ CFU mL^{-1}).
4. Coliforms were not isolated from small herds instead of medium (3.1%) and large (4.6%) herds. Occurrence of *S.aureus* was noticeably higher in medium size (19.1%) and large size herds (20.8%) than in small herds (12.6%).
5. Milk which is produced in small organic herds, meets the milk quality requirements more than milk from large herds, taking into account the following milk quality indicators: the total colony count, presence of coliforms and presence of *S. aureus*.

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JERUSALEM ARTICHOKE FLOUR FEEDING EFFECTS ON CALF DEVELOPMENT IN THE FIRST MONTHS OF LIFE

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Abstract
Studies have been conducted to find out the effect of the feeding of calves (*Bos Taurus*) with Jeruslem artichoke (*Helianthus tuberosus)* concentrate produced in Latvia containing the prebiotic – inulin. The study was performed on two groups of animals - a control group of 8 animals and test (prebiotic) group of 8 animals in summer (from June to August, 2013), and winter (from December, 2013 to February, 2014) in one of cow farm of Latvia, in the municipality of Bauska. Both groups were fed the whole milk, but the test group received additionally 12 g of Jerusalem artichoke powder (an average of 500 g kg^{-1} inulin) per day. The overall health status and physiological parameters (temperature, heartbeat and breathing frequency) of both animal groups before the study were of the normal range. After the experiment, we found out that the calves of the test group during both winter (one case) and summer seasons (seven cases), had fewer cases of diarrhea than the control (winter months four cases and summer months nine cases) group animals, the average daily weight gain (control group 0.53 g, prebiotic group 0.75 g) and the total weight gain (control group 29.42 kg, prebiotic group 42.13 kg) during 56 test days was significantly higher ($p<0.05$) than that for the control animals. We concluded that the use of Jerusalem artichoke flour concentrate when fed to the calves generally gives positive impact on the development and growth of the calves, improves the status of the gastrointestinal tract and the morphometric indicators.
Key words: calves, Jerusalem artichoke, inulin, weight growth.

Introduction

During recent years, dairy farmers worldwide have been looking for ways to improve the management techniques and find products allowing to increase the productivity of animals, at the same time improving animal welfare conditions and keeping them in a good health status. For decades antibiotics were used as feed additives to improve animal welfare and to obtain economic benefits. The European Union has introduced a ban on antibiotics as growth promoters in animal feed, from January 1, 2006 (Verdonk et al., 2005), but it is still possible to use antibiotics for the prevention of diseases. It becomes more and more obvious that the use of antibiotics in animal husbandry has to be significantly reduced because of the antimicrobial resistance. Direct animal-human contact or the contaminated food is the possible transmission route for the resistant bacteria between the animal and human populations (Mathur and Singh, 2005).

Therefore now it becomes more important than ever to find alternatives in order to keep a good animal health status, reduce illnesses, mortality, and continue to reach a high weight gain without using so many antibiotics. One of the potential alternatives for replacing antibiotics would be the supplementation of animal diets with prebiotics (Samanta et al., 2013). „Prebiotics" came into light only recently and were coined by G.R. Gibson and M.B. Roberfroid (1995). Prebiotics are carbohydrates (polysaccharides and oligosaccharides) and they may be defined as non-digestible food ingredients that stimulate the growth and/or activity of microorganisms in the digestive system and exert antagonism to *Salmonella sp*. and *Escherichia coli*, limiting their proliferation (Krol, 2011; Patel and Goyal, 2012). The possible potential positive effect of the use of prebiotics in the animal feeds was recognized already in the 1980s (Verdonk et al., 2005*)*. Since then, studies of the effects of the use of prebiotics in feed are still actual and will continue to be (Gaggìa et al., 2010; Grand et al., 2013). So J.G.M. Houdjik et al. (1998) has documented the use of prebiotics in diets for piglets, E. Flickinger et al. (2003) used prebiotics for pet animals, chicken (*Gallus gallus domesticus*), rabbits (*Oryctolagus cuniculus*) and pigs (*Sus domesticus*). With calves (*Bos Taurus*) most of the studies were carried out using prebiotics (Quezada-Mendoza et al., 2011) - mannaoligosaccharides (Krol, 2011) and fructooligosaccharides like inulin (Heinrichs et al., 2009; Masanetz et al., 2011). Inulin is a natural constituent of a wide range of plants and vegetables (Van Loo, 2007). Such plants as Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Cichorium intybus)* are rich in inulin. They have been used for industrial extraction of inulin already for a long time (Fleming et al., 1979). There are several studies performed on a single- stomach animals and birds feeding them inulin derived from Jerusalem artichoke which show the positive effect of inulin on growth and health of those animals (Kleessen et al., 2003; Valdovska et al., 2012). Nevertheless, there have been only limited studies to define the effects of the feeding of inulin to the calves. The aim of this study is to determine weather there is an effect on health condition, average daily gain and morfofunctional development of the gastrointestinal tract in calves

when feeding them for the first four months after the birth with the Jerusalem artichoke concentrate (inulin 485 g kg^{-1} – 501 g kg^{-1}) produced in Latvia.

Materials and Methods

The trial was carried out in two stages in one of the cow farm of Latvia in the municipality of Bauska. The first stage was performed in summer (from June to August, 2013), the second - in winter (from December, 2013 to February, 2014).

First after the birth calves were fed 2 liters of a fresh mother's colostrum by nipple bottle for 3 consequent days (2-3 times per day). The first feeding was started not later than 30 minutes after the birth of the calves. After 3 days they were switched to the whole bulk milk up to 6 L per day depending on the calve's age.

The actual trial started when the calves were 4 weeks old and has been stopped 56 days later, and then a planned slaughter of animals was performed.

The research groups consisted of randomly selected animals. At the beginning of the study all calves were examined and fsllowing data was collected: heart rate, respiratory rate, body temperature, fecal score and *habitus*. About 23 ± 5 days old healthy male calves with the initial body weight of 50 ± 5.0 kg were grouped. During the research stage in summer calves were kept in calf houses with separated pens with natural ventilation. The total area of each pen for 4 calves was 7.5 m^2. During the second stage in winter the calves were kept in individual pens. Pens were cleaned manually one time per day.

During the entire research the calves were given up to 3 L whole milk 2 times a day and had free access to meal feed, hay and fresh water. Two weeks after the start of the trial calves additionally had free access to a fodder, made on the farm from wheat flour, which did not contain any antibiotics, prebiotics, probiotics or chemotherapeutics.

Fodder chemical composition is shown in Table 1.

Sixteen crossbreed calves were split into groups: 8 calves for summer time stage [summer control (SC), n=4; summer prebiotic (SP), n=4] and 8 - for winter time stage [winter control (WC), n=4; winter prebiotic (WP), n=4]. The whole milk, hay and fodder used to feed the experimental, as well as the control calves had the same nutrition value and quality. The only difference was that the milk feed for prebiotic group calves contained prebiotics. All SP and WP group animals were fed 12 g of Jerusalem artichoke concentrate with inulin averagely 500 g kg^{-1}, the composition of the concentrate is given in Table 1.

Daily fecal scoring was done according to Larson's scale, where 0 – normal, firm but not hard; 1- soft, does not hold form, piles but spreads slightly; 2 – runny, spreads readily to about 6 mm depth; 3 – watery, liquid consistency, splatters (Larson et al., 1977).

Body weight, heart rate, respiratory rate and temperature were measured every two weeks. The weight was determined by special measuring tape (we-Bo tape) by measuring calf heart girth behind the front legs. Body temperature was measured rectally with rectal digital thermometer, designed for veterinary use. The heartbeat was measured by placing phonendoscope at the left side of costal cavity and counted heart beats per minute.

The first stage was performed in summer from June to August, 2013, when the environmental temperatures ranged from +10 °C to + 29 °C degrees, second - in winter from December 2013 to February 2014 when environmental temperatures ranged from +7 °C to -15 °C degrees.

The animals were slaughtered after 56 days. Immediately after slaughtering, the gastrointestinal tract was removed from the carcass. The following morphometrical measurements were taken: the total weight of the gastrointestinal tract, the weight of

Table 1

Chemical composition of concentrated feed and Jerusalem artichokes flour for study animals

Flour name	Composition (g kg^{-1} Dry Matter basis)						Composition (g mg^{-1} Dry Matter basis)			
	Dry matter g kg^{-1}	CP	NDF	ADF	Strach	Inulin	Free glukouse	Free fructose	Sacch-arose	Nucleic Acids
Concen-trated feed	882	142	481	34	655	-	-	-	-	-
Jerusalem artichoke	948-956	171	-	-	628-645	485-501	8	26	106	21

CP - Crude protein
NDF - Neutral Detergent Fiber
ADF - Acid Detergent Fiber

the rumen without the feed masses, the weight of abomasum without the
feed masses, the length of the abomasum and rumen.
For statistical analysis the average ± standard error was calculated and a t- test for paired samples in *Microsoft Excel* and *R-Studio* programs was used. P-values less than 0.05 were considered to be statistically significant.

Results and Discussion

In this study, we wanted to determine the feeding effect of locally produced Jerusalem artichoke concentrate on the calf's body. Generally, Jerusalem artichoke flour contain on average 10 g kg^{-1} inulin, specially designed technology allows to increase the amount of inulin to 485 g kg^{-1} - 501 g kg^{-1} (Fleming and Groot Wassink, 1979; Valdovska et al., 2012) - so it is easier to add it to the feed material in our study – whole bulk milk.

The following table (Tab.2) shows that the heart rate, respiratory rate and the temperature of both groups were within physiological reference range. In this study two of the summer stage control group animals were under 7 days long antibiotic treatment, because one calf had purulent rhinitis. In general, calve's health was good.

When the respiratory rate was analyzed for the summer group calves separately from the winter group calves, there was a difference between a 4 weeks old calf ($p<0.01$) and the one of 6 and 12 weeks ($p<0.05$). WP and SP calf respiratory rate proved to be significantly ($p<0.01$) higher in 6 and 8 weeks old calf and ($p<0.05$) in 11 weeks old calf. In this study respiratory rate was higher during summer stage.

SP and SC group calves body temperature was higher than that of the WP and WC group calves. There is no statistically significant difference in temperature measurements between calves treated with prebiotics and the control group animals. The increase of temperature and the significantly increased respiratory rate in summer stage could be explained through higher environmental temperature (+22 °C). It is well known that an increase in environmental temperature may increase the animal's body temperature.

It is possible to read in literature about an increased heart rate of a calf at an early age, which decreases when calf becomes older (Westervelt et al., 1979). In this study it was not observed. It could be explained through the constant inhibitory effect of *n. vagus* center on heart rate when the calf is 4 weeks old. Increased heart rate was detected in all animals when calves were 12 weeks old and we measured the heart rate in the slaughter house. The increase of heart rate in a stressful situation has been reported by other authors (Westervelt et al., 1979; Mohr et al., 2002).

There were no records of hard watery diarrhea (3 marks) in any group.

During the first week of the study (calves were five to six weeks of age) fecal score in SC and SP group was mostly soft or almost soft (average of 0.75 and 0.50 marks; Fig. 1). At seven weeks of age two WC group calves were detected with watery feces (averagely 1.63 marks), it might refer to a disfunction of gastrointestinal tract. At the age of seven weeks fodder was started to be fed to the calves and it could make impact on the gastrointestinal tract. Next week all the SC group animals were observed soft or watery fecal masses at an average of 1.7 marks, meanwhile

Table 2

Physiological fundamentals of the control and prebiotic groups of animals during winter and summer

Groups, old week	Respiratory rate (breaths min^{-1})		Heart rate (beats min^{-1})		Temperature (°C)	
	Summer	Winter	summer	winter	summer	Winter
Control						
4 week	36 ± 3.3	24 ± 0.01	104 ± 7.3	105 ± 5.8	38.9 ± 0.31	38.8 ± 0.27
6 week	33 ± 2.0	29 ± 1.9	111 ± 11.4	113 ± 1.6	39.1 ± 0.61	38.8 ± 0.49
8 week	31 ± 3.9	28 ± 5.6	117 ± 6.8	113 ± 1.6	39.2 ± 1.00	38.7 ± 0.36
10 week	30 ± 5.2	28 ± 3.3	117 ± 3.8	125 ± 1.6	39.1 ± 0.38	39.4 ± 0.23
12 week	33 ± 2.0	29 ± 1.9	130 ± 5.2	141 ± 3.3	38.9 ± 0.41	39.0 ± 0.67
Prebiotic						
4 week	33 ± 3.8	29 ± 4.3	113 ± 3.8	121 ± 3.7	38.5 ± 0.4	38.4 ± 0.19
6 week	32 ± 3.3	25 ± 1.6	119 ± 6.0	110 ± 6.8	39.1 ± 1.12	38.4 ± 0.22
8 week	33 ± 2.0	26 ± 1.7	123 ± 5.1	117 ± 4.9	38.8 ± 0.43	39.1 ± 0.07
10 week	32 ± 3.3	32 ± 2.8	111 ± 5.1	116 ± 6.5	38.7 ± 0.24	38.3 ± 0.22
12 week	36 ± 3.3	28 ± 2.8	136 ± 3.7	146 ± 5.0	38.7 ± 0.14	38.3 ± 0.07
Reference limits (Mohra et al., 2002)	15 – 40			86 - 125	38.0 – 39.5	

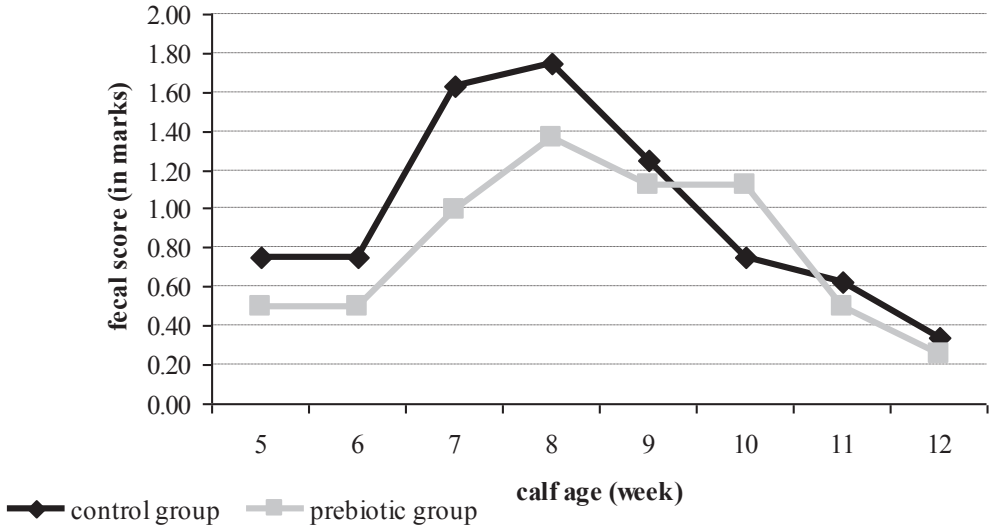

Figure 1. Calf fecal score (summer groups).

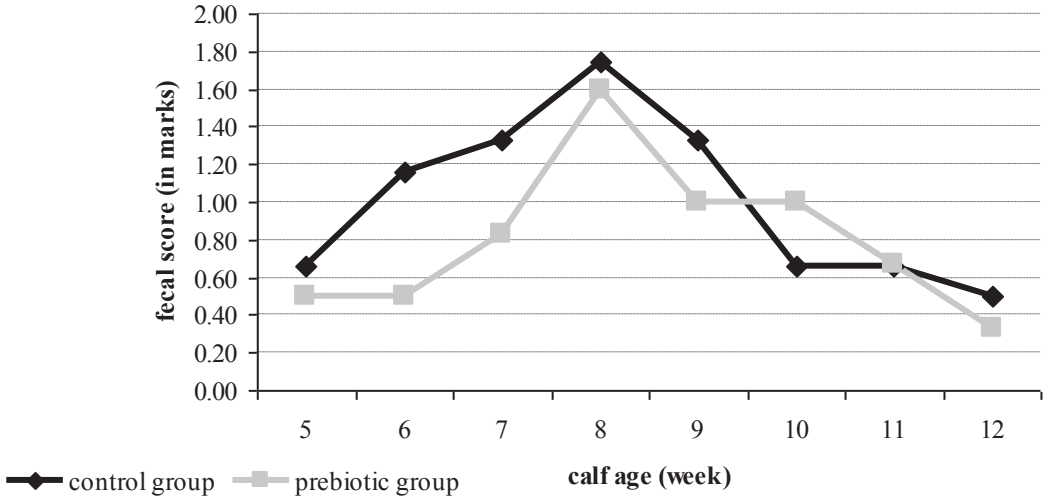

Figure 2. Calf fecal score (winter groups).

the fecal masses of the SP group animals were normal or soft at an average of 1.3 marks. Starting from the 10th week of life the consistency of feces in all animals became more rigid and at 12 weeks of age in the SC group it was 0.36 marks, but in the SP group 0.29 marks (Fig. 1). In this study we observed that at 11-12 weeks of age calves begin to eat more hay than before and this could stabilize the digestive processes.

This study shows that the SP group calves had less watery feces than those of the SC group - this could prove that feeding inulin might improve the function of gastrointestinal tract.

During the research winter stage the consistency of animal feces was normal (WP) or soft (WC), but at 6 weeks of age the fecal score of the WP group animals was on average 0.5 but of the WC group 0.67 marks (Fig. 2). The fecal score of seven and eight weeks old calf of the WC group was on average from 1.33 to 1.83, but the fecal score of the WP group animals was from 0.83 to 1.66 - it means soft. The calves of both groups at an age of 11 and 12 weeks had normal or soft fecal consistency, but at 12 weeks of age the WC group animals had 0.5 marks and the WP group animals 0.33 marks.

In summary we could conclude that the prebiotic group animals during both winter and summer stages had less watery feces than the control group animals (Fig. 1 and 2). In the middle of the study (at 7, 8 and 9 weeks of life), when animals were offered new feed materials (fodder and hay) the fecal masses became softer and the incidence of observed diarrhea increased. The calves additionally fed with

Table 3

Calf total live weight growth dynamics

Groups	Average weight of the animal (kg) at the research day			Live weight gains (kg) at the time period (day)			Average daily body weight gain (kg)
	1	28	56	1-14	1-28	1-56	
Control	53.6±5.71	71.9±10.38	83.0±11.18	7.9±5.36	18.3±4.67	29.4±5.47	0.53±0.183
Prebiotic	54.3±3.28	75.4±10.15	96.4±11.50	10.0±4.00	21.1±6.87	42.1±8.21	0.75±0.201

Table 4

Calf gastrointestinal growth performance

Groups	Average cold carcass weight (kg)	Average total mass of gastrointestinal tract (kg)	Rumen mass		Abomasum mass	
			empty (kg)	relative (%)	empty (kg)	relative (%)
Control	43.57±8.16	14.20±1.49	1.14±0.26	2.61±0.74	0.55±0.10	1.25±0.10
Prebiotic	49.14±8.07	14.44±1.57	1.29±0.32	2.62±0.80	0.62±0.11	1.27±0.15

prebiotics showed a relatively stable gastrointestinal tract organ activity at this age, especially the WP group.

Starting from the 10th week of life, fecal consistency becomes more rigid, which could be explained through the development of a stable ruminant digestive system which continues up to 11 weeks of life. Similar results were also obtained by other authors, who found the gastrointestinal tract stabilization after feeding prebiotics (Flickinger et al., 2003; Heinrichs et al., 2009; Król, 2011; Grand et al., 2013).

Hereafter we analyzed the growth rate and the development of the gastrointestinal tract organs of the control and the prebiotic group calves. Since there was no significant difference between the body weight gain at the summer and winter stages, we analyzed both stages together.

Comparing the results of the growth dynamics of the live weight of the calves from the first research day to 56 days, we found that the weight gain of the calves in 56 days additionally fed with inulin was significantly higher ($p<0.05$) than in the control group animals (Tab. 3.) The milk added inulin increased the relative cold carcass weight by 6% compared to the control group (Tab. 4).

During the first rearing period (0-28 study days) the relative body weight gain of the prebiotic group animals was by 5% higher but during the 0-56 study day period even by 23% higher than of the control group animals. N. Stolić et al., (2012), who carried out the research feeding prebiotics (mannan-oligosaccharides) to the calves, got similar results.

In all stages of the research the weight of the prebiotic group calves was higher than to the control animals.

The average daily weight gain of a prebiotic group animal was by 0.277 kg higher compared with the control group and this difference was statistically significant ($p<0.05$).

For the prebiotic group calves, all indicators show a slightly better developed gastrointestinal tract than in control animals, but these differences did not prove significant ($p>0.05$). We can conclude that the additional feeding with inulin can accelerate the development of the gastrointestinal tract.

Since the prebiotics are known to be active mainly in the intestinal canal, rather than in the stomach, it could be the main reason why the changes in gastric morphometric measurements are not significant. Research in this area should be continued.

This is only the first step of the study showing the feeding effects of the prebiotic inulin on the calf health and weight gain. The study is certainly significant and persuaded us to continue research on locally produced Jerusalem artichoke flour concentrate usage for this purpose.

Conclusions

1. The additional feeding of the Jerusalem artichoke flour containing prebiotic inulin to the calves at 4-12 weeks of age improves the gastrointestinal functional capability by reducing the incidence of diarrhea after new feed materials both in winter and summer periods.

2. The animals additionally fed with inulin at the age of 12 weeks showed significantly higher ($p<0.05$) live weight gain (control group on average 83.00 kg, prebiotic group 96.43 kg) and significantly higher average daily weight gain ($p<0.05$) the control group 0.53 g, but prebiotc group 0.75 g.The carcass weight was by 5.72% higher than of the control group animals.
3. The study shows that the Jerusalem artichoke flour concentrate feeding generally improves the gastrointestinal morphometrical indicators thereby showing the positive impact of prebiotic inulin on the gastrointestinal tract development and growth.

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STAPHYLOCOCCUS SPECIES IN DIFFERENT AGE GROUPS OF PIGS IN LATVIA

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Abstract
Antibiotic resistance of *Staphylococcus* is increasing worldwide. New antibiotics are used in big amounts in the meat production more and more. As a zoonosis, *Staphylococcus aureus (S. aureus)* is found in various species of animals and people, especially in those, who are working on commercial swine farms and slaughterhouses. The aim of the study was to find out the occurrence of *S. aure*us in different age groups of pigs on commercial swine farms in Latvia. Microbiological samples (n=702) including nasal, rectal, milk and air samples were collected during October 2013 from three closed farms of different sizes and several age groups of pigs and investigated with microbiological standard methods. *S. aureus* was found in all swine farms. The occurrence of *S. aureus* in Latvian pig farms was 41% and the highest occurrence of *S. aureus* was among 3-3.5 month old piglets. *S. aureus* was 1.8 times more frequently found in nasal than in rectal samples, and only in 28.38% of pigs *S. aureus* was in both - nasal and rectal samples. *S. aureus* was found also in sow milk in 13% of samples and in 3 samples of air (n=23).
Key words: *staphylococcus, S. aureus,* pig farms.

Introduction

*Staphylococcus aureu*s is an opportunistic pathogenic Gram positive bacteria and the causative agent of a wide panel of infections ranging from superficial lesions to life-threatening septicaemia (Charlier et al., 2009; Boucher et al., 2010). It is no longer a human healthcare-associated problem, but is now a community-associated problem (Morgan, 2008). Methicillin resistant *Staphylococcus aureus* (MRSA) were found to colonize and infect various animal species including horses, cows, dogs and cats, rabbits and other companion animals. A special focus has been put on the isolation of MRSA from pigs in several countries including the Netherlands, Denmark, France, Canada, the USA and Singapore. However, in the majority of reports pigs were colonized, but were not infected by MRSA (Kock et al., 2009). According to the data of European Food Safety Authority (EFSA), in 2009 MRSA CC398 (MRSA genome type that is common in animals and people) was found in 39% percent of pigs in Dutch slaughterhouses and in 13% of fattening pigs in Germany, and one of the most frequent hosts of this MRSA CC398 type are pigs.

Currently, *S. aureus* occurrence and antibiotic resistance in the world increases and with it also the concern about *Staphylococcus aureus* and MRSA colonization in swine farms in Europe and spreading from animal to human. Besides all European country data about the prevalence of *S. aureus* in pigs, no investigations have been made in the situation of the Latvian pork industry. Therefore, the aim of the study was to find out the occurrence of *S. aureus* in commercial swine farms in Latvia.

Materials and Methods

The collection of microbial samples took place in October 2013 in three pig farms from three different regions of Latvia – Kurzeme, Vidzeme and Riga. The description of each pig farm is given in Table 1. The body condition of swine was scored according to Stockmanship standards (Carr, 1998). Evaluation of animal welfare, hygiene, and microclimate conditions in the pig farms was based on Council Directive 2008/120/EC of 18 December 2008, laying down minimum standards for the protection of pigs and microclimate standards according to M. Muirhead suggestions (Muirhead et al., 2013).

Sampling

Pigs were divided into four groups: pre-weaned piglets with sows, 3-3.5 months old piglets, 4-4.5 months old piglets and fattening pigs (shortly before slaughter) (see Table 2). Nasal and rectal samples were collected from each group. Also, from each farm milk samples (n=69) and air samples (n=23) were collected. In total, 305 pigs and 702 microbiological samples were investigated. Nasal and rectal samples were collected with sterile transport swabs (Meus, IT). Milk samples were collected in 50 mL amount sterile tubes without preservative. Air samples were collected using Baird-Parker Agar plates according to Koch's sedimentation method (Boucher et al., 2010). All microbiological samples were stored at +4 °C and the first isolation was made during 24 hours after the sample collection.

Microbiological examination

The research was performed at the Institute of Food and Environmental Hygiene of Latvia University of Agriculture Faculty of Veterinary Medicine. Samples from transport swabs were transferred on Baird-Parker Agar with egg yolk supplement (Becton, Dickinson, USA), and incubated at 37 °C for 24 hours according to LVS EN ISO 6888-1:1999 A1:2003 'Microbiology

Table 1

Characterisation of pig farms

Pig farm	Number of sows	Number of fattening pigs	Health conditions	Hygiene and welfare conditions	Antibiotic usage
A	250	1500	Somewhat thin (score 2.5) and thin sows (score 2), reduced fertility and birth rate, cannibalism	Some dirty cages and pens, slatted floors, no available straw and environment enrichment	For treatment
B	1200	8000	Good health and condition (score 3.5)	Dirty and wet pens of fattening pig groups, slatted floors, concrete solid floor with straw in 4-4.5 month old group.	For treatment and prophylaxis
C	20000	12000	Scars and purulent lesions on joints and phalanx, weak and thin (score 2) piglets, sows in normal condition (score 3), cannibalism.	All pens dirty and wet, no available straw and environment enrichment, too cold for piglets (24 °C in newborn piglet pens), slatted floors.	For treatment

Table 2

A number of investigated pigs, milk and air samples in each farm

Group of pigs/sample type	Number of investigated pigs/samples		
	Farm A	Farm B	Farm C
Pre-weaned piglets with sows	32	32	32
3-3.5 month old piglets	15	25	24
4-4.5 month old piglets	24	24	24
Fattening pigs	25	24	24
Milk	18	25	26
Air	6	9	8

S. aureus and other species - Part 1: Technique using Baird-Parker agar medium - Amendment 1: Inclusion of precision data'. After incubation, positive colonies were inoculated on Mannitol Salt Agar plates (Biolife, IT) at 37 °C for 24 hours. Rabbit Coagulase plasma (Becton Dickinson, USA) slide coagulase test was made with colonies from Mannitol Salt Agar plates. Coagulase positive colonies with positive reaction on MSA plates were determined as *S. aureus*-like and were inoculated on CHROMagar Staph aureus plate (Becton Dickinson, USA) at 37 °C for 24 hours. Isolates were confirmed to be *S. aureus* by examining previous tests. Samples were categorised positive, if at least one *S. aureus* positive colony-forming unit was isolated.

Results and Discussion

The Genus *Staphylococcus* consists of a variety of opportunistic pathogens of variable relevance in veterinary medicine. The most clinically relevant staphylococcus in veterinary medicine is the coagulase positive Staphylococcus aureus. A noted property of staphylococci is their ability to become resistant to antimicrobials (Weese and Duijkeren, 2009; Brown et al., 2005).

Staphylococcus aureus is an important cause of food poisoning, pneumonia, wound and nosocomial bacteremia. It is one of the natural components of microflora and may exist in environment, on skin and in mucus. Most animals may be colonized with *S. aureus*, but only recently MRSA strains were isolated from several food production animals, including pigs, cattle, chicken and other animals (Boucher et al., 2009; Weese and Duijkeren, 2009).

In our research, *S. aureus* was found in all three pig farms. Results showed that 41% of tested pigs were *S. aureus* positive. *S. aureus* was found in 34% of nasal samples and in 19% (Figure 1) of rectal samples; in addition, in 28.4% of pigs *S. aureus* was found in both – nasal and rectal samples, but in 19.8% of cases - only in nasal samples and in 5.9% of cases - in rectal samples.

Hypothetically our investigation is similar to Belgian MRSA research (Dewaele et al., 2013), because MRSA come from *S. aureus* species isolates and in Belgian research the highest sensitivity to

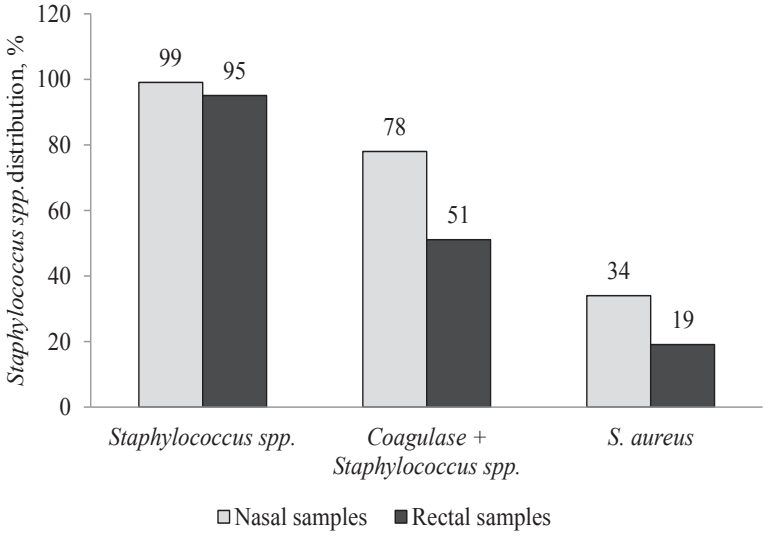

Figure 1. Comparison of *Staphylococcus spp.* distribution in nasal and rectal samples from pigs %.

determine MRSA carriage was found in the samples taken from nares 0.83, but from rectum - only 0.47. In I. Dewaele study, the best combination for sample taking is nares and perineum (sensitivity 0.96), nares and skin (sensitivity 0.92) and nares and rectum (sensitivity 0.89).

Housing conditions of fattening pigs have considerably changed in the past decades from extensive systems with large space allowance, substrate and/or outdoor housing, to intensive husbandry systems that have been developed for large scale production of pork. In these intensive husbandry systems, fattening pigs are housed with high housing density and without substrate. However, the intensive husbandry systems cause welfare problems for pigs. The main reason of these welfare problems is that the intensive housing conditions do not fulfil the internal need of the pig to perform species-typical behaviour. Pigs housed in barren environments show more abnormal agonistic behaviour, more manipulative social behaviour and have a higher level of aggression than pigs housed in pens with straw bedding, and it was concluded that these behaviours indicate welfare problems (Beattie et al., 1995; Jonge De et al., 1996). Stress caused by housing and management of pigs may not only affect animal welfare, but also the acceptance of the product by the consumer and the productivity. For example, stress caused by mixing of unfamiliar pigs reduced the growth rate for weeks (Ekkel, 1996).

This study has found that there is a difference between *S. aureus* occurrence in pig farms (see Figure 2). *Staphylococcus spp.* and coagulase positive

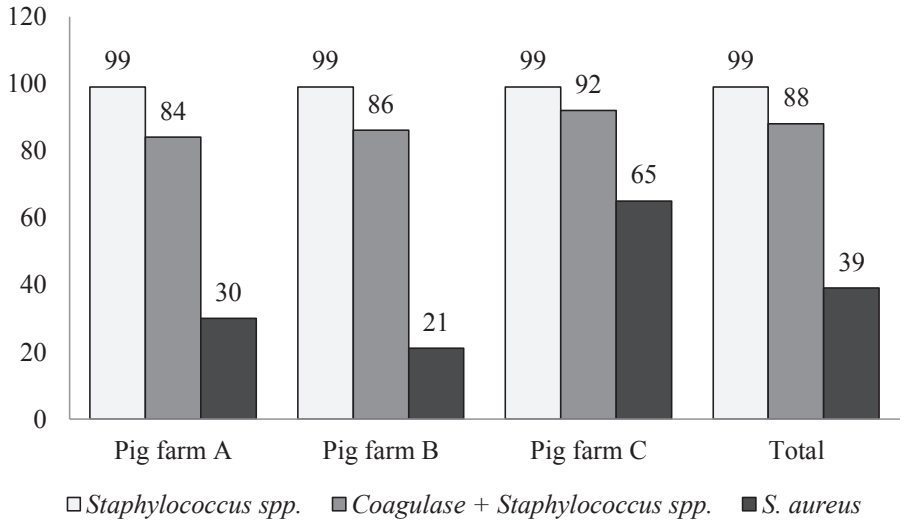

Figure 2. *Staphylococcus spp*. distribution in pig farms %.

staphylococcus as environmental microorganisms were highly spread in all three pig farms (in total, S*taphylococcus spp. w*ere found in 99% of nasal samples but in 78% of nasal samples – coagulase positive staphylococcus), but *S. aureus* was three times less spread in the pig Farm B. One of the reasons, that explains these rates, is antibiotic usage for prophylactic purposes in Farm B, but in Farms A and C - only for treatment. In addition, conditions of animal welfare and hygiene were best in Farm B. *S. aureus* occurrence was highest in the pig Farm C, where pig breeding, animal welfare and hygiene conditions were worst. In this farm, despite our country regulations and Council Directive 2008/120, sows were kept all the time in cages (during gestation, artificial insemination and gestation period) and in farrowing crates during piglet suckling period. There was no strain available for pigs in this farm. In addition, in pig Farm C pigs were more stressful than in Farm A and C and cannibalism signs were seen (bite wounds on ears, ducks, and neck).

Weaning and forming of new groups is a stressful time for all species of animals and can result in negative effects on the neonate after weaning. Stressful events such as weaning, forming of new groups and transportation can weaken immune function (Hickey et al., 2003) and reduce growth rates. During this time, piglets are also more susceptible to illness due to a compromised immune system and insufficient nutrient intake (Kuller et al., 2004).

In our research the high occurrence of *S. aureus* (see Figure 3) was seen in 4 - 4.5 and 3 - 3.5 months old piglet groups, and in Farm C in these groups the occurrence of *S. aureus* is 28-37%, - higher than in pre-weaned group. However, other researchers (Smith et al., 2009) have found a trend that MRSA occurrence decreases when pigs from young piglets reach adult age.

In our research, we observed a tendency, that *S. aureus* distribution in pig farms in different age groups mostly differ because of animal welfare and hygiene conditions. In 3 - 3.5 months old piglet group, *S. aureus* occurrence is one of the highest (92% in Farm C and 63% in Farm A). During that period in all farms pigs were transported from the piglet barn to pig fattening barn. In Farm B in the age group of 3 - 3.5 months old piglets, *S. aureus* were not found. In Farm B that group was provided with strain and other environment enrichment things, consequently, stress signs were low and cannibalism signs were no evident. In Farm B pre-weaned piglets got antibiotics less than other groups, and therefore *S. aureus* occurrence was the highest there. Nevertheless, *S. aureus* occurs despite the usage of antibiotics in a non-stop regime in low dosages.

To the authors' knowledge, this is the first examination of *S. aureus* occurrence in sows' milk in Latvia. We found *S. aureus* in 13% samples of milk, usually in sows with signs of mastitis, and that is one of the ways how a sow can infect newborn piglets, therefore not always, when sows were infected with *S. aureus,* the microorganism appeared in milk. Comparing our data to other researchers' data about dairy cow herds (Sommerhauser et al., 2003), where occurrence of *S. aureus* in milk samples varied from 9-27% depending on herd, our data includes in this interval one of lowest rates and it shows, that *S. aureus* occurrence in sows' milk is similar to occurrence of S. *aureus* in cows' milk.

S. aureus was found only in 3 of 23 air samples in pig groups with the highest *S. aureus* occurrence,

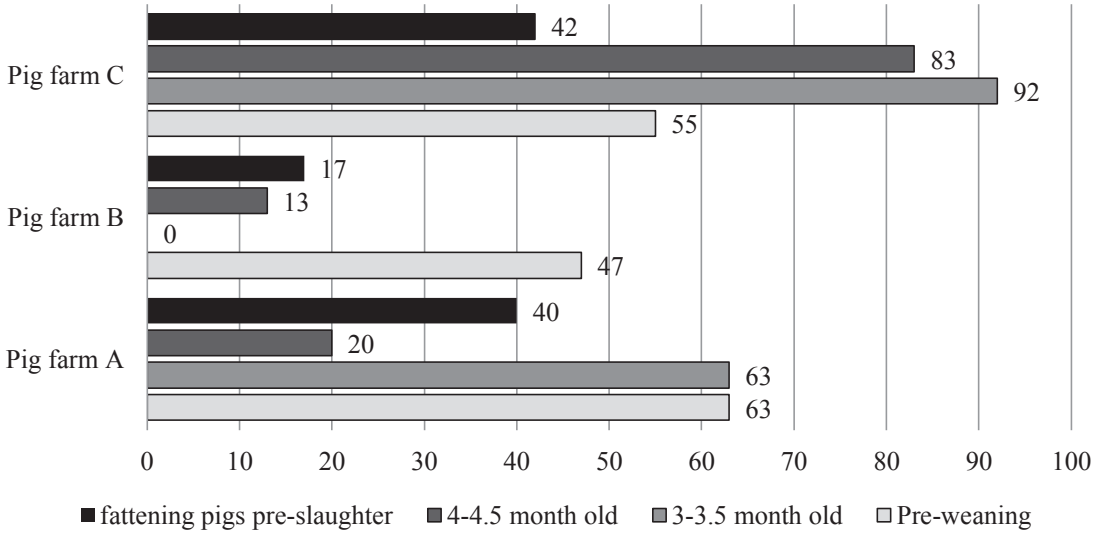

Figure 3. *S. aureus* distribution % in different pig age groups in pig farms.

but in other researchers' investigation MRSA was found in 21 samples of 24 (Schulz et al., 2011) and in 23 samples of 27 (Friese et al., 2011). In J. Schulz et al. research occurrence of MRSA in the investigated farms in pig nasal samples was 80%, but in our study *S. aureus* occurrence was only 34%. That shows a tendency: the higher the occurrence of microorganism in the herd population, the more spread it is in the environment, including air.

Further investigations are necessary to find MRSA in the isolated *S. aureus*.

Conclusions

1. Occurrence of *S. aureus* in the Latvian pig farms was 41% and the highest occurrence of *S. aureus* was among 3-3.5 months old piglets.
2. *S. aureus* was 1.8 times more frequently found in nasal than in rectal samples, and only in 28.4% of pigs *S. aureus* was found in both - nasal and rectal samples.
3. *S. aureus* was found in sows' milk and air in 13% of samples.

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ALARIA SPP. EPIZOOTIOLOGICAL SITUATION IN WILD BOAR IN LATVIA

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Abstract
During the controls for *Trichinella* in wild boar meat, *Alaria spp*. mesocercariae in the examined samples are diagnosed. There does not exist a routine veterinary inspection for diagnosis of alariosis (*Alaria spp*.) in Latvia. The aim of the study was to determine *Alaria spp*. mesocercariae distribution in wild boar (*Sus scrofa*) meat in Latvia. Laboratory examination was performed in Latvia University of Agriculture Faculty of Veterinary Medicine laboratory of Parasitology, from 2010 to 2013. Meat samples were examined using artificial digestion method, which in regulation No 2075/2005 is considered an official detection method for *Trichinella*. In total, 1233 wild boar meat samples from different hunting regions of Latvia were examined. The territory of Latvia was divided into four regions – Kurzeme, Zemgale, Vidzeme and Latgale. For examination we used meat samples from wild boar pillars of diaphragm. Samples were taken from wild boars of different age and gender. The prevalence of infection in the examined wild boar meat samples from all regions was 8.2%, but the intensity of infection 2.8.
Key words: *Alaria spp.*, alariosis, wild boar, prevalence of infection, intensity of infection.

Introduction

To provide consumers with appropriate food, official veterinary inspection is made in abattoirs and hunted wild animals. The most well known and actual parasitological disease, which potentially can invade humans is trichinellosis (*Trichinella*). The legislation of EU member states regulates the official meat inspection for *Trichinella spp*. detection. During the examination of wild boar meat for *Trichinella,* frequently *Alaria alata* mesocercariae are detected.

Alariosis is a widely distributed infection in the world, which in definitive hosts can reach prevalence of infection up to 95% (Paulsen et al., 2012). Fluke development occurs with three different hosts. Definitive hosts are animals of *Canidae* family (dog (*Canis lupus familiaris*), wolf (*Canis lupus*), red fox (*Vulpes vulpes*), raccoon dog (*Nyctereutes procyonoides*) and polar fox (*Vulpes lagopus*) etc.), and the second intermediate hosts - amphibians and their tadpoles (Riehn et al., 2012). Relevant part in *Alaria* development is played by paratenic hosts, which can be various animals from different classes – reptiles (*Reptilia*), birds (*Aves*) and mammals (*Mammalia*), for example, animals from orders of rodents (*Rodentia*), carnivores (*Carnivora*) and ungulates (*Artiodactyla*), possibly humans, too (Paulsen et al., 2012). Definitive hosts are invaded with *Alaria* by consuming both intermediate host and paratenic host (Акбаев и др., 1998). Definitive hosts with faeces excrete to the environment parasite eggs, which at appropriate external environmental temperature, 21 °C – 27 °C become infective in 11 to 12 days. At the beginning the first stage of larvae – miracidium develops in the egg. Miracidium hatches from the egg and thereafter penetrates freshwater snail. In the snail miracidium continues its development, transforming into the second stage of larvae – cercaria. Cercaria goes through the snail and by actively moving penetrates the second intermediate host – a frog or tadpole, where it develops into metacercariae (Möhl et al., 2009; Трусова, 2009). Carnivores get infected by consuming a frog or tadpole, but here is a possibility, that the intermediate host is consumed by omnivores instead of carnivores, for example, wild boars (*Sus scrofa*), moles (*Talpa europaea* (L.)), birds (*Aves*), mice (*Muridae*), thereby becoming a paratenic host. *Alaria* larvae – mesocercariae in the paratenic host, after digestion get free in the intestines, perforate intestinal wall and migrate to different parts of organism, mostly to subcutaneous tissue where localise and do not continue their development but keep sustained possibility to invade. *Alaria* mesocercariae accumulate in the paratenic host (Гладкова и др., 1970). As soon as such a paratenic host is eaten by the definitive host, *Alaria* will develop to a mature trematode and life cycle will be finished (Трусова, 2009). It should be noted that in the Institute of Food Safety, Animal Health and Environment – 'BIOR' a study was carried out about *Alaria alata* distribution in different host animals in Latvia – the study includes information about foxes, raccoon dogs and wild boar. This study shows that *Alaria alata* is prevalent in wild carnivores in Latvia which may be a potential source of environmental contamination (Esīte et al., 2012).

According to the Federal Institute for Risk Assessment in Germany (BfR), consumers may become invaded with *Alaria* by consuming inappropriately cooked wild boar meat. The symptoms and course of disease depends on species and amount of consumed larvae (Wild boar meat may contain the harmful *Alaria alata*, 2007). In carnivores and omnivores alariosis is characterised by digestive system disorders and myositis (stage of larvae). In the case of severe invasion, the following blood chemical parameter changes are observed – increased alkaline phosphatise, increased ALAT (alanine amino

transferase) and ASAT (aspartate amino transferase) concentration, decreased protein amount, as well as decreased water content in muscles (Трусова, 2009). Information about changes in blood chemical parameters in wild boars in Latvia is not available, and we also do not have any broader studies about alariosis epizootiological situation in wild boar population, therefore the objective of our study was to determine the epizootiological situation of *Alaria spp*. invasion in wild boars in Latvia.

Materials and Methods

The research was made in Latvia University of Agriculture Faculty of Veterinary Medicine Institute of Food and Environment laboratory of Parasitology, from year 2010 to 2013. Material for research and diagnosis in cooperation with hunting collectives was obtained from different regions of Latvia. The territory of Latvia was divided into four regions – Kurzeme, Zemgale, Vidzeme and Latgale. 241 samples were received from Kurzeme region, 916 samples from Zemgale, 71 from Vidzeme, and five samples from Latgale. Overall, 1233 samples of wild boar pillars of diaphragm were examined. Until examination the samples were stored at temperature + 2 °C - + 4 °C. All obtained samples, i.e. wild boar pillars of diaphragm, were examined using artificial digestion method, which in regulation No 2075/2005 is defined as an official method for *Trichinella* diagnosis. For examination we took 50 g large samples from pillars of diaphragm. We diagnosed *Alaria* on a light microscope in 10×10 magnification. Statistical data processing was carried out with "Microsoft Excel – 2010." We calculated the prevalence of infection % (IE) and intensity of infection (II), as well as t-test. The prevalence of infection was calculated using equation 1, the intensity of infection – equation 2.

$$IE = \frac{Number\ of\ positive\ samples}{Number\ of\ examined\ samples} \times 100 \quad (1)$$

where

IE – prevalence of infection is ration of positive samples and total number of examined samples, in percentage, %.

$$II = \frac{Numberoflarvae}{Numberofpositivesamples} \quad (2)$$

where

II - intensity of infection is ratio of detected number of larvae and total number of positive samples.

Results and Discussions

Overall, from all four regions 1233 samples from wild boar pillars of diaphragm were examined and *Alaria* mesocercariae were diagnosed in 101 samples (Table 1).

In Kurzeme region the prevalence of infection in received 241 samples was 7.9%. From the region of Zemgale we received the most of wild boar meat samples, in total 916. Out of them 77 were positive and the prevalence of infection was 8.4%, which is significantly higher ($p>0.05$) if compared to the invasion in other regions. Whereas in the meat samples received from Vidzeme only in four samples *Alaria* mesocercariae were found and the prevalence of infection 5.6% was the lowest in comparison to other regions. The least number of samples were received from the region of Latgale, the obtained number of samples was only five and *Alaria* was diagnosed only in one examined sample. In the examined wild boar meat samples from all regions of Latvia, *Alaria* mesocercariae prevalence of infection was 8.2%. Other country studies show similar data. In Germany in wild boar meat *Alaria* mesocercariae prevalence of infection reached 11.5% (Riehn et al., 2012), in Austria – 6.7% (Paulsen et al., 2012), but in Croatia during a similar study the prevalence of infection was 1.8% (Urosevic et al., 2012). It should be noted that in the research carried out in the Institute of Food

Table 1

Distribution of obtained and examined samples by region

Region	Kurzeme	Zemgale	Vidzeme	Latgale	Total
Examined samples (number)	241	916	71	5	1233
Positive samples (number)	19	77	4	1	101
Larvae (number)	47	229	5	1	282
II	2.5	3.0	1.2	1.0	2.8
IE (%)	7.9	8.4	5.6	20	8.2

II – intensity of infection
IE – prevalence of infection, %

Safety, Animal Health and Environment – 'BIOR" in the period from 2009 to 2011 in wild boar meat the prevalence of infection was 19.4% (Esīte et al., 2012).

Intensity of infection in the examined wild boar meat positive samples from all regions was 2.8. The highest intensity of infection 3.0 ($p>0.05$) was observed in the region of Zemgale. In the positive 77 wild boar meat samples the detected number of mesocercariae was 229. Comparatively high intensity of infection 2.5 was discovered in the 19 positive samples from the region of Kurzeme. Low intensity of infection 1.2 was in samples from Vidzeme and 1.0 – from Latgale. In one positive sample number of *Alaria* mesocercariae varied from one to eight. In the Institute of Food Safety, Animal Health and Environment – "BIOR" similar to our study, in 50 g wild boar pillars of diaphragm, the number of mesocercariae were from one to 26 (Esīte et al., 2012). Whereas in a study carried out in Austria, when using for examination 35 g pillars of diaphragm, the diagnosed mean number of mesocercariae was 4.5 (Chmurzyńska et al., 2013). In the research done in France it is mentioned, that from eight examined regions, from year 2007 to 2009 the intensity of infection increased from 62 to 94 (Portier et al., 2011).

In recent years *Alaria* has been increasingly diagnosed in wild boar meat samples, for that reason study has to be continued.

Conclusions

1. *Alaria* mesocercariae in the examined wild boar meat samples were diagnosed in all regions of Latvia.
2. The highest prevalence of infection relative to the examined samples was found in the regions of Zemgale (IE 8.4%) and Kurzeme (7.9%).
3. The highest intensity of infection was diagnosed in Zemgale (II 3.0) and Kurzeme (II 2.5), but the least in samples from Latgale (II 1.0).

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GOAT KIDS STOMACH MORPHOLOGICAL DEVELOPMENT DEPENDING ON THE MILK TYPE

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Abstract
In goats (*Capra)* the growth and functional development of certain parts of the multi-chambered stomach continue during the first few months after birth. The aim of this research was to clarify morphofunctional changes in the kids abomasa and rumina and live weight gain during the first 8 weeks of life. Research was performed in two parts. In the first part (P1) we used Saanen breed kids which were all kept in the same conditions and in the second part (P2) we used Saanen breed kids which were separated in two groups with different feeding diets. In P1 the stomach contents of the goat kids were collected after slaughter (on day 1, 17, 25 and 30), weighed full and empty. The gastrohromoscopical method proved that in the newborn kids the area where the abomasal pH is 3.0 and lower is about 10% of the surface of the abomasum, but in age of three weeks it is composing 80% of the mucosal surface of the abomasum. In P2 were two groups – in the first group (MMG) kids which were fed with dairy (mother) milk and lived with mothers, the second group (MRG) kids were fed with milk replacer and lived separate from mothers. The stomach contents were collected after slaughter(at day 45 and 60).We confirm that the most important age of stomach development and kids growth in postnatal period are the first 45 days. During this period the most significant differences can be observed. On day 60 there are no significant differences ($p>0.05$) between MMG and MRG stomach development.
Key words: goat kids, gastric development, weight gain.

Introduction

In goats (*Capra)*, like in other ruminants, growth of the stomach parts and their functional development continues during the first months after birth. Studies have proved that different feeding factors and environmental conditions can influence the development of the ruminant stomach parts in kids (Hamada, 1975; Church, 1979; Smith and Sherman, 1994). However, studies should be continued in this field because not only the goat keeping and feeding management changes (feed components, way of feeding, frequency and duration) but also research methods (Sanz-Sampelayo et al., 2000; Khan et al., 2007; Fernandes et al., 2012; Mahouachi et al., 2012; Ripoll et al., 2012).

Recently, investigations are carried out on issues whether the kids of different cross- breeds need a different amount of feed, vitamins and macro-elements during their intensive growth and development period. Attention is paid not only to the growth and development stimulation by choosing the optimum amount of feed for each breed or cross-breed of goats, but also to the amount and quality of the obtained products (meat and milk yield) (Fernandes et al., 2012; Mahouachi et al., 2012; Ripoll et al., 2012). The authors, investigating identification mini-boluses influence on the underdeveloped goat forestomach in the first month of life, point out that we lack modern studies on the growth and development of different stomach parts (Pinna et al., 2006; Carnes et al., 2009; Castro et al., 2010). By studying literature, we established that studies on the functional activity changes of the abomasum during the first month of postnatal life are scarce.

Goat kids weight gain is one of the quality indicators as well as economically important for breeders. There are findings about body weight and possibilities to increase meat production, meat quality, carcass characteristics and tissue composition, including fatty acids and cholesterol contents, carcass yield, feed intake and digestibility by crossbreeding (Potchoiba et al., 1990; Sen et al., 2004; Mekonnen et al., 2014; Ozcana et al., 2014).

Therefore, the aim of the present study was to determine if the feeding of milk replacer, which was intended for calves, changed both the functional activity of the abomasa in goats and the weight ratio of the ruminant stomach parts during the first month of postnatal development, as well as to clarify morphofunctional changes in the kids' abomasa and rumina and live weight gain during the second month of life.

Materials and Methods

Research was performed in two parts, both of which were carried out in one farm in Latvia, Zemgale region, during the months of February to April in 2013.

In total 40 goat kids were used in the research. In the first part of the study (P1) 20 kids of Saanen breed were kept and fed identically. The investigation took place from the animal birth till the 30th day of postnatal life. All kids after birth lived with their mothers and the first seven days were fed on colostrum (*ad libitum*) but on day eight they were weaned. After weaning, kids were placed into two cotes 10 per each, and four times a day by using nipple buckets they were fed with calf milk replacer (with the fallowing content of nutrients - crude whey protein 220 g kg^{-1}, lysine 17 g kg^{-1} fat,

160 g kg^{-1} and 380 g kg^{-1} lactose). During investigation, a control slaughter was carried out: at the age of one day (6 hours after birth; n=5), 17 days (n=5), 25 days (n=5), and 30 days (n=5). To find out the development of the multi-chambered stomach, its morphometric analysis – length and width of abomasum (Hamada, 1975) and weight measurements - were carried out as described by other authors (Church, 1979; Khan et al., 2007; Castro et al., 2010). After macroscopic examination, ingest was removed from the stomach, and the following measurements of stomach were made: total weight, weight of each stomach part using scales CAS Model:SW-1 with accuracy 10 x 0.005 kg - after which weight percentage of full abomasal and forestomach parts was calculated (Castro et al., 2010).

To evaluate the functional condition of abomasum, gastrochromoscopic examination was carried out by using 3 g kg^{-1} of Congo red as an indicator. The method described by other authors was used in order to detect the parietal cell activity (Harinder, 2005; Cellorama, 2007). The abomasum was opened, cleaned and spread. Then, 3 g kg^{-1} of indicator Congo red was sprayed on the clean mucosa, and in 1-2 minutes the result was evaluated. The indicator coming into contact with hydrochloric acid producing parietal cells changed its colour from red to dark blue-violet. The area of the active (hydrochloric acid producing) parietal cells was calculated as percentage ratio of the abomasal surface.

In the second part (P2) of research, the age of two kids groups were 15 days (n=20). In the first group (MMG) were kids which were fed with dairy (mother's) milk *ad libitum* and lived with mothers (n=10), second group (MRG) kids were fed with foregoing calf milk replacer using nipple buckets, and lived separate from mothers in cote (n=10). Drinking water and hay were easy accessible for all goat kids which were involved in this research.

The stomach contents (*reticulum, rumen, abomasum, omasum*) of the goat kids were collected after slaughter on day 45 MMG (n=5), MRG (n=5), on day 60 MMG (n=5), MRG (n=5). All gastric parts were weighed full and empty after washing with 9 mlL^{-1} NaCl (abomasum and reticulorumen with omasum), and immediately processed for anatomical analyses. After full stomach weighing we detected the length and width of rumen and length of abomasums (Hamada, 1975; Church, 1979; Khan et al., 2007; Castro et al., 2010). The relative stomach weight was calculated by relating the body weight and concrete weight of stomach separately for each kid.

The data obtained in the research were statistically processed by using R Studio programme. Mean arithmetic value and the standard error were calculated.

Results and Discussion

In P1 research, analysis of adapting processes in the stomach of kids in postnatal ontogenesis we performed starting at 6 hours from birth until the animal reached the age of 30 days.

As kids are developing, the relation between weights of the gastric parts changes - the rumen in seventeen days old kids is 33.5% and the abomasum - 48.8% of the total gastric weight. After day 25 this relation changes in to the favour of the rumen – which then already reaches a 49% mark of the sum weight of the stomachs, the weight of abomasa then decreases to 33.4%. Similar dynamics are found by other authors (Sanz-Sampelayo et al., 2000; Khan et al., 2007; Carnė et al., 2009; Castro et al., 2010; Mahouachi et al., 2012).

We found that new born kids have 5-10% stomach area where the indicator Congo red solution changes colour from red to dark blue-purple. And it shows that on the first day after birth in fundal glands area there are only 10% of all parietal cells are developed and functional. Moreover, it is proved that at this age the level of enzymes and hormones involved in the process of curdling and processing of colostrum is sufficient in calves and kids (Moschopoulou et al.,

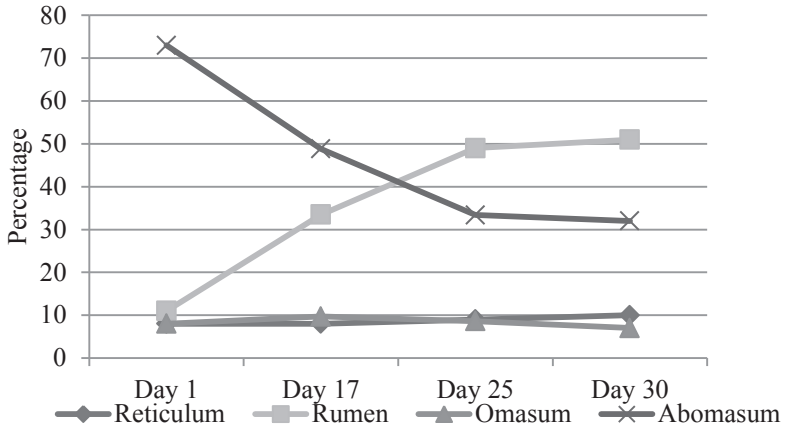

Figure 1. The weight ratio of percentage in the various kids' stomach compartments.

Table 1

The size of rumen and abomasum in kids fed on calves milk replacer (MGR) or mother's milk (MMG)

	Lenght (cm)				Width (cm)			
Group	Day 45		Day 60		Day 45		Day 60	
	mean	Stdev	mean	Stdev	mean	Stdev	mean	Stdev
Rumen								
MMG	21	1.1	23	0.8	23	0.8	25	1.0
MRG	19	0.7	22	1.2	22	0.6	25	1.7
Abomasum								
MMG	-	-	-	-	22	0.9	26	1.3
MRG	-	-	-	-	18	0.7	25	1.2

2006; Khan et al., 2007). The level of the hydrochloric acid secretion in the abomasum, that was determined in kids immediately after birth, is exactly as it is needed for digestion and absorption of the most important constituent parts of colostrums (Mahouachi et al., 2012).

The mucosal surface of the abomasum significantly increases ($p<0.01$) and by the time they reach 25 days of age it composes 70-72%, but at the age of 30 days 80%. This indicates that when the animals are 25 days old, the fundal glands in the abomasal mucosa are already fully developed and produce hydrochloric acid which provides pH 3.0 and lover acidity level of abomasa. Such pH is necessary for pepsin activation in the abomasum. Furthermore, during this period not only hydrochloric acid reaction increased in the stomach juice of kids but also the enzyme secretion (chymosin and pepsin) (Smith and Sherman, 1994; Winden et al., 2002; Moschopoulou et al., 2006).

In P2 research, analysis of clarify morphofunctional changes in the kids abomasa and rumen and live weight gain during the second month of life.

After the control slaughtering on day 45 and 60 we determined the size of rumen and abomasum in MRG and MMG.

Results show that the average length of rumen on day 45 in MMG group kids was 21 ± 1.1 cm, in MRG group it was 19 ± 0.7 cm, but on day 60 the average length in MMG and MRG was 23 ± 0.8 cm and 22 ± 1.2 cm respectively. Rumen width on day 45 in MMG group kids was 23±0.8 cm, in MRG groups it was 22 ± 0.7 cm, but on day 60 all kids rumina width reached 25 ± 0.8 cm. Our data show that between 15 and 45 days of age feeding on milk replacer increases speed of rumen development compared to feeding with mother's milk. It is possible that kids' limited access to milk replacer contributed to reinforcement of the combined feed and hay intake, and feeding with roughage feed promotes rumen development (Van Soest, 1994; Suárez et al., 2007; Mishra et al., 2013). Kids who stayed with their mother, could reduce hunger with mother's milk at any time, which does not promote roughage feed intake.

In kids which were fed with mother's milk the length of abomasum (*curvature minor*) on day 45 was 22 ± 0.9 cm, but in milk replacer group at day 45 was 18 ± 0.7 cm.

On day 45 kids who were fed with mother's milk showed higher abomasal length compared with kids who were fed with milk replacer. Normally mother's milk bypasses proventriculus and goes directly into the abomasum; perhaps it contributes to the development of this part of stomach. It must be admitted, that the abomasum and rumen size on day 60 between groups did not show any differences. At this age goat kids start intensively feed on hay and silage, which likely contributed to the development of multi-chamber stomach.

In the second part of research we also calculated the relative weight of the stomach.

Weight gain from day 45 till day 60 in MMG was 0.044 g day^{-1}, but in MRG it was 0.0073 g day^{-1} and it shows that for the live weight it is very important to feed kids on mother's milk.

After slaughtering we weighed all multi-stomach parts together and separately – full and empty. In MRG the full abomasal weight was 112 ± 15.5 g, but empty 30 ± 2.54 g and the full weight of rumen was 1174.4 ± 39.70 g, empty 144 ± 11.22 g while in MMG full abomasal weight was 255.6 ± 5.50 g, but empty 69 ± 4 g, and the full weight of rumen was 1739 ± 131.7 g, empty 225.4 ± 7.05 g ($p<0.05$).

The relative stomach weight on day 45 was 6.01 ± 0.004% in MRG and 4.98 ± 0.005% MMG, while on day 60 in MRG it was 5.57 ± 0.001% and in MMG 5.88 ± 0.11%. The relative weight of stomach was significantly higher ($p<0.05$) in kids which from day

Table 2

Live weight and the weight of the stomach in kids fed with calves milk replacer (MRG) or mother's milk (MMG) at 45 and 60 days of age

Group	Live weight (kg)				Weight of the stomach (g)				Relative stomach weight (%)			
	Day 45		Day 60		Day 45		Day 60		Day 45		Day 60	
	mean	Stdev	mean	Stdev	mean	Stdev	mean	Stdev	mean	Stdev	mean	Stdev
MMG	9.3	0.18	9.9	0.56	1978	94.3	2055	62.3	4.98	0.01	5.6	0.01
MRG	7.8	0.26	7.9	0.37	1339	54.7	1476	184.4	6.01	0.01	5.9	0.11

15 to day 45 were fed on mother's milk (MMG). On day 60 this difference is not significant.

In general, we can confirm that mother's milk at early age (up to 45 days of life) can significantly accelerate the growth of kid and multi-chamber stomach, where the animals are provided with sufficient amount of hay and roughage. This is important when the kids are grown for meat production. Reaching 60 days of age, this difference is not significant between groups.

Conclusions

In conclusion, as kids grow the weight relations between individual gastric parts change. One day old kids abomasal weight makes up to 80% of gastric total weight, whereas in 25 day old up to 33.4%. The gastrohromoscopical method proved that in newborn kids the area of pH≤3.0 it is about 10% of the surface of the abomasum,however, with time it significantly increases and by the time kids reach 30 days of age it is composing 80% of the abomasal mucosal surface.

During the second part of research we confirmed that the most important age of stomach development and kids growth is approximately 45th day of age when the most significant differences can be observed. On day 60 there are no significant differences in stomach development between goat kids' groups. Differences in weight gain were observed between groups – live weight gain in MMG was six times higher than in MRG.

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OVERVIEW OF *LISTERIA MONOCYTOGENES* CAUSED ABORTIONS IN CATTLE IN LATVIA IN 2013

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Abstract
Listeria monocytogenes is a pathogen that causes infectious diseases in animals and foodborne infection in humans. The aim of this retrospective study was to analyse *L. monocytogenes* caused abortions in cattle in Latvia in 2013, and to describe the potential reasons of these abortions. In total, 186 cattle abortion cases were investigated. The pathological material from aborted foetuses included samples of brain, heart, liver, spleen, kidneys and lung, liquid of stomach and liquid from thoracic and abdominal cavities. These samples were tested with bacteriological methods according to ISO 11290-1:2007. *L. monocytogenes* was found in 44 (23.7%) cases of cattle abortions. Positive cases were more distributed in the central and the south-eastern parts of Latvia, because in this territory winters tend to be wetter and colder than in other parts of Latvia. Seasonality was observed of *L. monocytogenes* caused abortions and the majority of cases occurred during spring and autumn, when the cattle were fed with silage. All abortion cases were observed in the second and the third trimester of the gestation. *L. monocytogenes* caused abortions occurred in cattle of different ages, but significantly ($p<0.05$) more often – in 3 years old cattle.
Key words: *Listeria monocytogenes*, cattle, abortions, Latvia.

Introduction

Genus *Listeria* consists of various species, but only two of them are pathogenic to animals – *L. monocytogenes* and *L. ivanovii* (Husu et al., 1990; Frances et al., 1991; Czuprynski et al., 2010) and *L. monocytogenes* also for humans. *L. monocytogenes* is a facultative intracellular pathogen that is associated with severe foodborne infections in humans and may cause infectious disease in many different animal species, especially in farm ruminants – cattle, sheep and goats (Nightingale et al., 2004). Bacteria of genus *Listeria* are widely distributed in different environments – in soil, water, sewage, on the plants, in the intestinal tract of various animal species, and also in farm environments. Listeriosis in animals can be observed in different clinical forms, such as, infections of central nervous system, uterine infection, gastroenteritis (Bartt, 2000; Siegman-Igra et al., 2002; Drevets and Bronze, 2008), and sometimes *L. monocytogenes* can cause keratoconjunctivitis and mastitis (Erdogan et al., 2001). Uterine infection usually results in late-term abortions or stillbirth and septicemia of neonates. The symptoms of neural form of listeriosis include encephalitis, unilateral facial paralysis, circling and excessive salivation. Eye infection is associated with direct inoculation of the eye with *L. monocytogenes* present in feed, especially in silage (Nightingale et al., 2004). Contaminated forage, most often poor quality or damaged silage, can cause outbreaks of listeriosis among the animals (Low and Donachie, 1997). *L. monocytogenes* usually enters the animal through ingestion, then the pathogen is disseminated via haematogenous spread to the viscera, brain, and gravid uterus (Ryser and Marth, 2007). In pregnant animals, listeria can localize in the placentomes and enter the amniotic fluid, subsequently the foetus aspirates the pathogen, which multiples and kills the foetus in the late-term of gestation (Timoney et al., 1988). Different factors can predispose to listeriosis infection – season, immune suppression, for example, pregnancy, and management practices, including feeding silage. Listeriosis in ruminants has a seasonal character, and it mostly occurs in winter or early spring and is associated with feeding of damaged silage (Ryser and Marth, 2007; Hellström, 2011). Faecal shedding by asymptomatically infected livestock poses a risk for contamination of farm environment and raw food at the pre-harvest stages (Zundel and Bernard, 2006). Faecal shedding may reflect levels of *L. monocytogenes* in animal feed. Rodents also are *L. monocytogenes* carriers, therefore can be a potential risk factor for faecal contamination of animal feed (Ryser and Marth, 2007).

In good-quality silage, prepared from grass, whole crop cereals, maize, or leguminous plants, which may or may not be wilted (dried to optimum moisture content) in the field before ensiling, the onset of anaerobic conditions stimulates the indigenous or added lactic acid bacteria to multiply quickly. As these bacteria expand to populations as high as 10^9 CFU g^{-1} within 48 h, they convert the plant sugar to lactic acid, causing conditions for rapid decrease in pH of silage (McDonald et al., 1991). Commonly, a pH of well-preserved and well-prepared silage is lower than 4.5 and such acidic conditions inhibit the growth of microorganisms that reduce the quality of silage, including *Listeria.* Higher dry-matter silage usually tends to have a higher pH, but in these silages the level of available water is lower and this may help to minimize the growth of *Listeria*. Grass silage in

cooler, wetter climate countries tends to have lower sugar levels and higher moisture contents and this results in a poorer, slower fermentation. Therefore in countries with warmer climates, these silages may be more susceptible to *L. monocytogenes* contamination and growth than grass and maize silage. Although *Listeria* dies in a well-fermented silage, if the pH increases before all bacterial cells are killed, the surviving *Listeria* cells will multiply (Ryser and Marth, 2007). D.R. Fenlon et al. (1996) showed that *L. monocytogenes* could survive over one year in bags that were used to wrap big bales and stored for reuse. This indicates that *L. monocytogenes* may survive in silage and can be a potential source of infection in cattle.

The aim of the present study was to analyse *L. monocytogenes* caused abortions in cattle in Latvia in 2013, and to describe the potential reasons of these abortions.

Materials and Methods

This was a retrospective study, which was done on the basis of investigation results of the Institute of Food Safety, Animal Health and Environment 'BIOR', obtained after the bacteriological tests of pathological material from aborted foetuses. Cattle abortion cases were investigated within the annual plan of Food and Veterinary Service of Latvia.

The pathological material from aborted foetuses included samples of brain, heart, liver, spleen, kidneys and lung, liquid of stomach and liquid from thoracic and abdominal cavities. The bacteriological investigation was done according to the ISO 11290-1:2007 with some modifications. For organ samples, 10 – 25 g of organ tissue were aseptically transferred into sterile stomacher closure bags and ½ Frazer broth (Biolife, Italy) was added for enrichment to achieve a 1:10 dilution, then the sample was homogenized in ½ Frazer broth by stomaching (BagMixer® 400, Interscience, France) at a normal speed for 60 s. After incubation at 30 °C for 24 h, 1.0 mL of enrichments in ½ Frazer broth were transferred into Frazer broth (Biolife, Italy) and 0.1 mL of enriched broth were plated onto ALOA medium (Biolife, Italy). Frazer broth and ALOA medium were incubated for 24 h at 30 °C and 37 °C, respectively. After 24 h incubation, 0.1 mL of enriched Frazer broth were plated onto ALOA medium and incubated for 24 h at 37 °C. For liquid samples direct plating onto ALOA medium were performed with subsequent incubation for 24 h at 37 °C. Small and round (0.5 – 1 mm in the diameter) colonies in blue-green colour, surrounded by a characteristic opaque halo on ALOA medium were considered as *L. monocytogenes* and were plated onto sheep blood agar (Biolife, Italy) and incubated for 24 h at 37 °C. Small, round colonies (0.5 – 1 mm in the diameter) of grey or greyish white colour, which displayed haemolysis on the sheep blood agar were considered as *L. monocytogenes*. Gram staining, catalase tests and commercial identification system Crystal GP (Becton, Dickinson and Company, USA) were performed for the identification and confirmation of *L. monocytogenes*.

According to the Latvian Environment, Geology and Meteorology Centre (LEGMC), the meteorological data – average air temperature and average rainfall – were used to analyse the seasonal influence on *L. monocytogenes* caused abortions.

In order to calculate the age significance in cattle *L. monocytogenes* caused abortion cases, t-test (Microsoft Excel 2007) at the confidence level 95% was performed.

Results and Discussion

In 2013 *L. monocytogenes* were isolated in 44 cases (23.7%) out of 186 investigated aborted foetuses. This is the highest rate of *L. monocytogenes* caused abortions in cattle during the last three years in Latvia. *L. monocytogenes* caused abortions were detected in 22.9% and 16.1% of bacteriologically investigated cattle abortions in 2011 and 2012, respectively (Šteingolde et al., 2013).

Cattle abortion cases due to *L. monocytogenes* were observed mostly in spring (Fig. 1). This could be explained by the fact that listeriosis cases have a seasonal character and more often are observed during indoor season – when animals are fed with silage (Ryser and Marth, 2007). Switching cattle from grazing to a diet of silage, mostly during the indoor season, leads to increased faecal shedding of *L. monocytogenes* (Husu, 1990). Therefore, also in the autumn were observed more abortion cases due to *L. monocytogenes*, than in the summer months. According to the LEGMC data, the April of 2013 was colder and wetter comparing with the previous years, and this could be a potential reason why that pasture season started later than in other years. Therefore, the relatively high number of listeric abortion cases in May 2013 was associated with a more prolonged silage feeding period than in 2011 and 2012. Only two cases of *L. monocytogenes* caused abortions in cattle were observed during the May 2011 and 2012 (Šteingolde et al., 2013).

The gestation period when cattle aborted was known in 30 cases out of total 44 cases. Our study proved the viewpoint that abortions caused by *L. monocytogenes* usually occur in the late-term of gestation (Timoney et al., 1988; OIE manual, 2008). Abortion cases during the first trimester of gestation were not observed in this study. Most often (63%) *L. monocytogenes* caused abortions were observed in the third trimester of gestation: in 11 cases abortions

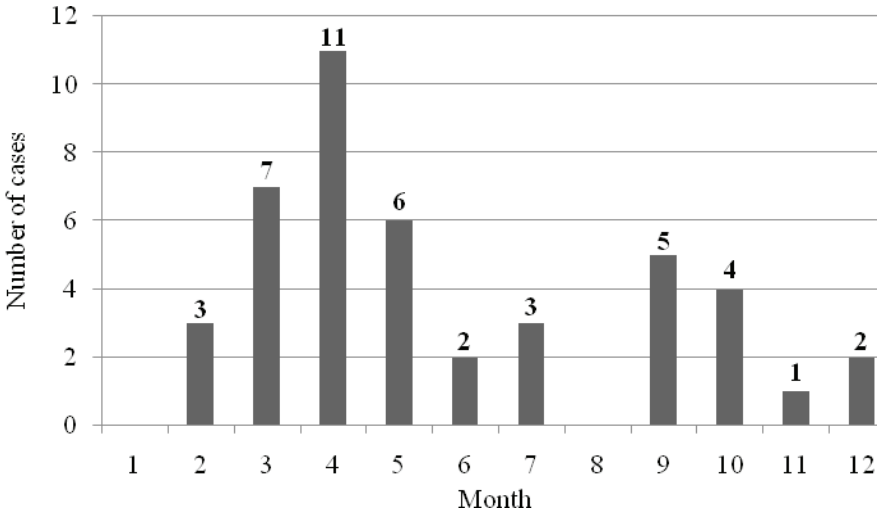

Figure 1. Occurrence of *L. monocytogenes* caused abortions in cattle in 2013: 1 – January; 2 – February; 3 – March; 4 – April; 5 – May; 6 – June; 7 – July; 8 – August; 9 – September; 10 – October; 11 – November; 12 – December.

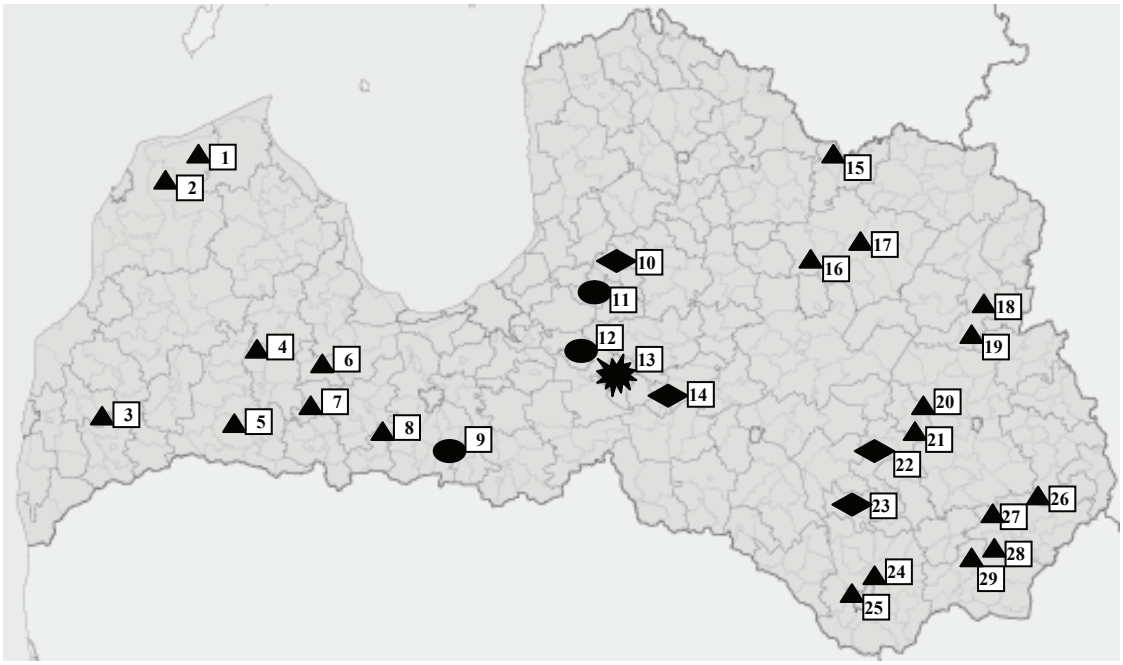

Figure 2. Illustrative distribution of *L. monocytogenes* caused abortions in cattle during the year 2013: ✹ - 5 cases in region; ◆ - 3 cases in region; ● - 2 cases in region; ▲ - 1 case in region.

1 – Ances region
2 – Popes region
3 – Bunkas region
4 – Gaiķu region
5 – Kursīšu region
6 – Jaunpils region
7 – Zebrenes region
8 – Tērvetes region
9 – Sesavas region
10 – Siguldas region
11 – Allažu region
12 – Ogresgala region
13 – Lielvārdes region
14 – Aizkraukles region
15 – Gaujienas region
16 – Druvienas region
17 – Galgauskas region
18 – Tilžas region
19 – Krišjāņu region
20 – Viļānu region
21 – Galēnu region
22 – Saunas region
23 – Rožkalnu region
24 – Daugavpils region
25 – Kalkūnes region
26 – Ezernieku region
27 – Andrupenes region
28 – Skaistas region
29 – Kombuļu region

were observed in the 7th month of gestation, in 7 cases abortions occurred in the 8th month of gestation and in 1 case – in the 9th month of gestation. The remaining 37% of *L. monocytogenes* caused abortions were observed during the second trimester of gestation: in 3 cases abortions occurred in the 5th month of gestation and in 8 cases – in the 6th month of gestation. This could be associated with a tendency that in small herds cattle parturitions are planned mostly in spring. Late-term gestation, changes in farming and feeding methods – pasture season changes to indoor season, movement of pregnant cattle to parturition premises in larger herds are stress factors that also can predispose to *L. monocytogenes* caused abortions.

The age of aborted cattle was also analysed. The age was known in 43 of total 44 aborted cattle cases. Significantly the most frequent age of aborted cattle was 3 years – in 35% of cases. Out of a total of 43cases, cattle were 4 years old in 26% of cases, 2 years old – in 14%, 5 years old in 9%, 6 years old in 9%, and in a very few cases – 5% and 2% – cattle were 8 and 7 years old, respectively. This observation indicated that younger cattle are less resistant to *L. monocytogenes* than older, but there is a need for further studies to confirm this observation.

L. monocytogenes caused abortions were observed in the whole territory of Latvia (Fig. 2). These abortion cases were more distributed in the central and the south-eastern parts of Latvia. These regions of Latvia tend to have colder winters than other parts of the country, but the highest rainfall rate tends to be at the northern and the south-western seacoasts and in the eastern part of central Latvia. According to the LEGMC data, the lowest rainfall rate is observed in the southern part of Latvia. Moist weather in the central part and colder weather in the south-eastern part of Latvia could be contributing factors for *L. monocytogenes* presence in silage and subsequent abortion cases, because silage in cooler, wetter climate countries tends to have lower sugar levels and higher moisture contents and that may promote the growth of *L.monocytogenes* in the silage (Ryser and Marth, 2007).

Conclusions

1. *L. monocytogenes* caused abortions in Latvia in 2013 were observed in 23.7% of bacteriologically investigated abortion cases, and these cases were more distributed in the central and south-eastern parts of Latvia.
2. *L. monocytogenes* caused abortions occurred more often in spring and autumn that was associated with the indoor season, when the cattle were fed with silage. Higher rate of *L. monocytogenes* abortion cases in May 2013, possibly, was associated with a prolonged silage feeding period due to colder and wetter spring, especially in April, than in previous years.
3. All abortion cases in cattle due to *L. monocytogenes* were observed in the late-term of gestation – in the second and third trimester of gestation.
4. *L. monocytogenes* caused abortions occurred in cattle of different ages, but significantly more often – in 3 years old cattle ($p<0.05$).

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CORTICOSTEROID-INDUCED ALTERATION IN LIVER FUNCTION IN DOGS AND ITS DECREASE POSSIBILITIES

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Abstract
Nowadays excessively used corticosteroids in veterinary medicine induce steroid hepatopathy in dogs (*Canis lupus familiaris*). The objective of this study was to determine the possibility of the hepatoprotectants to decrease the corticosteroid-induced alteration in such dogs' blood serum enzymes as alaninaminotransferase (ALAT), gammaglutamyltranferase (GGT), alkaline phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP). The study took place in private veterinary clinics in Riga, Latvia, during 2013, with the permission of dogs' owners. Twenty eight animals, which received corticosteroids due to present diagnosis, were divided into two groups. In the first group long-lasting corticosteroid methylprednisolone acetate injection was used once, while in the second group the hepatoprotectants were used after the injection of corticosteroids. It was discovered that after 14 and after 30 days of hepatoprotectants use, blood enzymes were significantly lower ($p<0.05$) than in dogs that did not receive hepatoprotectants. In both groups the enzyme values did not reach the reference limits. The study is set to investigate further if and when the values reach the reference limits.
Key words: dogs, corticosteroids, liver, blood serum enzymes, hepatoprotectants.

Introduction

Corticosteroids are widely used in veterinary medicine. These medications are used for animals with allergic and anaphylactic reactions, in shock, with autoimmune diseases or in other conditions (Badylak and van Vleet, 1981; Lucena et al., 1999; Abraham et al., 2006). Despite the fact that using corticosteroids can cause some complications such as osteoporosis, *diabetes mellitus*, hypertension, cataracts, iatrogenic hyperadrenocorticisms and others, they are used excessively and being overdosed (Levine et al., 2008).

Local and systemic corticosteroids are used in veterinary medicine, both causing changes in different organ systems of the animal, especially in the endocrine system and in the liver morphofunctional condition (Badylak and van Vleet., 1981; Abraham et al., 2006). It is proved that corticosteroids can cause changes in 2-3 days after the beginning of the therapy. Injectable and long-acting corticosteroids cause more complicated changes in comparison to orally used and local corticosteroids (Dillon et al., 1980). Corticosteroid-induced alteration in the liver morphofunctional condition is called steroid hepatopathy. This is a specific pathology only in dogs (*Canis lupus familiaris*) (Fittschen and Bellamy, 1984). It is known that some blood serum enzymes indirectly reflect liver morphofunctional condition, but histological findings of the liver biopsy reflect it directly. Increased values of such blood serum enzymes as alaninaminotransferase (ALAT), aspartataminotransferase (ASAT), gammaglutamyltranferase (GGT), alkaline phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP) are specific for the steroid hepatopathy. The alkaline phosphatase value increases in the serum because of glycogen raised deposit in the liver and vacuolization of hepatocytes (Badylak and van Vleet, 1981; Lucena et al., 1999). This process is caused by corticosteroids – an isoenzyme of alkaline phosphatase that is specific only to dogs, produced at hepatocytes (Dillon et al., 1980). Increased AP value is one of the most used biochemical indicators in the diagnostics of the liver disease (Center et al., 1992). The possibility to distinguish corticosteroid-induced AP increase from liver pathology induced AP increase has greater differential diagnostic value. The safe differential method is thermic processing of AP isoenzymes – corticosteroid-induced AP isoenzyme is thermostable (Teske, 1999; Feldman and Nelson, 2004).

Different solutions have proved capable of protecting the dog's body and especially its liver against the negative corticosteroid alteration. There exists a description of the evaluation of the aminoacid S-adenosylmethionine's influence on systemic and hepatic effects on prednisolone in dogs (Center et al., 2005), the efficiency of a butafosfan and vitamin B12 ('Catosal') on biochemical and hematological blood parameters in dogs treated with dexamethasone (Deniz et al., 2009) and others. Even though the hepatoprotectants are included in various health supplements, their efficiency towards protecting or reversing corticosteroid-induced changes in dog liver are insufficiently investigated.

The aim of the study is to investigate the corticosteroid-induced alteration in the liver function in the dogs and the possibility to decrease liver alterations by the use of hepatoprotectants.

Materials and Methods

The study took place in private veterinary clinics in Riga, Latvia, during 2013, with the permission of dogs'

owners. Twenty eight dogs of various age, weight, breed and gender were used in the present study. All dogs had a confirmed disease and were treated with corticosteroids. The animals were divided into two groups conditionally: in the first group dogs received only an injection of corticosteroids, in the second dogs received hepatoprotectants after the injection. For this study we selected a long-acting corticosteroid – methylprednisolone acetate - 40 mg mL^{-1} in intramuscular route once on the first day of the study in a dose of 0.1 mg kg^{-1}, but as hepatoprotectective agent – 'GlutaMax', which contains the essence of silymarin (*Silybum marianum*) – 133 mg in each pill, meant for 15 kg of bodyweight; the essence of curcuma (*Curcuma longa*) – 33 mg for each 15 kg of bodyweight; and the essence of artichoke (*Cynara scolymus*) – 66.6 mg, choline, lecithin, B group vitamins, zinc. These were used once per day *per os* from the first day of study for 30 days.

To estimate the hepatoprotectant influence on dog's condition and the possibility to protect the liver against corticosteroids impact, the following blood serum enzymes were determined – alaninaminotransferase (ALAT), gammaglutamyltransferase (GGT), alkaline phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP) values (Hoffmann et al., 1977). The concentration of the enzymes increases because of the hepatotoxic drug influence on hepatocytes (Fittschen and Bellamy, 1984).

The hepatoprotectant 'GlutaMax' was used because of its unique composition. Silymarin has antioxidant, hepatoprotective, antifibrotic, and anti-inflammatory effects (Flatland, 2003; Johnson, 2008). The essence of this plant is used in the treatment of experimentally induced mushroom hepatotoxicity (Vogel, 1984). Silymarin is used in human medicine as additional treatment of acute viral hepatitis, alcoholic liver disease and toxin or drug-induced hepatitis (Johnson, 2008). The essences of curcuma and artichoke have antioxidant and anti-inflammatory effects and are also used for liver detoxication. Choline regulates the metabolism of fats, works against fatty degeneration of liver. N-acetylcysteine provides a ready source of glutathione to detoxify toxic intermediates. N-acetylcysteine should also be considered for the treatment of any severe toxinrelated hepatic injury (Johnson, 2008). Lecithin decreases the necrosis of hepatocytes. Zinc is used to decrease hepatic copper content in breeds with hepatic copper accumulation and chronic liver diseases. In other canine hepatobiliary disorders zinc is suggested because of its antioxidant and antifibrotic effects (Johnson, 2008).

A day before corticosteroid use blood samples were collected from each animal *v. jugularis*. To separate the serum we centrifuged the blood on 1,300 rounds per minute for 10 minutes (Gulbis, 2011). We analyzed the serum not more than 15 minutes after the separation. GGT, ALAT and AP were determined in serum by biochemical analyzer 'MINDRAY BS-120'. Corticosteroid-induced enzyme thermostable alkaline phosphatase was determined by Teske method (Teske, 1999), the blood serum was handled for 15 minutes in temperature 60 °C, therefore only thermostable isoform will be presented into blood serum.

To analyze the data the programs MS Excel and '*RStudio*' were used. P-values less than 0.05 were considered to be statistically significant. For the comparison of blood serum enzymes values T-test was used.

Results and Discussion

Before the study (day 0) blood serum enzyme values were defined for each dog. It was found that serum enzymes as ALAT, GGT, AP and cAP values were similar to each other ($p>0.05$) and were within reference limits (Table 1).

Table 1

The values of some blood serum enzymes in dogs during the study

Parameters	Reference limits (Geffre et al., 2009)	Groups	Using of hepato protectant	Day 0	Day 14	Increase from Day 0 (times)	Day 30	Increase from Day 0 (times)
ALAT	10 – 100 U L^{-1}	1.	Not used	69.2±8.0	206.4±28.8	3.0	177.6±19.5	2.6
		2.	Used	66.3±6.7	157.5±8.6	2.3	141.3±8.6	2.0
GGT	0 – 9.6 U L^{-1}	1.	Not used	5.0±0.7	19.6±4.4	3.9	17.8±3.5	3.6
		2.	Used	4.6±0.8	12.3±1.7	2.7	11.0±1.3	2.4
AP	23 – 212 U L^{-1}	1.	Not used	59.2±5.5	412.8±75.3	7.0	329.2±46.7	5.6
		2.	Used	63.2±6.1	274.0±20.5	4.0	221.2±18.1	3.5
cAP	-*	1.	Not used	12.5±2.2	265.1±41.4	21.0	210.6±32.7	16.8
		2.	Used	13.4±2.1	205.2±22.5	15.0	158.4±17.1	11.8

*Corticosteroid-induced alkaline phosphatase (cAP) does not have reference limits, because in healthy body it could not exist.

Fourteen days after the injection of long-lasting corticosteroid methylprednisolone acetate all dogs from the group one had a very high enzyme activity. The mean value of ALAT (206.4 ± 28.8 U L^{-1}) was approximately three times higher than on day 0 (69.2 ± 8.0 U L^{-1}); the mean value of GGT increased 3.9 times (19.6 ± 4.4 U L^{-1} versus 5.0 ± 0.7 U L^{-1}); the mean value of AP increased approximately seven times from the day 0 (412.8 ± 75.3 U L^{-1} versus 59.2 ± 5.5 U L^{-1}), but cAP mean value in serum was 21 times higher than on the day 0 (265.1 ± 41.4 U L^{-1} and 12.5 ± 2.2 U L^{-1}, respectively) (see Table 1).

The second purpose of this study was to find out how effectively the hepatoprotectant 'GlutaMax' can decrease the alteration in the biochemical parameters on the serum, which was caused by methylprednisolone acetate. It became apparent that all the investigated enzyme values in blood serum obtained from the dogs from group two, which were using hepatoprotective agents after the long-lasting corticosteroid injection were significantly lower ($p<0.05$) than these in the dogs from group one (see Table 1).

The mean value of ALAT in dogs from the group two on day 14 increased to 157.5 ± 8.6 U L^{-1}, which was only 2.3 times higher than ALAT value on day 0 and significantly lower than the value from the animals of the group one – 206.4 ± 28.8 U L^{-1} ($p<0.05$) (see Table 1). The mean value of GGT in the group two increased to 12.3 ± 1.7 U L^{-1},compared with 19.6 ± 4.4 U L^{-1}, as seen in group one (see Table 1 and Figure 2). The increase of the mean value of AP was 274.0 ± 20.5 U L^{-1}, and this was only four times higher than in the same group on day 0 in comparison with group one where the increase was 7 times higher (see Table 1). Corticosteroid-induced AP (cAP) mean value in group two on the fourteenth day was also high – 205.2 ± 22.5 U L^{-1}, that was 15 times higher than on day 0, but in group one the same value was 21 times higher than on day 0 (see Table 1).

The mean value of ALAT in dogs from group one serum on **day 30** increased to 177.6 ± 19.5 U L^{-1}, which is only 2.6 times higher than on day 0 (not 3 times as it was in the same animals' serum on day 14). The mean value of GGT in the first group on day 30 was 3.6 times higher (17.8 ± 3.5 U L^{-1}) than in the same animals' serum on the day 0, which was significantly higher ($p<0.05$), but lower than on day 14 (see Table 1). The mean value of AP on the day 30 in dogs from the group one was 5.6 times higher than on day 0 and it was 329.2 ± 46.7 U L^{-1}. But this increase was significantly lower ($p<0.05$) than the increase on day 14 (7 times) (see Table 1). The cAP mean value on day 30 in dogs' from the group one had increased 16.8 times, compared with 21 times on day 14 (see Table 1).

It should be noted that corticosteroid-induced thermostable AP in serum was found in small amounts (12.5 ± 2.2 U L^{-1}) even on the day 0. This can be explained with stress condition because of the veterinarian presence and blood collecting from the animal. It is acknowledged that it is higher glucocorticoid, e.g. cortisol level in blood when animal is in stress (Feldmann et al., 1994). It is experimentally proven that there are small amounts of thermostable alkaline phosphatase even in healthy dogs' blood as the authors describe it with being in stress condition (Hoffmann et al., 1977; Fukui et al., 2006).

It should be noted that the mean values of ALAT, GGT, AP and cAP in the serum of the dogs from the second group on day 14 and also on day 30 because of influence of hepatoprotectant 'GlutaMax' were significantly lower. That indicates that the negative effect of long-acting methylprednisolone acetate is decreased. Every enzyme mean value was lower compared to the same mean values from the animals of the first group, but none of the enzymes achieved the reference limits (see Table 1).

It can be concluded that the hepatoprotectant 'GlutaMax' used for 30 days, and even for 14 days, after one injection of long-lasting methylprednisolone acetate in dosage of 0.1 mg kg^{-1} bodyweight in dogs, significantly decreases corticosteroid-induced great increase of value of such enzymes as ALAT, GGT, AP, cAP in dogs serum.

In regard to these enzyme values on the day 30, it should be noted that all of them had the tendency to decrease in comparison to the fourteenth day of the study (see Table 1).

The results of our study prove the fact of corticosteroid-induced negative effects on liver functional condition (Badylak and van Vleet, 1981; Lucena et al., 1999; Abraham et al., 2006). These negative effects have been reflected by enzymes ALAT, GGT, AP and especially cAP significant increase in blood serum. The hepatoprotectant 'GlutaMax' could not completely prevent these functional failures of liver during this study. The question consists of how long time do we need to reverse the values of these enzymes in dogs' serum to reference limits in order to show that the negative effects of corticosteroids are completely gone. It would be desirable to confirm complete reversing by histological and immunohistochemical findings. The investigations on this direction are ongoing.

Conclusions

1. The corticosteroid methylprednisolone acetate statistically significantly increases the values of alaninaminotransferase (ALAT), gammaglutamyltranferase (GGT), alkaline

phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP) in dogs' blood serum.
2. The hepatoprotectant 'GlutaMax' in dosage of 1 pill for 15 kg of bodyweight, used 30 days after the one injection of long-lasting methylprednisolone acetate for dogs, significantly decreases the values of alaninaminotransferase (ALAT), gammaglutamyltranferase (GGT), alkaline phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP) in dogs' blood serum.

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CLINICAL OUTCOME OF CUTANEOUS MAST CELL TUMORS IN DOGS

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Abstract
A prospective study was performed on 15 client-owned dogs to assess the clinical outcome after surgical excision of canine cutaneous mast cell tumors (MCTs) depending on histologic grade and completeness of surgical margins. The surgical margins were evaluated as complete, close or incomplete if they were more than 3 mm, from 1 to 3 mm or less than 1 mm wide, respectively. Survival time for dogs with low grade MCT (based on grading by M. Kiupel et al.) was 409 days compared with only 60 days for dogs with high grade tumor. Dogs with high grade tumors had significantly shorter survival time and worse prognosis than dogs with low grade tumors (p=0.013). Complete excision was associated with lower possibility and longer time to tumor recurrence, as well as longer survival, however, marginal surgical border status did not have a significant impact on time to tumor recurrence and survival. It was also found that tumor duration but not tumor size had a significant impact on surgical margin status (p=0.047). Tumor size significantly affected survival time with larger tumors being correlated with shorter survival (p=0.03). The results of our study suggest that grade, tumor size and recurrence are significant factors for MCT prognostication.
Key words: Canine, mast cell tumor, grade, surgical margins, recurrence, survival.

Introduction

Mast cell tumors are second most common malignancy in dogs after soft tissue sarcoma group (Morris and Dobson, 2001). The prognosis for dogs with MCT varies from good to poor, depending on various factors, including histopathologic grade, clinical stage, metastasis at the time of diagnosis, anatomic location of the tumor (Gieger et al., 2003), completeness of surgical margins, systemic signs, breed, plasma histamine concentration (Ishiguro et al., 2003), mitotic activity (Preziosi et al., 2004; Thompson et al., 2011) and vessel density within the tumor (Preziosi et al., 2004). As various immunohistochemical assays for proliferation markers are found to be sensitive in prognostication of human tumors, a large number of them have been studied in veterinary cancer patients. Argyrophylic nucleolar organizer region (AgNOR) count (Scase et al., 2006), presence of proliferating cell nuclear antigen (PCNA), ki-67 nuclear staining (Scase et al., 2006; Séguin et al., 2006), c-kit expression (Takeuchi et al., 2013), DNA ploidy, abnormal p53 tumor suppressor gene expression were found to be prognostic for canine cutaneous MCT. Unfortunately, none of immunohistochemical markers is prognostically as valuable and as cost-efficient as histologic evaluation of tumor for histopahologic grade and evaluation of surgical margins. There are several systems for MCT grading, where some macroscopic and microscopic features are correlated with biologic behavior and clinical outcome of the tumor. The most commonly used grading systems are following – MCT grading system according the recommendations by A.K. Patnaik et al. (1984) with three grades where grade I tumors are associated with longer survival time compared with grade III tumors; and MCT grading system by M. Kiupel et al. (2011) with two grades where low grade tumors have a better prognosis than high grade tumors. As tumors in veterinary patients have not been previously studied in Latvia, the purpose of this study was to assess retrospectively the clinical outcome of dogs with MCT depending on histopathologic grade and completeness of surgical excision and to find out if delayed surgeries predominate in Latvia.

Materials and Methods

Fifteen client-owned dogs were included in this study. The animal use in this study was permitted by Food and Veterinary Service of Latvia (Food and Veterinary Service license No. 45 for animal use in the experiment). Cutaneous MCTs were diagnosed in all dogs between May 2011 and June 2013 on the basis of histological examination of excised tumor.

Signalment, breed, sex, age, weight, tumor location, tumor duration, tumor size, histological grade, completeness of surgical excision, postoperative medical therapy, date of recurrence, date of death, cause of death and metastasis status were recorded.

Clinical examination, complete blood count and serum biochemistry screen before surgical excision of tumors were performed by local veterinarians at three veterinary clinics of Latvia. Thoracic radiographs, abdominal radiographs and abdominal ultrasound were performed when deemed necessary.

Histological examination of all tumor biopsies was done by a Board-certified veterinary pathologist (Ilze Matīse – Van Houtana, the American College of Veterinary Pathologists) at the Pathology laboratory of Faculty of Veterinary Medicine at the Latvia University of Agriculture. The histological grade of MCT and completeness of surgical margins were evaluated during histological examination of tumor. The tumors were graded by the use of both best known grading systems – according A.K. Patnaik et

Table 1

Histologic classification of mast cell tumors in dogs

Feature	Patnaik grade*	Kiupel grade*			
	I	II	III	Low	High
Location	Dermis	Dermis / subcutis	Dermis / subcutis	NS	NS
Cells	Uniform population	Moderate anisocytosis	Marked anisocytosis	NS	NS
Granularity	Distinct, obvious	Variable but visible	Variable, may be absent	NS	NS
Nucleus	Uniform, round	Round to indented	Marked anisokaryosis	Rare bizarre nuclei or rare karyomegaly	Karyomegaly; 3 bizarre nuclei / 10 HPF
Nucleolus	Inconspicuos	Small but obvious	Prominent	NS	NS
Mitoses	None	0-2/HPF	3-6/HPF	0-6/10 HPF	7/10 HPF
Binucleated cells	NS	Occasional	Common	NS	NS
Multinucleated cells (3 or more nuclei)	Absent	Absent	Present	0-2/10 HPF	3/10 HPF
Stromal changes	None	Edema, necrosis, hyalinized collagen	Hemorrhage, edema, necrosis, hyalinized collagen	NS	NS

NS – not specified
HPF – high power field (400 ×)
Karyomegaly – nuclear diameters of at least 10% of neoplastic mast cells vary by at least 2-fold
*Patnaik grade – Patnaik et al., 1984; Kiupel grade – Kiupel et al., 2011.

al. (1984) with 3 grades, in which grade I has the best prognosis and grade III has the worst, and M. Kiupel et al. (2011) with 2 grades, in which low grade tumors are associated with good outcomes and high grade tumors have worse prognosis (Table 1). Following the recommendations of B. Séguin et al. (2001), the surgical margins were considered as complete if there was at least 3 mm wide band of healthy tissue between tumor cells and surgical margins. If the tumor cells were at the distance from 1 to 3 mm of surgical margins, the surgical margins were considered close, but if tumor cells reached or even crossed the surgical margins, the surgical margins were defined as incomplete. Surgical margins were marked with India ink marking dye immediately after excision or after formalin fixation for better orientation and evaluation of surgical margins during histological examination.

Follow-up information was obtained through direct contact with the owners and patients. The staging of dogs was performed three, six and twelve months after surgery through clinical examination of an animal and surgery area, examination of regional lymph nodes through palpation and fine needle aspiration. Twelve months after surgery abdominal ultrasound was performed for evaluation of metastases. Surgery was the only treatment for all dogs. End points of study were at least twelve months after surgery or death.

The disease control rate was defined as the percentage of dogs without evidence of local and distant tumor recurrence at a specific time after surgery. Recurrences were further characterized as local or distant. Time to local recurrence was defined as the interval between the excision of MCT and evidence of tumor regrowth at the place of surgery. Time to distant recurrence was defined as the interval between the excision of MCT and evidence of tumor regrowth at a different cutaneous location. Survival time was defined as the interval from the date of MCT excision to the date of death.

Statistical analyses were performed by t-test for continuous variables and Pearson chi-square analysis for categorical variables. To estimate time to recurrence and overall survival, the Kaplan-Meier method was used. All statistical tests were performed using computer software SPSS Version 17. Values of $p<0.05$ were considered significant.

Results and Discussion

Fifteen dogs with cutaneous MCTs were included in this study. Two dogs were mixed breed, remaining 13 dogs represented ten breeds: Golden Retriever (n=2), Labrador (n=2), French Bulldog (n=2), and one from each of the following: Weimaraner, Boxer, Dachshund, American Staffordshire Terrier, Chinese Crested Dog, Doberman and Sharpei. In the recent study by J. Warland and J. Dobson Boxers, Labradors, Golden Retrievers and Staffordshire Bull Terriers were found to be predisposed to MCT development (Warland, Dobson, 2013). There were 8 females (2 spayed and 6 sexually intact) and 7 males (1 castrated

and 6 sexually intact) in our study. The median age of the dogs at the time of surgery was 8.2 years (range, 4.1 to 13.6 years). The median age and sex distribution were in agreement with findings in other populations of dogs with this type of tumor (Gieger et al., 2003; Scase et al., 2006). The median weight was 32.8 kg (range, 6.7 to 53.0 kg).

Five of 15 tumors were located on the trunk, 5 on the hind limbs, 3 on the forelimbs, 1 was on the tail and 1 on the head. According to previous studies, 9 – 61% of MCTs are located on the trunk, 29 – 45% on the extremities and 10 – 20% on the head or neck (Weisse et al., 2002, Ishiguro et al., 2003; Thompson et al., 2011). There is higher prevalence of limb location in our study and lower prevalence of head/ neck location of MCTs, compared with previous studies. The median tumor size was 4.3 cm (range, 0.7 to 10.0 cm) in diameter. The median tumor duration (the number of days between tumor detection by the owners and surgery) was 121 days (range, 14 to 360 days). Previously reported median MCT diameter was 1.9 – 3 cm with median duration of 2.5 months (Gieger et al., 2003; Séguin et al., 2006). Our results present the tendency of delayed surgeries in Latvia, which can be the reason of the increased size of tumors at the time of surgery. The mean size of M. Kiupel et al. low grade tumors was 3.2 cm in diameter compared with 5.9 cm for high grade MCTs, however, the difference was not significant. We found that tumor duration has a significant impact on the surgical margin status (p=0.047). There was no statistically significant correlation between tumor size and surgical margins; however, the tumor size had a significant impact on the survival time (p=0.03).

According to A.K. Patnaik et al. grading system, 2 of the dogs had grade I, 9 grade II, and 4 grade III MCT (Table 2). Our results agree with the previous clinical trials, where grade I MCT represented 6 – 31%, grade II - 44 – 79% and grade III - 15 – 29% of all MCT cases (Gieger et al., 2003; Preziosi et al., 2004; Takeuchi et al., 2013). According to M. Kiupel et al. grading system, 9 dogs in our study had low grade and 6 had high grade MCT. Similar proportion was found in a recent study by Y. Takeuchi et al. (2013), where 60% of dogs represent low grade MCT and 40% - high grade. These high grade tumors are associated with poor prognosis (Kiupel et al., 2011; Takeuchi et al., 2013) and additional treatment (chemotherapy) is indicated in these cases.

Five of 12 tumors were excised completely, 3 of 12 tumor excisions were done marginally and 3 of 12 surgeries were incomplete. For 3 missing tumors the evaluation of surgical excision could not be done because only a part of tumor was submitted for histological examination.

Five of 15 dogs had local tumor recurrence. Two of 4 MCTs recurred locally after an incomplete initial surgery; time to local recurrence was 30 and 34 days. Recurrence of tumor has been observed in 2 of 3 cases with marginal excision, recurrence time was 30 and 228 days. The association between completeness of surgical excision and tumor control rate is shown in Figure 1. Six months after initial surgery the disease

Table 2

Histologic grade, status of surgical margins and clinical outcomes of MCT in 15 dogs

Number of dog	Patnaik grade*	Kiupel grade*	Status of surgical margins	Tumor duration, days	Local recurrence, days after surgery	Distant recurrence, days after surgery	Survival time if not alive, days
1	2	low	complete	14	-	621	691
2	2	low	marginal	-	-	228	-
3	2	low	incomplete	-	-	-	-
4	2	low	complete	-	-	-	-
5	1	low	complete	-	-	277	367
6	2	high	incomplete	-	-	-	-
7	2	low	unknown	360	-	90	291
8	2	low	complete	30	-	-	-
9	3	high	complete	-	30	-	69
10	3	high	incomplete	21	30	-	76
11	3	high	marginal	-	30	-	60
12	2	high	unknown	180	59	-	59
13	2	low	marginal	180	-	-	-
14	1	low	unknown	-	-	-	-
15	3	high	incomplete	60	34	-	34

*Patnaik grade – Patnaik et al., 1984; Kiupel grade – Kiupel et al., 2011.

control rate was 80% for complete surgery, 67% for marginal and 50% for incomplete. Twelve months after surgery the disease control rates were 60%, 33% and 50%, respectively. Complete excision was associated with lower possibility for tumor recurrence; however, marginal status did not have a significant impact on time to tumor recurrence and survival in our study. All dogs with local tumor recurrence were euthanized due to rapid progression of MCT. Median survival time for these dogs was 60 days (range 34 – 76).

The M. Kiupel et al. low grade and high grade tumor control rates at 12 months after initial surgery were 67% and 17%, respectively (Figure 2). Median time to tumor recurrence was 304 days for Kiupel low grade and 27 days for high grade MCT. Survival time for dogs with M. Kiupel et al. low grade tumors was 409 days compared with 60 days only for dogs with high grade MCT. M. Kiupel et al. high grade tumors had significantly shorter survival time than low grade tumors (p=0.013). Our results are similar to those published recently which reported median progression free survival in dogs with low and high M. Kiupel et al. grades 553 and 84 days, respectively (Takeuchi et al., 2013).

Four of 15 dogs had distant tumor recurrence. Median time to distant tumor recurrence was 304 days (range 90 – 621 days). This proportion of dogs that developed additional MCT is slightly higher than that of the study by A.M. Simpson et al. (2004) in which the incidence of distant MCT development

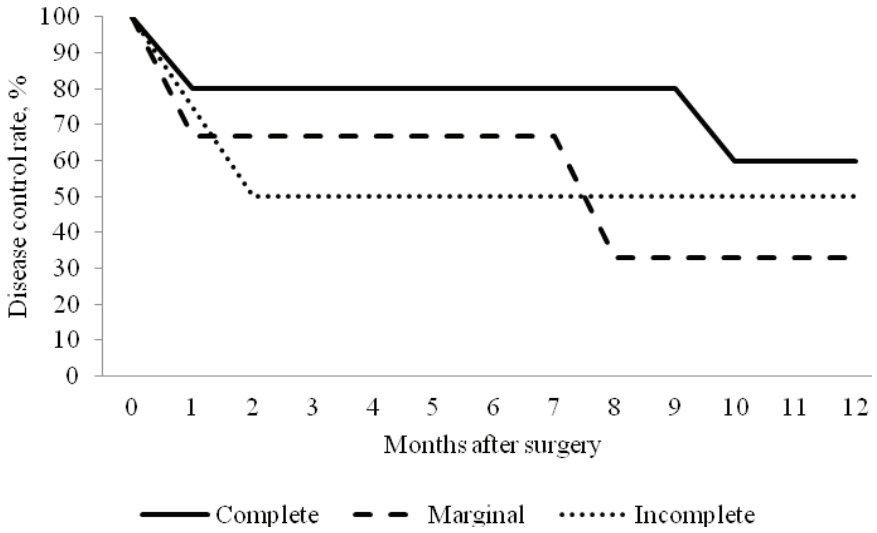

Figure 1. Association between completeness of surgical excision and disease control rate in 12 dogs with cutaneous mast cell tumors.

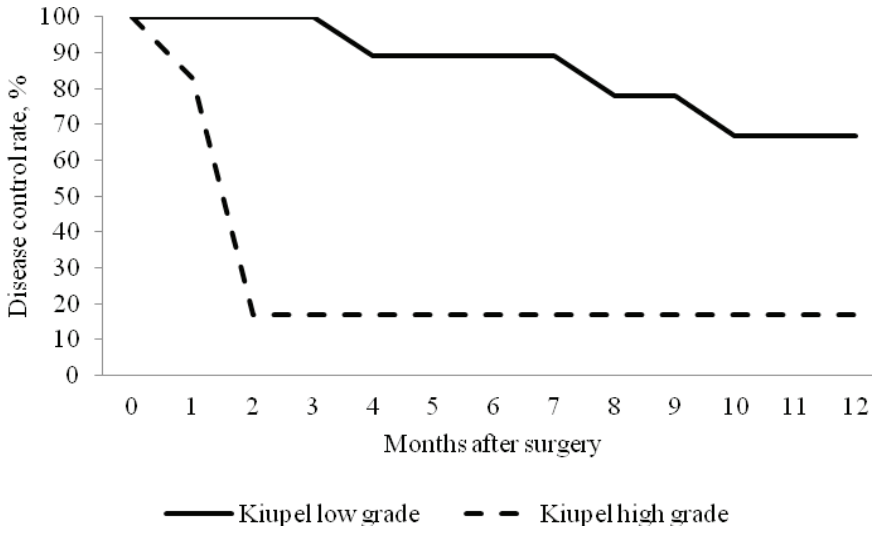

Figure 2. Association between tumor grade and disease control rate in 15 dogs with cutaneous mast cell tumors.

was 24% within 362 days. The higher incidence of distant recurrence in our study can be associated with the prospective character of our study and decreased possibility to miss out valuable clinical information. Three of 4 dogs with distant recurrence were euthanized. The reason of euthanasia for two of those dogs was progression of recurred MCT, survival time for them was 291 and 691 days. Hepatic and splenic metastases were found during abdominal ultrasound at the time of tumor recurrence. One of the dogs with distant tumor recurrence was euthanized due to another type of malignancy. Five of 15 dogs had local tumor recurrence within approximately 37 (range 30 – 59) days after surgery.

Survival time for dogs with distant tumor recurrence was 491 days, compared to dogs with local tumor recurrence survival time averaged only 60 days. Tumor recurrence was significantly correlated with survival (p=0.001). Total recurrence rate was 57%. Overall survival time was 206 days (range, 34 to 691 days).

Conclusions

The tumor control rate 12 months after initial surgery was 67% for M. Kiupel et al. low grade and only 17% for high grade tumors. Median time to tumor recurrence was 304 days for low grade and 27 days for high grade MCT. Survival time for dogs with low grade tumors was 409 days compared with 60 days for dogs with high grade MCTs. M. Kiupel et al. grade was significantly associated with survival time (p=0.013). Tumor control rate at 12 months was 60% for complete surgical excision, 33% for marginal and 50% for incomplete one. Median time to MCT recurrence and median survival time was 326 and 376 days for complete excision, 129 and 60 days for marginal and 32 and 55 days for incomplete excision, respectively. Complete excision is associated with lower possibility and longer time to tumor recurrence, as well as longer survival, however, marginal status does not have a significant impact on time to tumor recurrence and survival according to our study. We found that tumor duration has a significant impact on surgical margin status (p=0.047), and that tumor size has a significant impact on survival time (p=0.03). We conclude that surgical excision of MCT must be done as soon as tumor is detected, and histologic evaluation of the tumor gives essential information for clinician about tumor prognosis and the necessity of additional treatment. However, because of the relatively small number of dogs included in this study (n=15), further investigation is warranted to validate these recommendations.

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THEORETICAL EVALUATION OF HYDROTREATED VEGETABLE OIL APPLICATION IN DIESEL ENGINES

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Abstract
A lot of different EU directives and regulations set the targets to decrease greenhouse gas emissions, to increase the share of renewable energy, and to improve energy efficiency. Biofuel usage is directly linked to all of these problems. Since the first generation food-based biofuels should not receive public support after 2020, investigations of next generation biofuels are topical. Hydrotreated vegetable oil (HVO) is one of the most promising next generation biofuels in the near future. This article deals with the results of mathematical modelling to determine the main diesel engine operating parameters (power, torque and fuel consumption) running them on HVO and its blends with fossil diesel fuel. The modelling results of the car *Opel Insignia 2.0 CDTi* show that every 5% of HVO in fuel blend reduces maximum power and torque of around 0.38% while raising specific fuel consumption by volume of around 0.10%. Analyzing the most realistic scenario in the near future – 7% HVO and 93% fossil diesel blend, the predicted fuel consumption increase (0.14%) and power and torque decrease (0.54%) is inconsiderable for vehicle exploitation, and HVO seems to be a promising biofuel to replace biodiesel in fuel blends and to promote reaching the EU targets.
Key words: biofuels, hydrotreated vegetable oil (HVO), mathematical modelling, power, torque, fuel consumption.

Introduction

A number of directives and regulations define biofuels use in the EU, for example, the Biofuels Directive, the Renewable Energy Directive, the Fuel Quality Directive etc. One of the latest documents – the Communication of the European Commission 'A policy framework for climate and energy in the period from 2020 to 2030' – was published on 23 January 2014.

Analyzing the EU targets 20/20/20 to be attained by 2020, i.e., greenhouse gas emissions reduction (20%), share of renewable energy (20%), and improvements in energy efficiency (20%), it was stated that the certain progress has been achieved (A policy framework …, 2014):

- greenhouse gas emissions in 2012 decreased by 18% relative to emissions in 1990 and are expected to reduce further by 24% and 32% in 2020 and 2030;
- the share of renewable energy has increased to 13% in 2012 as a proportion of final energy consumed and is expected to rise further to 21% in 2020 and 24% in 2030 respectively;
- the EU had installed about 44% of the world's renewable electricity (excluding hydro) at the end of 2012;
- the energy intensity of the EU economy has reduced by 24% between 1995 and 2011 whilst the improvement by industry was about 30%;
- the carbon intensity of the EU economy fell by 28% between 1995 and 2010.

The future of EU transport development should be based on alternative, sustainable fuels. The Commission has therefore not proposed new targets for the transport sector after 2020 (current target is 10% renewable energy). Based on the lessons of the existing target and on the assessment of how to minimise indirect land-use change emissions, it is clear that the first-generation biofuels have a limited role in decarbonising the transport sector (Biofuels Policy and Legislation, 2014) and the EU Commission has already indicated, that food-based biofuels should not receive public support after 2020 (A policy framework …, 2014).

At present the major part of the biofuels consumption in the world and in the European Union, including Latvia, is formed by the first-generation biofuels – biodiesel, bioethanol and rapeseed oil. Lately relatively little has been done to research the latest generation fuels and the results obtained are contradictory. The same inconsistency exists also in the classification of biofuels in the so-called generations (Carriquiry et al., 2011). The prevailing standpoint is that the first-generation biofuels commonly are derived from oil, starch or sugar containing plants that can be used in food. The second-generation biofuels are produced from non-food raw materials such as wood, straw, green grass, organic waste etc. Algae, microbes, cellulose and sea weed is a stock for the third-generation biofuels, but the fourth-generation biofuels in future will be produced from genetically modified plants (Carere et al., 2008; Scragg, 2009; Third and Fourth Generation ..., 2010; Demirbas, 2011a; Demirbas, 2011b; Nigam and Singh, 2011; Singh et al., 2011).

Hydrotreated vegetable oil (HVO) is one of the most promising next generation biofuels in the near future. It can be produced from the triglycerides based biomass such as vegetable oil, animal fat, waste cooking oil and algae (No, 2014).

Hydroprocessing of vegetable oils allows easy transformation of fatty acid triglycerides into

Table 1

Fossil diesel, biodiesel and HVO properties

Parameter	Fossil diesel	Biodiesel	HVO
Density, kg m^{-3}	820 … 850	860 … 900	775 … 785
Viscosity, mm^2 s^{-1}	2.2 … 3.5	3.5 … 5.0	2.5 … 3.5
Cloud point, °C	-5 … -30	-5 … 15	3 … -30
Distillation, °C	340 … 350	350 … 375	180 … 320
Lowest heating value, MJ kg^{-1}	42.5 … 43.0	37.5 … 38.0	43.8 … 44.0
Cetane number	51 … 60	50 … 65	80 … 99
Sulphur content, mg kg^{-1}	< 12	< 1	» 0
Oxygen content, mg kg^{-1}	» 0	» 11	» 0

hydrocarbons. Three most important reactions take place during processing (Šimáček et al., 2010):

- hydrogenation of double bonds present in unsaturated chains of bonded fatty acids;
- hydrodeoxygenation – removal of oxygen atoms from carboxylic group in the form of water;
- hydrodecarboxylation – elimination of carboxylic group in the form of carbon dioxide.

The main fossil diesel, biodiesel and HVO properties are summarized in Table 1 (Aatola et al., 2008; Šimáček et al., 2009; Arvidsson et al., 2011; Lapuerta et al., 2011; Bezergianni and Dimitriadis, 2013; Pinto et al., 2013; No, 2014; Kim et al., 2014).

It can be seen from the data of Table 1 that HVO is not oxygenated fuel and the density of it is lower than that of fossil diesel and biodiesel. HVO has ultra-low sulphur content, high cetane number and heating value which is very beneficial in fuel for combustion ignition (CI) engines.

Analysis of more than 50 different investigations of HVO application was performed in the Republic of Korea (No, 2014). It was concluded that HVO has a higher oxidation stability than biodiesel, but shows poorer low-temperature performance than fossil diesel.

Emission characteristics of neat HVO and blends of HVO with fossil diesel are widely investigated by many researchers. Most of these studies show that HVO generally reduces NO_x emissions compared to conventional diesel and biodiesel. Performing investigations in Finland (Aatola et al., 2008) the test engine was a turbocharged 8.4 liter 6-cylinder 4-stroke direct injection heavy duty diesel engine. The engine was equipped with a common-rail fuel injection system and a charge air cooler. No exhaust gas recirculation (EGR) or exhaust after treatment device was used. The nominal power of the engine was 225 kW at 2200 min^{-1}. The results of the investigations show that the use of hydrotreated vegetable oil enables reductions in CO, total hydrocarbons (THC), and NO_x emission, and in engine smoke without any changes to the engine or its controls.

However, only a few studies have been conducted on the spray and burn characteristics of neat HVO and blends of HVO with fossil diesel in CI engine conditions (No, 2014), as well as on the engine power and torque measurement, and fuel consumption determination.

Most of the studies investigating the use of HVO fuel are done by testing engines on the benches, but rarely – the car or tractor in general. For example, a 1.5 liter DOHC diesel engine was used for engine dynamometer test to evaluate the differences of performance using biodiesel and HVO blends with fossil diesel fuel (Kim et al., 2014). HVO and biodiesel blended diesel show decreases in the power – the more biodiesel or HVO is blended, the more power decreased, for example, blending 2% of biodiesel to fossil diesel the power loss was approximately 1.4%, blending 20% – approximately 2.5%, but blending 50% – more than 5%. Blending the same volume of HVO to fossil diesel the power loss was accordingly 0.7, 1.8 and 1.2%. Volume-based lowest heating values (LHVs) of biodiesel and HVO are less than that of fossil diesel. It is common that the maximum power decreases when lower caloric value fuels are used. Biodiesel blended diesel shows the increase of fuel consumption when the blending ratio goes up (approximately from 1 to 8%), but HVO show a slight decrease of fuel consumption while their blending ratios increase (up to 1%) (Kim et al., 2014).

The aim of this research is to perform mathematical modelling to determine the main diesel engine operating parameters (power, torque and fuel consumption) running them on HVO and its blends with fossil diesel fuel.

Materials and Methods

The first step to reach the aim of this investigation is creating the mathematical model to perform thermodynamic calculations of diesel engine operation, to construct engine's effective power and torque curves, and to calculate fuel consumption.

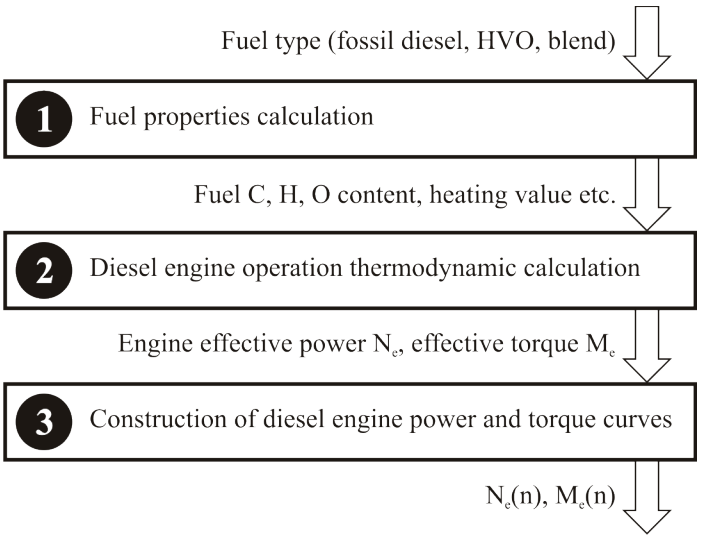

Figure 1. Model's block diagram.

Instead of creating a new model it was decided to modify an existing one that was developed in the doctoral thesis 'Rapeseed Oil Fuel Application in Diesel Engines and Logistics' (Dukulis, 2013) and described in the publication 'Development of the Model for Running the Diesel Engine on Rapeseed Oil Fuel and Its Blends with Fossil Diesel Fuel' (Dukulis and Birkavs, 2013).

This model was created in *ExtendSim* environment. Since the model is provided to evaluate the rapeseed oil fuel usage in diesel engines, the first module that calculates fuel's properties, for example, content of carbon (*C*), hydrogen (*H*) and oxygen (*O*) in fuel blend, heating value etc., have to be transformed substantially. The second module performs engine operation thermodynamic calculation, but the third one – constructs engine effective power and torque curves (Fig. 1). Last two modules do not need significant changes.

If the fuel blend percentage is known, the content of carbon (*C*), hydrogen (*H*) and oxygen (*O*) in fuel blend in fuel mass fractions can be calculated from the relationships (Šmigins, 2010):

$$C = \frac{\sum_{i=1}^{n} C_{cont-i} \cdot m_i}{\sum_{i=1}^{n} m_i}, \quad H = \frac{\sum_{i=1}^{n} H_{cont-i} \cdot m_i}{\sum_{i=1}^{n} m_i},$$

$$O = \frac{\sum_{i=1}^{n} O_{cont-i} \cdot m_i}{\sum_{i=1}^{n} m_i}, \qquad (1)$$

where

m_i – i_{th}-fuels content in blend, mass %;

C_{cont-i} – content of carbon in i_{th}-fuel, mass parts;

H_{cont-i} – content of hydrogen in i_{th}-fuel, mass parts;

O_{cont-i} – content of oxygen in i_{th}-fuel, mass parts.

The content of carbon, hydrogen, and oxygen in fossil diesel fuel is already known for a long time (the average values for modelling are assumed 0.870, 0.124, and 0.006 respectively). Since HVO is a relatively new fuel, a lot of researchers around the world investigate physicochemical properties of HVO depending on hydrotreatment temperature and catalysts. The average values are – 0.848 for carbon, 0.150 for hydrogen, and 0.002 for oxygen (Lapuerta et al., 2011; Pinto et al., 2013; Bezergianni et al., 2014).

The lower heating value Q_{lower} (J kg^{-1}) for any fuel can be calculated using classical relationship:

$$Q_{lower} = (33.91 \cdot C + 103.01 \cdot H - 10.89 \cdot O) \cdot 1000. \qquad (2)$$

The model blocks for determination of the fuel blend content and lower heating value are shown in Figure 2. Performing test simulations of these blocks, the calculated (theoretical) lower heating value for pure HVO is 44185 J kg^{-1}, but for fossil diesel fuel – 42209 J kg^{-1}. Comparing these values with the data from the Table 1, coincidence is close.

The second model's module 'Diesel engine operation thermodynamic calculation' determines engine's effective power and torque based on several fuel content sensitive parameters, for example, the theoretical amount of air required for combustion of 1 kg fuel, inlet pressure, temperature and pressure of fresh air-fuel mixture, residual gas pressure and coefficient, cylinder filling factor, pressure and temperature at the end of the compression stroke, the total and individual amount of combustion products, combustion products temperature etc. The

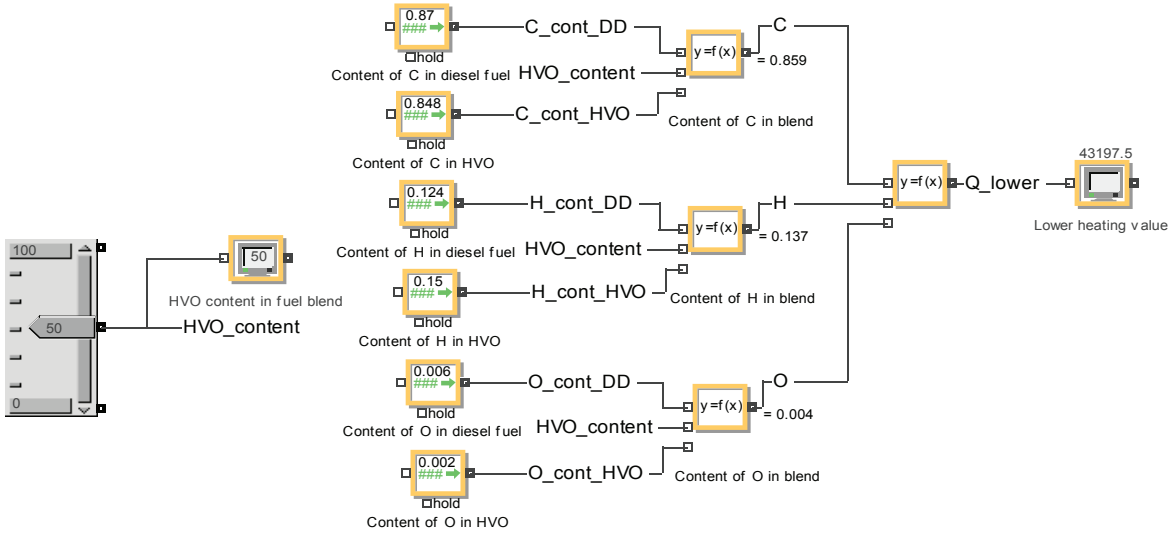

Figure 2. Fuel blend content and lower heating value determination blocks.

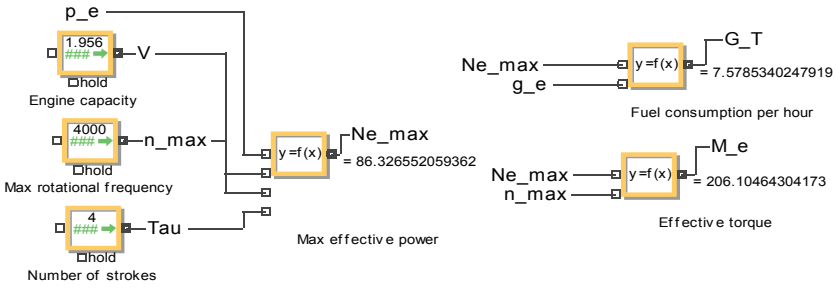

Figure 3. Engine power, fuel consumption and torque calculation blocks.

thermodynamic calculation of diesel engine operation is based on the classical relationships, given in the various sources of information (Internal Combustion Engine Handbook, 2004; Xin, 2011), but the existing model (Dukulis, 2013) was supplemented with a possibility to enter coefficients specific to the certain engine, depending on whether the engine is turbo-charged or not, with direct injection or precombustion chamber etc.

Since the diesel engine operation thermodynamic calculation module consists of about one hundred blocks only a few of them are shown in this article. The output parameters from the second module are: a maximum effective power N_{emax} (kW) at engine crankshaft rotational frequency n_{max} (min^{-1}), fuel consumption per hour G_T (kg h^{-1}), and an effective torque M_e (N m) at the same crankshaft rotational frequency n_{max} (Fig. 3).

If the maximum effective engine power N_{emax} and engine crankshaft rotational frequency n_{max} at which this power is developed are known, the engine effective power at any engine crankshaft rotational frequency can be determined according to the empirical relationship (Pommers and Liberts, 1985):

$$N_e = N_{e\max} \cdot \left[X \cdot \frac{n_e}{n_{\max}} + Y \cdot \left(\frac{n_e}{n_{\max}} \right)^2 - Z \cdot \left(\frac{n_e}{n_{\max}} \right)^3 \right], \quad (3)$$

where

N_e – engine effective power at engine crankshaft rotational frequency n_e, kW;

n_e – engine crankshaft rotational frequency at the point to be determined, min^{-1};

X, Y, Z – the empirical coefficients describing the engine type ($X + Y - Z = 1$).

If the value of the engine power in the full crankshaft rotational frequency range is known, the torque can be calculated using formula:

$$M_e = 9549 \cdot \frac{N_e}{n_e}. \quad (4)$$

Engine modelling studies are carried out for the same vehicle that is planned to be used later in the experimental studies, i.e., the passenger car *Opel Insignia 2.0 CDTi* (year of production – 2011, engine working capacity or volume – 1956 cm^3; compression ratio – 16.5).

Results and Discussion

In order to facilitate the input of variables and view the simulation results, a separate panel or window is set up. The essential elements of the model are 'cloned' in this window. Figure 4 shows an example of the car *Opel Insignia 2.0 CDTi* modelling.

The maximum power 94.49 kW for the car *Opel Insignia 2.0 CDTi* using diesel fuel is reached at 4000 min^{-1}, but maximum developed torque is 299.87 N m. Comparing acquired power and torque modelling values with the data given by motor vehicle manufacturers (128 hp or 96 kW and 300 N m respectively), differences do not exceed 2%. Such cut-off for modelling studies is permissible and does not interfere with the identification of differences among operating motor vehicles with various fuels.

The results of investigation 'Key properties and blending strategies of hydrotreated vegetable oil as biofuel for diesel engines' carried out in Spain, Colombia and USA (Lapuerta et al., 2011) show that a compromise between lubricity and cetane number would lead to a recommendation for low or medium HVO concentrations, and blends with concentrations above 50% would not be recommended in unmodified diesel engines. In colder regions, like in Latvia, especially in winter time cold flow properties of fuel blends also have to be considered. Every 10% of HVO in fuel blend deteriorate Cold Filter Plugging Point and Cloud Point temperatures accordingly by approximately 4.0 and 1.5 °C (Lapuerta et al., 2011). That is why in modelling studies blends of HVO with a diesel fuel in 5, 7, 10, 15, 20, and 25 vol.% are analysed. Additionally 7% blend is chosen because such amount of biofuel blend to fossil diesel is planned to be introduced in the fuel market in Latvia

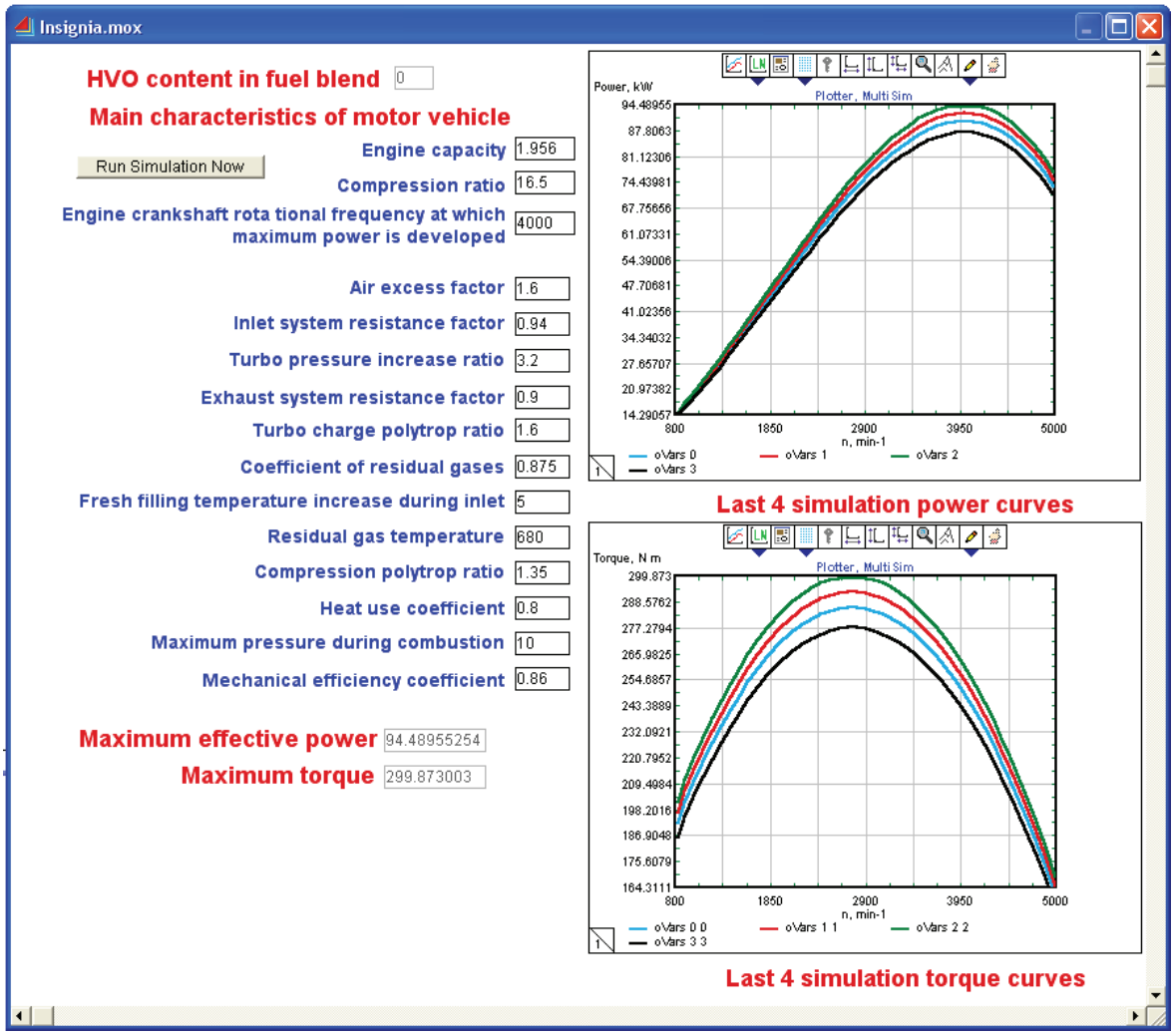

Figure 4. Example of the window for variables input and viewing of the simulation results.

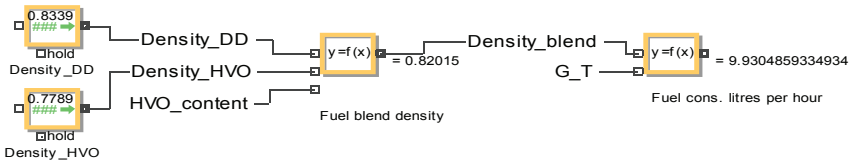

Figure 5. Fuel blend density and fuel consumption calculation blocks.

from 1 April 2014. Unfortunately, for now it could not yet be HVO because the Biofuel Law of Latvia, unlike many other countries, disclaim HVO as biofuel. But more than likely this problem legislatively will be solved in the near future.

Simulation results show, that the power and torque reduction for the car *Opel Insignia 2.0 CDTi* is linear – every 5% of HVO in fuel blend reduces maximum power and torque of around 0.38%, reaching the maximum power and torque difference for 25% HVO and 75% fossil diesel blend – 1.91%. Considering that 7% HVO and 93% fossil diesel blend is the most realistic scenario in the near future, predicted power and torque decrease (0.54%) is inconsiderable for vehicle exploitation.

Another important parameter is fuel consumption. In the case of HVO, the greater lower heating value (in modelling studies 4.5% higher than that of pure diesel fuel) makes reductions in specific fuel consumption when blends are used. Simulation shows that every 5% of HVO in fuel blend reduces specific fuel consumption of around 0.23%, reaching the maximum hourly consumption (kg h^{-1}) difference for 25% HVO and 75% fossil diesel blend – 1.15%. However, such interpretation on a mass basis is incorrect. The HVO fuel, despite having a greater heating value, has lower density. Since both diesel injection systems and fuel dispensing systems deliver fuel by volume, it is the volume-based heating value, and not the mass-based one which directly affects the engine specific fuel consumption. That is why additional blocks were added to the model recalculating hourly fuel consumption from kg h^{-1} to l h^{-1} (Fig. 5).

Recalculating hourly fuel consumption to l h^{-1} (at engine crankshaft rotational frequency n_{max} when the maximum effective engine power N_{emax} is developed) specific fuel consumption reduction transforms to the small increase – every 5% of HVO in fuel blend raises specific fuel consumption by volume of around 0.10%. For 25% HVO and 75% fossil diesel blend comparing with pure diesel fuel increase is 0.50%, but for pure HVO – 2.32%. Examining the most realistic scenario in the near future – 7% HVO and 93% fossil diesel blend, the predicted fuel consumption increase (0.14%) is inconsiderable for vehicle exploitation. It means that from both main operation viewpoints – dynamics and economy, HVO seems to be a promising biofuel to replace biodiesel in fuel blends and to promote reaching the targets estimated by EU directives and regulations.

Conclusions

1. An original mathematical model suitable to predict diesel engine operating parameters running them on HVO and its blends with fossil diesel fuel is developed using *ExtendSim* software.
2. Modelling results show that the reduction of engine power and torque, and the raise of specific fuel consumption for a car *Opel Insignia 2.0 CDTi* are linear – every 5% of HVO in fuel blend reduce the maximum power and torque of around 0.38%, at the same time raising specific fuel consumption by volume of around 0.10%. Using pure HVO the predicted fuel consumption increase is about 2.32% comparing with the diesel fuel.
3. Examining the most realistic scenario for the near future – 7% HVO and 93% fossil diesel blend, the predicted fuel consumption increase (0.14%) and power and torque decrease (0.54%) is inconsiderable for vehicle exploitation and from the theoretical point of view, HVO seems to be a promising biofuel to replace biodiesel in fuel blends and to promote reaching the targets estimated by EU directives and regulations.
4. In next studies it is necessary to carry out experimental investigations of vehicles to confirm the obtained modelling results.

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EFFECT OF IGNITION TIMING ON EMISSIONS OF SPARK IGNITION ENGINE USING E85 FUEL

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Abstract
This experimental study assesses the influence of ignition timing on emissions from a production four cylinder port injection spark ignition engine. The aim of this research was to evaluate the necessity of ignition timing correction when the regular gasoline vehicle is being adapted for the use of E85 fuel. Tests were conducted in the Alternative Fuels Research Laboratory of Latvia University of Agriculture in December 2013. The engine was fuelled with the ethanol-gasoline blend E85 or the commercial gasoline A95. The engine was tested within a vehicle in a chassis dynamometer in steady state conditions, which resemble driving at 50 km h^{-1} and 90 km h^{-1}. The original engine control unit was replaced with a programmable one. Engine-out and tailpipe exhaust gas samples were taken and analysed with a FTIR-type analyser AVL SESAM. Carbon monoxide (CO), unburned hydrocarbons (HC), nitrogen oxides (NOx), acetaldehyde and unburned ethanol emission volumetric share is presented. CO, HC and acetaldehyde emissions were not affected by variation of the ignition timing within the tested range. NOx and ethanol emissions were reduced with the ignition timing retard. The emissions of CO, HC and NOx were reduced, when the engine was fuelled with the E85 fuel, comparing with the gasoline use. Ignition timing, optimized for the gasoline, was found suitable for the E85 fuel from the emission analyses point.
Key words: ethanol, ignition timing advance, emissions.

Introduction

The biofuels are increasingly used as the energy source in the light passenger vehicles. The biofuels are considered as renewable energy source and contribute to the reduction of the greenhouse gas emissions (Costagliola et al., 2013). The ethanol has been known as a suitable fuel for the spark ignition engines since the 19th century. Currently all commercially available gasoline in Latvia is blended with the ethanol in 0.05 $m^3 \cdot m^{-3}$ volume ratio. One of the benefits of ethanol use as a fuel is the reduction of depletion of non-renewable energy resources. By adding ethanol, oxygen is blended into the fuel which favours the combustion of the gasoline, reducing toxicity of the emissions. The use of pure ethanol as the fuel is problematic due to the engine cold start difficulties below 13 °C. Gasoline on ratio 0.15 $m^3 \cdot m^{-3}$ is added to anhydrous ethanol to facilitate cold starting and act as a denaturing agent. Resulted fuel is designated E85 and is available in the service stations in many parts of the world, including Latvia. Due to the physiochemical differences of gasoline and ethanol, the use of E85 requires vehicle adaptation. Specially designed production vehicles (Flexible Fuel Vehicles or FFV) can use gasoline, E85 or mixture of both in any ratio. It is possible to convert regular gasoline vehicle for use of E85 fuel. Normally such converting is limited to increase of the fuel supply. Ignition parameters are usually left unchanged.

Engine tailpipe emissions of the vehicle, converted to use of E85, must be as low as possible and certainly within legal limits. Depending on the vehicle production date, different standards apply for acceptable level of certain, so-called 'regulated' emission gas components. Currently those emission gas components are nitrogen oxides (NOx), carbon monoxide (CO), total hydrocarbons (HC), non-methane hydrocarbons (NMHC) and particulate matter (PM). Ignition timing is known to be one of the factors that affect engine emissions (Heywood, 1998).

Combustion of the air-fuel mixture in the combustion chamber must occur in a certain moment of engine operating phase, to push piston downwards and efficiently convert chemical energy into mechanical energy. Due to the delay of ignition and duration of the combustion, ignition spark must be supplied with certain advance, depending on the engine design and operating conditions. Peak cylinder pressure and therefore temperature of combustion and exhaust gases are affected by spark timing. Timing, which provides maximal peak cylinder pressure, also provides maximal brake torque (MBT) (Heywood, 1988). Depending on the engine design, conditions of MBT may not provide optimally balanced emissions. Usually in production engines ignition timing is retarded from MBT. Retarded timing lowers burning temperature and increases temperature of exhaust gases. Lower burning temperature can decrease NOx emissions. Increased exhaust gas temperature can reduce hydrocarbon emissions (Heywood, 1988).

K. Silaipillayarputhur and S.A. Idem (2011) found that combustion duration increases with the engine speed. As combustion duration increases, engine output power decreases, because the cycle diagram further deviates from ideal Otto cycle. If the combustion duration increases, ignition advance must be also increased to maximize engine output

power. Delay of ignition and combustion flame speed is different for ethanol and gasoline. Laminar flame speed of regular gasoline is 33 m s^{-1} and 39 m s^{-1} for ethanol (Hara and Tanoue, 2006; Turner et al., 2011). Therefore, using ignition advance parameters, which are optimal for gasoline operation, will lead to non-optimal operation on fuel with high ethanol content, such as E85.

C. Sayin (2012) evaluated the impact of varying spark timing on the performance and emissions of a gasoline engine. He found a decrease of brake thermal efficiency and increase of brake specific fuel consumption, emissions of CO and hydrocarbons (HC), when gasoline with higher research octane number (RON) than the requirement of an engine was used at a nominal spark timing. The increase of ignition advance for gasoline with higher RON boosted engine power and decreased emissions of CO and HC, and decreased fuel consumption.

T. Topgül et al. (2006) studied the effects of gasoline and gasoline-ethanol blends on a single cylinder spark ignition engine performance and emissions. Experiments were conducted at a constant speed in wide open throttle mode. They concluded that using E60 fuel, ignition timing had to be retarded by 4 degrees, comparing to the use of pure gasoline E0, to achieve maximal brake torque. The increase of ethanol content in the fuel led to a decrease of exhaust gas temperature. CO emissions were reduced with an increase of ethanol content. They found increase of HC emissions with increase of ethanol content. Slight decrease of HC emissions was found when the ignition timing was retarded.

N. Türköz et al. (2014) conducted experiments on 4 cylinder carburettor SI engine using E85 fuel. They investigated the effect of ignition timing advance on the engine performance and emissions. They reported best engine performance and emissions when ignition timing was advanced by 4 degrees, comparing to use of pure gasoline. NOx emissions were increased with increase of ignition advance, CO and CO2 emissions were mainly unaffected and HC emissions increased with ignition retarding.

From the literature review, the impact of the spark timing on emissions of SI engine running on E85 fuel is not clearly studied. Some researchers are conducting experiments with carburettor engines, in wide open throttle mode. Wide open throttle operating conditions are rarely used in real life. Results of optimal ignition timing for high ethanol content fuel, comparing with gasoline are contradictory. The authors of this study investigated the effect of ignition timing advance on modern SI engine emissions in conditions, which resemble regular driving. The aim of this research is to evaluate the necessity of ignition timing fine tuning for E85 when the regular gasoline engine is being adapted for use with E85. Only the effect on emissions is investigated.

Materials and Methods

Tests were performed using two different fuels, purchased at the commercial fuel stations. Main properties of the test fuels were obtained from the certificates, provided by the fuel suppliers. Gasoline (A95) of EN228 standard had research octane number (RON) 95.4; motor octane number (MON) 85.9 and ethanol content 0.048 m^3 m^{-3}. E85 had RON and MON above 101; ethanol content 0.781 m^3 m^{-3}.

Conventional port injection SI engine was used. The specifications of the engine and the vehicle are listed in Table 1. The original engine control module (ECU) was replaced by a programmable ECU VEMS V3 to maintain the engine operating parameters within the required limits. The original narrowband oxygen sensor was replaced by a wideband sensor Bosch LSU 4.2. The original catalytic converter was retained.

Table 1

Main specifications of test automobile

Parameter	Value
Model	Renault Twingo
Identification number	VF1C068AE28944909
Date of production	30.04.2003
Engine	Type D7F 702, 4-cylinder 8-valve
Displacement volume, cm^3	1149
Piston bore / stroke, mm	69.0/ 76.8
Volumetric compression ratio	9.65
Gearbox	Type JB1 517, 5-gear manual
Gear ratios	Final drive 3.866; 4th gear 0.966; 5th gear 0.820

The engine was tested within the automobile. Load conditions were simulated in the laboratory on the edgy current roll type chassis dynamometer Mustang MD1750. The exhaust gas temperature (EGT) was measured in engine exhaust manifold. Engine exhaust emission gas samples were taken before the catalytic converter and from the tailpipe. Composition of exhaust gas was analysed with the Fourier transform infrared spectrometer (FTIR) AVL SESAM. A beam of wide spectrum infrared light is passed through the cooled and dried sample gas. The amount of energy absorbed at each significant wavelength is analysed. Signatures of absorbed wavelength and relation of absorbed energy to the volume of the specific gas component was recorded during factory calibration of the equipment. Composition and concentration of

the sample gas is determined using matrix calculation methods, comparing sample and calibration data. Measurement system allows for simultaneous measurement of 31 gas components with sampling rate 1 Hz. Results of the testing were expressed as a concentration timeline in volume unit share, parts per million (ppm). Volume shares of total HC and NOx were calculated by AVL SESAM system software. The results are not directly comparable to the results, obtained using other methods.

Wheel speed, power and torque were recorded using chassis dynamometer control software. The engine speed, exhaust gas temperature and air-fuel ratio were registered with ECU monitoring software VEMSTune.

Dynamometer was set at a constant speed mode, throttle opening was fixed to reach the required pressure in the inlet manifold. Variation of wheel power and torque between different test conditions was statistically insignificant and beyond the precision of chassis dynamometer measurement system.

Before testing, the ignition timing advance was mapped for maximal break torque (MBT) at stoichiometric air-fuel ratio for both fuels, E85 and gasoline. Detonation limits were not reached at chosen test conditions. Series of road tests were performed to find engine load conditions for two typical driving modes: at 50 km·h^{-1} and 90 km·h^{-1} steady driving on a flat road. All tests were performed at stoichiometric air-fuel ratio. Three test points of ignition timing advance, expressed in crank degrees before top dead centre (CA BTDC), at both driving modes were used:

- Timing set by vehicle producer for gasoline (nominal timing);
- Timing for maximal brake torque for E85 (MBT E85);
- Timing for maximal brake torque for gasoline (MBT A95).

Testing was conducted in steady state conditions, listed in Table 2.

Table 2

Test conditions

Parameter	Settings 1	Settings 2
Wheel speed, km×h^{-1}	50	90
Engine speed, min^{-1}	1859	2834
Gear	4th	5th
Wheel brake torque, N×m	23.50	39.60
Wheel brake power, kW	4.57	11.74
Ignition timing advance, nominal setting, degrees CA BTDC	31.5	31.0
Ignition timing advance, MBT E85, degrees CA BTDC	34.0	33.0
Ignition timing advance, MBT gasoline, degrees CA BTDC	36.0	37.0

The vehicle was driven on the chassis dynamometer in selected conditions for 340 seconds before the actual measuring started. Each test lasted 40 seconds and was repeated 5 times. Arithmetic mean of 5 valid test results was used as a result. Confidence intervals were calculated for 95% confidence level.

Results and Discussion

Temperature of the exhaust gases depends on an average temperature in the combustion chamber and timing of combustion in the engine cycle. Decrease of EGT is attributed to higher ignition timing advance (Fig. 1). Higher ignition advance increases maximal pressure and peak temperature in combustion chamber (Heywood, 1988). This effect does not directly reflect on EGT. As with higher timing advance combustion starts earlier in the engine cycle, larger part of oxidation reaction chain takes place inside the cylinder, which causes decrease of EGT. Ethanol has higher heat of evaporation comparing to gasoline (Turner et al., 2011). It can be attributed to the decrease of EGT when engine was tested with E85 fuel.

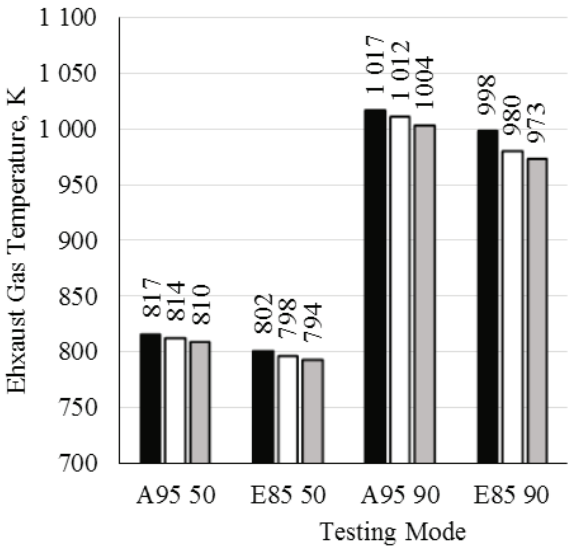

Figure 1. Temperature of engine-out exhaust gases: ignition timing: ■ 31.5…31, □ 34…33, ■ 36…37.

Hydrocarbon emissions consist of unburned fuel in gaseous state. HC emissions are largely caused by partial or complete misfire. Emissions of HC are known to increase in light load, low engine speed conditions (Sayin, 2012). Chain of oxidation reactions of hydrocarbons continues during exhaust stroke and outside of the cylinder. Therefore variations of HC emissions are related to variations of EGT (Heywood, 1988). HC emissions were insignificantly affected by changing of ignition timing at selected test conditions for both fuels (Fig. 2; Fig. 7).

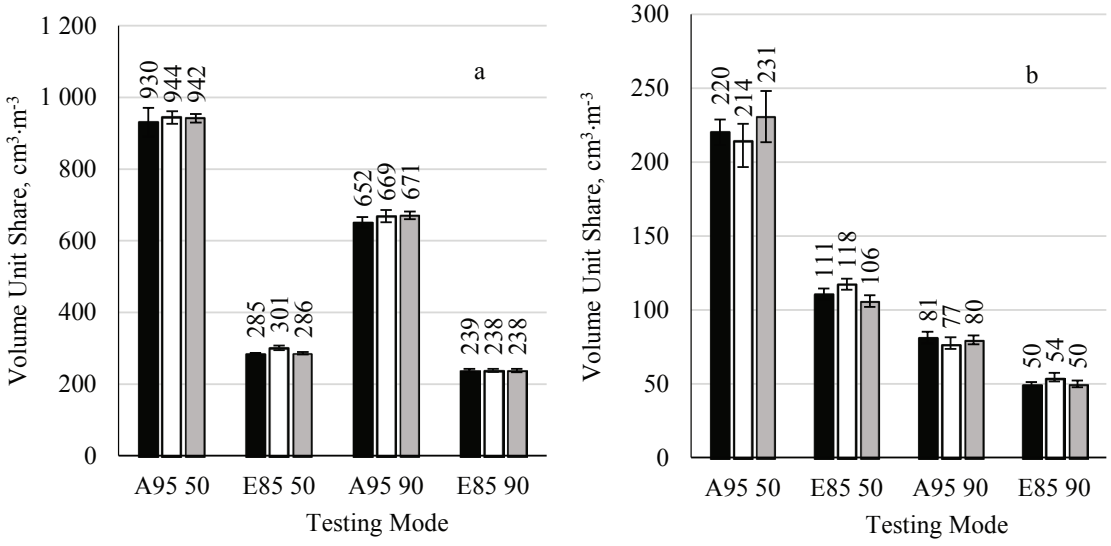

Figure 2. HC volume share:
a - engine-out, b - tailpipe; ignition timing: ■ 31.5…31, □ 34…33, ■ 36…37.

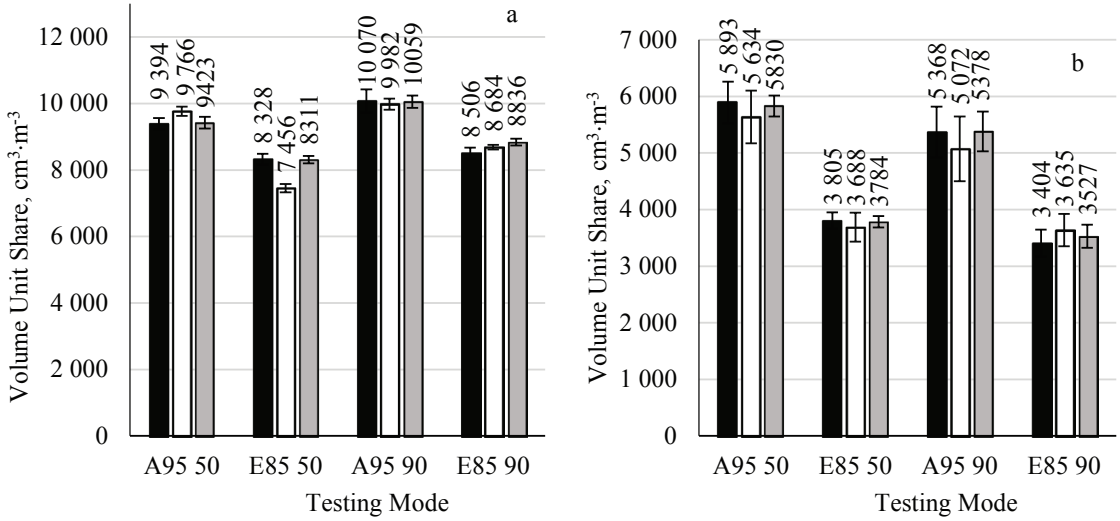

Figure 3. CO volume share:
a - engine-out, b - tailpipe; ignition timing: ■ 31.5…31, □ 34…33, ■ 36…37.

Apparently misfire rate did not change within the tested ignition timing range. Slight increase of exhaust gas temperature with ignition timing retard did not have effect on the reduction of HC emissions. HC engine-out emissions were reduced by 63-69% by volume, using E85 instead of gasoline (Fig. 7). Higher EGT, engine speed and load in 90 km h^{-1} test mode were attributed to the reduction of HC emissions.

CO formation is one of the steps of burning of hydrocarbons. Oxidation of CO to CO_2 requires relatively high temperature above 973 K. When the temperature in the combustion chamber falls as a result of advancement of expansion stroke, some amount of CO is not oxidised and forms part of exhaust gases (Heywood, 1988). The amount of CO emissions were insignificantly affected by variation of ignition timing within the tested range (Fig. 3; Fig. 7). The amount of CO engine-out emissions were reduced by 11-16%, comparing E85 use with gasoline.

The principal source of NO and NO_2 (which are summed as NOx) is the oxidation of nitrogen during the peak temperature phase at the beginning of the expansion stroke. Emissions of NOx are not a direct product of fuel combustion but rather a side effect. Ignition timing has a significant influence on the combustion temperature.

If the timing is retarded, ignition places combustion in a later point of engine cycle, when the piston has

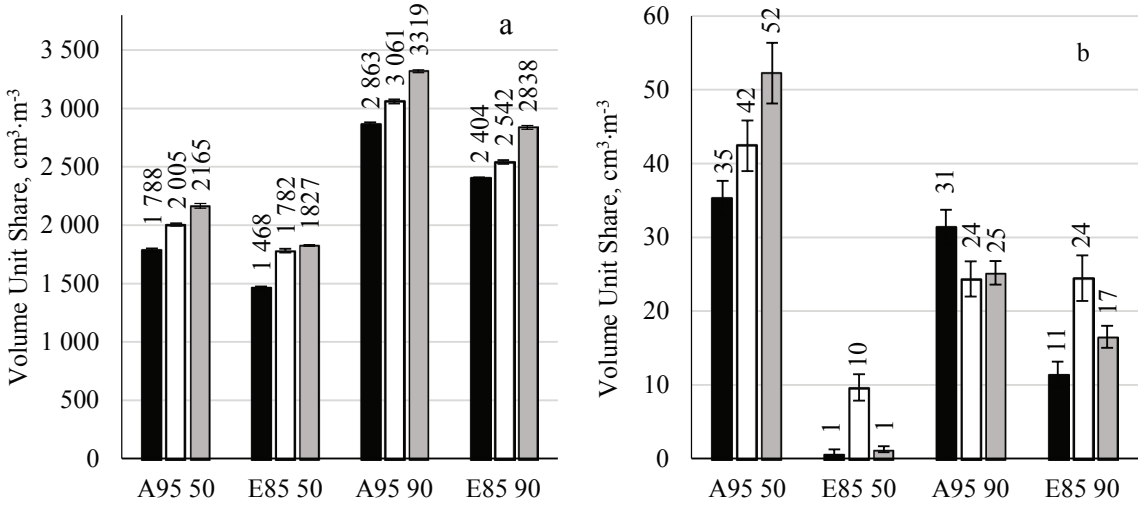

Figure 4. NOx volume share
a - engine-out, b - tailpipe; ignition timing: ■ 31.5…31, □ 34…33, ■ 36…37.

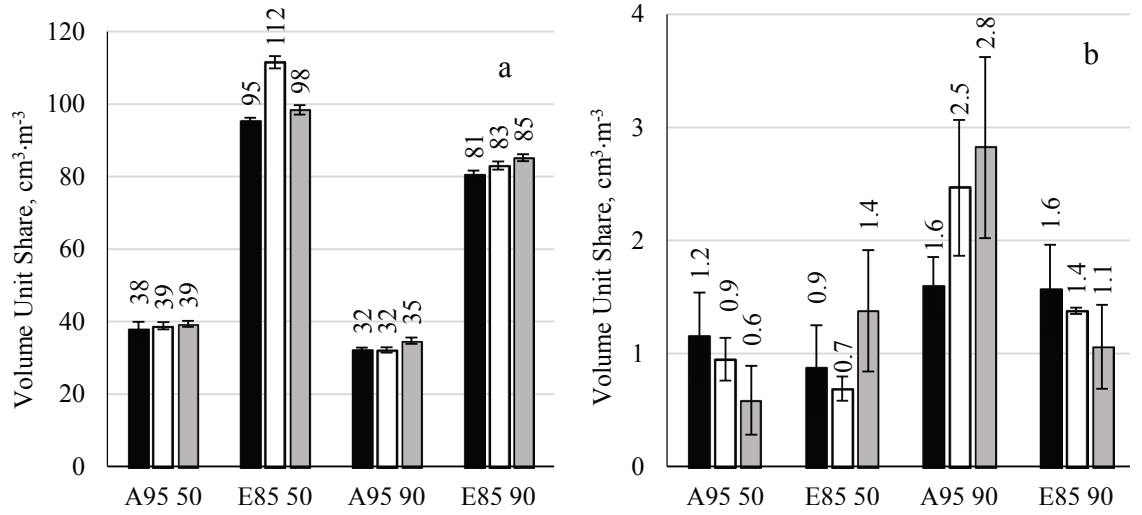

Figure 5. Acetaldehyde volume share:
a - engine-out, b - tailpipe; ignition timing: ■ 31.5…31, □ 34…33, ■ 36…37.

already started downward movement. Peak pressure and temperature is therefore reduced (Heywood, 1988).Test results showed a significant dependence of NO_x emissions on the spark timing (Fig. 4; Fig. 7). Retarding of ignition from MBT point decreased NOx emissions in all test conditions. Reduced NOx emission level was observed when E85 was used instead of gasoline. This can be explained by lower peak temperature in the combustion chamber. The original ignition timing, tuned for lower emissions using gasoline, was also effective when E85 was used. The reduction of NOx emissions using E85 instead of gasoline, at original spark timing (31 and 31.5 degrees CA BTDC accordingly) was noted: 16-18% for engine-out and 64-98% for tailpipe emissions.

Aldehydes in the engine combustion chamber are formed by cleavages of C-C or C-H bonds of hydrocarbons at high temperatures and partial oxidation of ethanol (Wagner and Wyszynski, 1996). The amount of acetaldehyde is known to raise with increase of ethanol content in fuel (Kumar et al., 2011). Test results demonstrated agreement with that in engine-out emissions (Fig. 5).

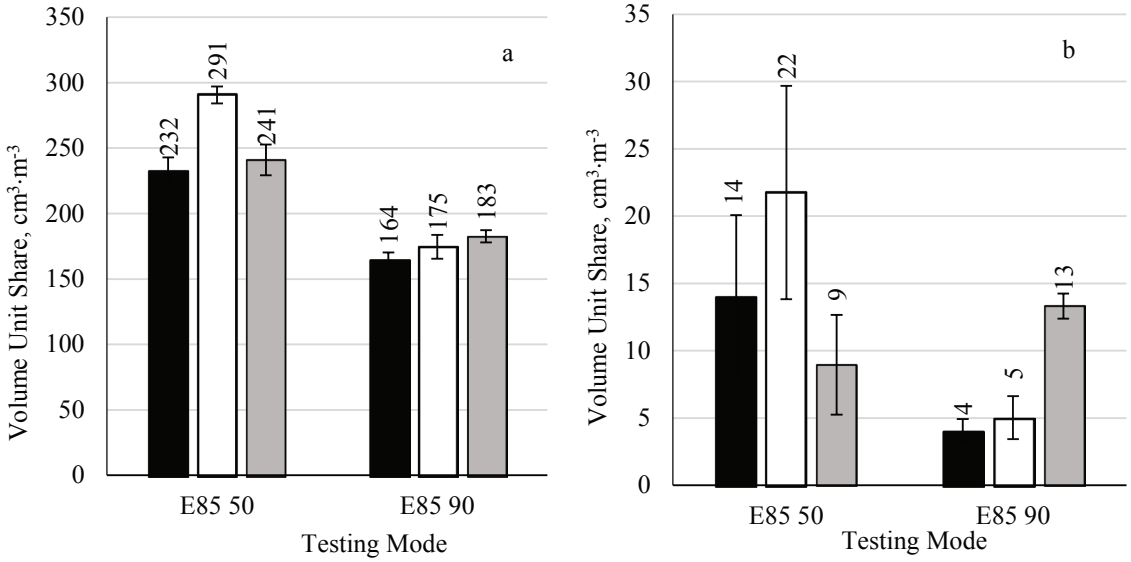

Figure 6. Unburned ethanol volume share:
a - engine-out, b - tailpipe; ignition timing: ■ 31.5…31, □ 34…33, ■ 36…37.

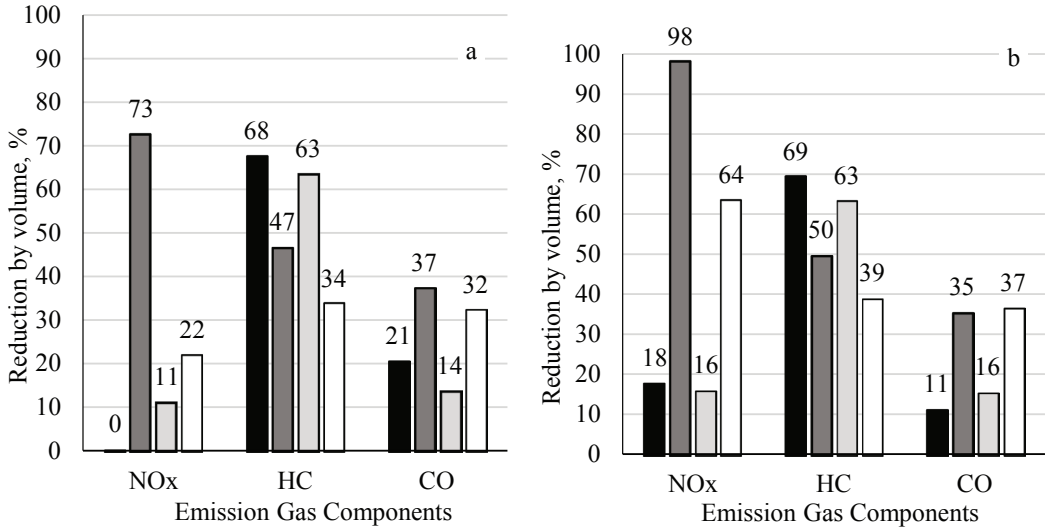

Figure 7. Comparison of reduction of regulated emissions using E85 with different ignition timing:
a - timing for MBT for E85 and nominal timing for gasoline, b - nominal timing for E85 and gasoline;
test conditions: ■ 50 km × h^{-1} engine-out, ■ 50 km × h^{-1} tailpipe,
■ 90 km × h^{-1} engine-out, □ 90 km × h^{-1} tailpipe.

Analysis of tailpipe emissions showed high efficiency of oxidising catalytic converter in the removal of acetaldehyde emissions. Emission levels at tailpipe did correlate directly with engine-out emissions of acetaldehyde. From the results it was concluded, that the ignition timing has no statistically significant effect on emissions of acetaldehyde.

Emissions of unburned ethanol were tested only with E85 fuel. Retarded ignition timing attributed to the reduction of ethanol engine-out and tailpipe emissions in 90 km h^{-1} test mode (Fig. 6). It can be explained with a higher average temperature in the combustion chamber and exhaust gases due to higher speed and load. When the exhaust gas temperature is high enough for oxidation of ethanol, the variation of temperature, caused by ignition timing, takes effect. Retarding of the ignition timing moves the start of ignition later in engine cycle, exhaust gas temperature is higher, and the amount of unburned ethanol is reduced in emissions.

Ignition timing for maximal brake torque using E85 in engine operating conditions, where detonations do not occur, have to be retarded, comparing to similar gasoline setup. Regulated emissions using E85 were found to be reduced, comparing to gasoline use in the tested steady state conditions. When adapting regular gasoline vehicle for the use of E85, the ignition timing for steady state operation can be left unchanged, at least from the emission analysis point. The impact of the ignition timing on the output parameters of cold start and dynamic driving using E85 is a subject for future work.

Conclusions

1. Volumetric share of the unburned hydrocarbons, carbon monoxide and acetaldehyde emissions was insignificantly affected by the variation of the ignition timing within the tested range.
2. The exhaust gas temperate increased with ignition timing retard and was higher when the gasoline was used, comparing to E85 use.
3. Nitrogen oxide emissions were reduced by 5-18% for engine-out and 25-42% for tailpipe emissions using E85 fuel at nominal spark timing comparing to maximal brake torque timing.
4. Unburned ethanol engine-out emissions were reduced by 6.3% in 90 km h^{-1} test mode at nominal spark timing comparing to maximal brake torque timing.
5. The emissions of the unburned hydrocarbons, carbon monoxide and nitrogen oxides were reduced in steady state test conditions using E85 fuel, comparing to the gasoline use.
6. The amount of acetaldehyde was increased up to 63% in the engine-out emissions using E85 fuel, when compared with the gasoline use.
7. Ignition timing, adjusted by the test vehicle's manufacturer for gasoline use, was found suitable for E85 from the emission analyses point.

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HEATING OF LOW - POWER INDUCTION MOTOR UNDER NO-LOAD MODE AND DIFFERENT COOLING CONDITIONS

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Abstract
The paper discusses heat transfer and the step response of a low-power induction motor to no-load mode under fan cooled and naturally cooled conditions. In Latvia University of Agriculture (LUA) in the electric drive laboratory the experimental tests were performed on a 1.1 kW totally enclosed fan cooled three phase induction motor with a fan mounted on a shaft for fan cooled conditions and with a fan taken off for naturally cooled conditions. The transient temperatures are measured in nine points of the stator end windings and in two points of the stator frame using thermocouples. Temperature is measured by using K type thermocouples and Pico-Log TC – 08 data logger. The current and voltage are measured by using Simple Logger II L562 two channel data logger. Measurement data are processed and archived using data loggers Pico-Log Recorder, Simple Logger II and Data View software. The experimental test results show that ventilation plays an essential role in the heating process of small power induction motors. Mathematical and virtual models of induction motor windings heating are represented to simulate the heating process of induction motor under no-load mode and different cooling conditions.
Key words: induction motor, no-load mode, heat dissipation, cooling conditions, modeling.

Introduction

Approximately 90% of the electric motors used in industry and homes are either three-phase induction motors or single-phase induction motors. Three-phase induction motors (IM) are used as drive motors in pumps, lifts, cranes, hoists, lifts, compressors, fans, driving lathe machines, crushers, mills, conveyors, etc. IM have high reliability and simple construction, but annual motor failure rate is conservatively estimated at 3-5% per year, and in extreme cases, up to 12% (Venkataraman et al., 2005). IM failures cause essential direct and technological losses, involving motor change and repair, as well as interruption of the production process.

IM failures may be classified as follows: 1) electrically related failures ~35%; 2) mechanically related failures ~ 31%; 3) environmental impact and failures related to other reasons ~ 34% (Venkataraman et al., 2005; Boldea and Nasar, 2010). Statistics of IM failure reasons show that many of them are caused by the overheating of the different parts involved in IM operation. The most sensitive part of IM to thermal overloads is the stator windings. The main limiting factor of an IM, is the stator windings temperature. Exceeding the temperature limit, results in acceleration of the oxidation process in insulation materials that eventually leads to IM being damaged. Because of this, an adequate protection system and accurate monitoring of IMs is very important to prevent thermal overloads.

Detailed description of experimental and analytical research methods and results of the transient heating of IM parts and thermal modelling is given in literature (Kylander, 1995; Zhang, 2010; Sniders and Gedzurs, 2012). The experimental investigations are performed on low-power totally enclosed fan cooled (TEFC) induction motor under stall, no-load, rated and overload conditions. The heating process of the IM is researched with different loads. The ambient temperature and cooling conditions are constant during all tests. Investigations of induction motors, protection systems and operation conditions have been performed in grain elevators. Investigations show that part of IM operates in enclosed dusty places (Gedzurs, 2013). Induction motor casings are covered by a layer of the dust, which decreases or blocks air flow from the cooling fan. That causes overheating of the IM parts due to lower heat transfer, even at loads lower than rated.

The objective of the study is to get experimental characteristics and thermal parameters during the heating process of the IM stator windings and frame under no-load fan cooled and naturally cooled conditions.

Materials and Methods

The heating process research was performed on a three phase induction motor: 4AX80A4Y3; 220/380 V; 4.9/2.8A; IP44; insulation class - B, m = 14.5 kg; P = 1.1 kW; n = 1400 min^{-1}; s = 0.67; $\eta = 0.75$; $\cos\varphi = 0.81$. Tests were performed in LUA in the electric drive laboratory. The block diagram of the experimental setup for conducting tests is shown in Figure 1. The test bench was fitted with laboratory measuring equipment – voltmeters (V), ammeters (A) and wattmeters (W) for monitoring of the three phase current, voltage and power. For temperature measuring of IM stator frame (casing) and windings, eleven miniature K-type thermocouples BK-50 (air probe – SE000) were installed. All thermocouples were connected to a data logger Pico-Log TC-08 with built in cold junction compensation (the accuracy of

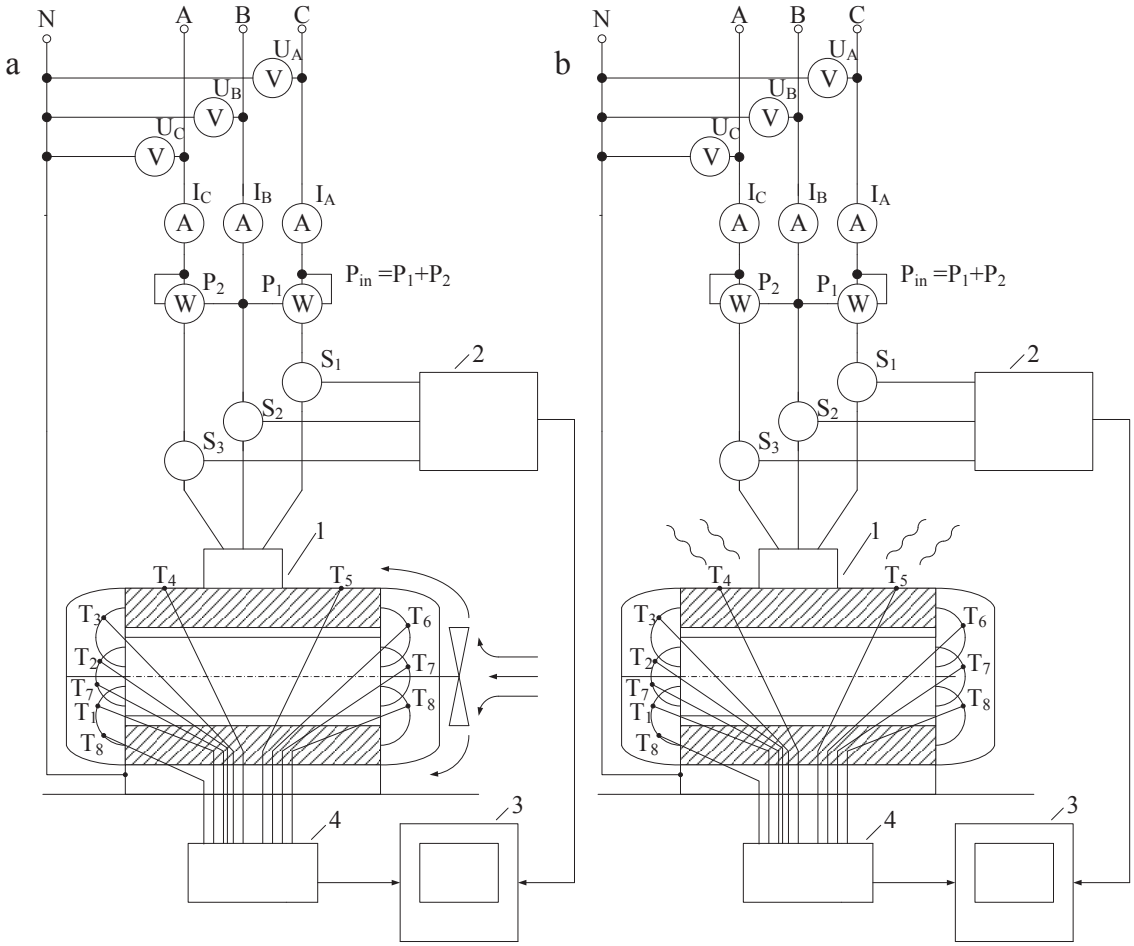

Figure 1. Experimental setup for induction motor heating process research: a - setup for fan cooling conditions; b - for naturally cooling conditions; 1 - Induction motor; 2 - Simple Logger II data logger; 3 - Computer; 4 - TC - 08 data logger; S_1 - current sensor; S_2,S_3 - voltage leads; T_1 ... T_{11} - thermocouples; A - ammeter; V - voltmeter; P - wattmeter; I - electric current; U - voltage; P - power.

temperature reading – ± 0.2% of temperature value ± 0.5 °C). The stator frame surface temperatures were measured in two points with thermocouples T_7 and T_8. They were mounted at the frame shaft side and fan side. The stator winding temperatures are measured for each phase (A, B, C) by nine thermocouples attached to the end windings. Six thermocouples (T_1, T_2, T_3, T_4, T_5, T_6) were placed at the shaft (drive) side end windings, other three thermocouples (T_9, T_{10}, T_{11}) - in the end windings at the fan side. The temperature of shaft side end windings was measured in 2 areas – near frame (T_1, T_2) and near rotor (T_3, T_4, T_5, T_6). The thermocouples were inserted into natural gaps in the end windings and bonded by thermal bandage. For measuring of IM stator current and voltage – the current sensor (current clamps 3XTA011AC), voltage leads and data logger Simple Logger II L562 (accuracy – current ± 0.5% of reading + 1 mV, voltage – ± 0.5% of reading +1 V) were used.

All heating tests were performed under cold initial conditions – the initial temperature of IM parts was equal to ambient temperature ($\theta_0 = \theta_a$). The input voltage and frequency were uniform with rated values (400V, 50 Hz) in all phases. The tests of the windings and frame transient temperatures were performed for two conditions of IM operation: 1) no-load with the fan on the shaft (fan cooled); 2) no-load without the fan (naturally cooled). Nonlinear regression method was used for the mathematical and statistical analysis of the data. To simulate the heating process of the IM windings MATLAB SIMULINK software was used.

Results and Discussion

Experimental test results of the IM thermal response to no-load and fan cooled conditions are shown in Figure 2. The initial temperature of the IM parts is equal to the ambient temperature $\theta_o = \theta_a$ = 24 °C. The temperature of IM parts reaches

steady-state value 50 minutes after the start of the test. The steady-state temperature of the shaft side end windings is 65 °C. The fan side end winding temperature is lower by 3 °C. The steady-state temperature of the shaft side stator frame temperature is 42 °C, 5 °C higher than the fan side stator frame temperature. The difference between the fan side and shaft side temperatures of the IM parts is due to different convection heat transfer and shows the efficiency of ventilation.

Figure 3 shows the results of the IM thermal response to no-load and naturally cooling conditions. The insulation class B allows continuous overheating of windings until θ_{max} = 120 °C. The test should be stopped when the temperature of windings reaches 120 °C. Switch-off point temperature of the shaft side end windings is 120 °C – frame side and 117 °C – rotor side. The lower temperature of the rotor side is caused by the ventilation effect of the rotor blades. A nonlinear regression equation (1) is used for the experimental data approximation. The standard error S of regression value for IM end winding heating under fan cooled conditions is S = ± 0.73 °C and for naturally cooled conditions S = ± 1.24 °C.

$$\theta(t) = \theta_{max} - \Delta\theta_{max} \cdot e^{-\frac{t}{T}} = (\Delta\theta_{max} + \theta_o) - \\ - \Delta\theta_{max} \cdot e^{-\frac{t}{T}} = \Delta\theta_{max} \cdot (1 - e^{-\frac{t}{T}}) + \theta_o , \quad (1)$$

where θ – temperature of IM part, °C;
θ_o – ambient temperature, °C;
θ_{max} – steady-state temperature of IM part, °C;
$\Delta\theta_{max} = \theta_{max} - \theta_o$ – maximum temperature raise, °C;
T –thermal time constant, min;
t - time, min.

The measured active power and current under no-load and fan cooled conditions is P_1 = 150 W; I_1 = 2.25 A, during no-load and naturally cooled – P_2 = 145 W; I_2 = 2.2 A. The measured active resistance of winding at 25 °C is R = 7.2 Ω. At no-load all active power converts to losses. No-load losses consist of electrical losses in the stator and rotor, magnetic losses and mechanical losses. No-load electrical losses in the rotor are small and can be neglected. Therefore, heating of windings is produced by electrical loses in the stator windings P_1 and can be calculated knowing the stator current and active resistance.

For simulation of IM stator winding heating under continuous no-load operation mode for fan -cooled and for natural convection cooling conditions, the mathematical model of transient heating is compiled. Non-stationary heating may be described by differential equation (2):

$$P_1 = C \cdot \frac{d\Delta\theta}{dt} + H \cdot \Delta\theta \quad (2)$$

where P_1 – electrical losses in stator windings, W;
$\Delta\theta = \theta - \theta_o$ - temperature raise, °C;
C – thermal capacity, $J \cdot °C^{-1}$;
H – heat dissipation, $W \cdot °C^{-1}$.

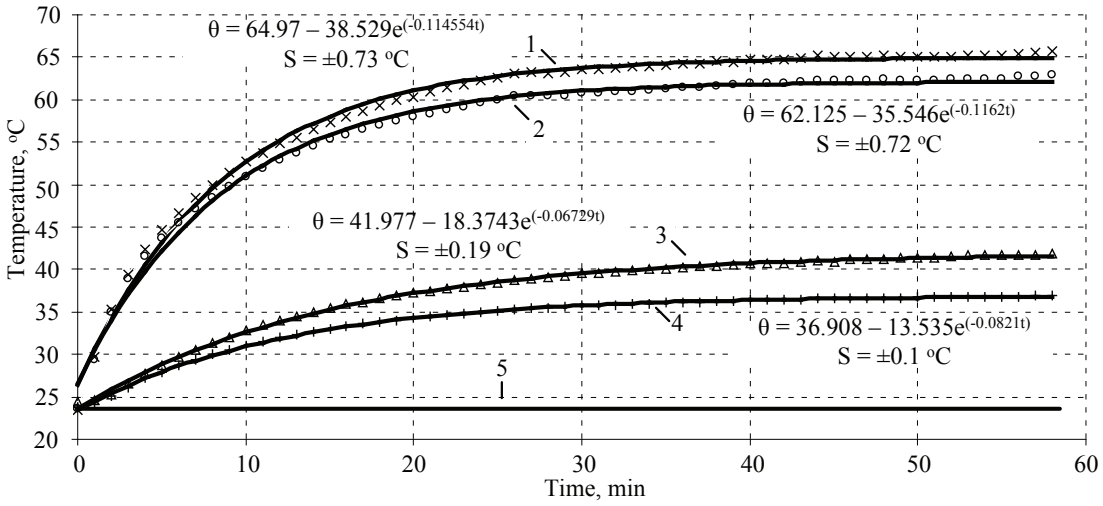

Figure 2. Response of induction motor parts temperature under no-load and fan cooled conditions:
1 - temperature of stator end windings - shaft side; 2 - temperature of stator end windings - fan side;
3 - temperature of stator frame - shaft side; 4 - temperature of stator frame - fan side;
5 - ambient temperature.

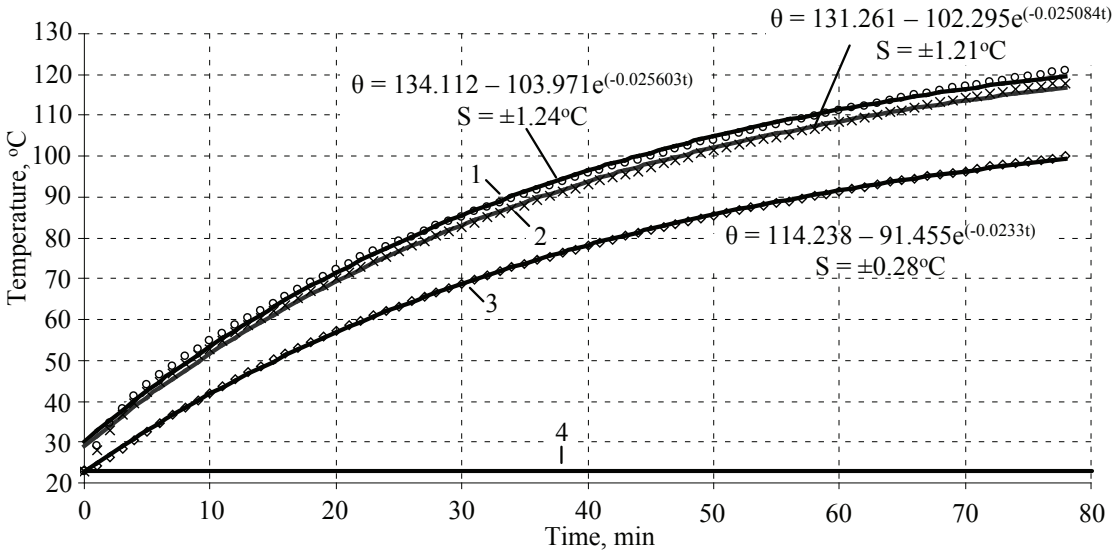

Figure 3. Response of induction motor parts temperature under no-load and naturally cooled conditions: 1 - temperature of stator end windings; 2 - temperature of stator end windings - inner side; 3 - temperature of stator frame; 4 - ambient temperature.

After modification of equation to (2), the differential equation in normal form has been obtained:

$$T\frac{d\Delta\theta}{dt}+\Delta\theta=K\cdot P_1, \quad (3)$$

where $T = C \cdot H^{-1}$ – IM thermal time constant, min;
$K = 1 \cdot H^{-1}$ – IM sensitivity coefficient, $°C \cdot W^{-1}$.

By solving differential equation (3), the heating process of stator windings can be described by exponential function (4):

$$\Delta\theta(t)=\Delta\theta_{max}(1-e^{-\frac{t}{T}})=K\cdot P_1(1-e^{-\frac{t}{T}}), \quad (4)$$

Using Laplace transforms to differential equation (4), an operational equation and transfer function is compiled for simulation in MATLAB SIMULINK:

$$T\cdot\Delta\theta(s)\cdot s+\Delta\theta(s)=K\cdot P_1(s), \quad (5)$$

where s – Laplace variable, min^{-1}.
$\Delta\theta$ (s) – Laplace transform of temperature raise, °C;
$P_1(s)$ – electrical losses in stator windings, W;

$$W(s)=\frac{\Delta\theta(s)}{P_1(s)}=\frac{K}{T\cdot s+1}, \quad (6)$$

Thermal capacity C, losses in windings P_1, heat dissipation and sensitivity coefficient K can be calculated for each of IM operation conditions using experimental tests results:

$$K_1=\frac{\Delta\theta_{1max}}{P_{11}}=\frac{38.5}{108}=0.35\ °C\cdot W^{-1};$$

$$H_1=\frac{1}{K_1}=\frac{1}{0.35}=2.8W\cdot °C^{-1};$$

$$C=T_1\cdot H_1=8.73\cdot 60\cdot 2.8=$$
$$=1469J\cdot °C^{-1};H_2=\frac{C}{T_2}=$$
$$=\frac{1469}{39.06\cdot 60}=0.627W\cdot °C^{-1}$$

$$P_{11}=3\cdot I^2\cdot R=3\cdot 2.25^2\cdot 7.2=108W;$$

$$P_{12}=3\cdot I^2\cdot R=3\cdot 2.2^2\cdot 7.2=103W$$

Using equation (6) and calculated thermal parameters of stator winding a virtual model in MATLAB SIMULINK has been developed (Figure 4). The following functional blocks are used: '$P_{1\ step}$'- step signal power losses generator; 'P_1', '$\Delta\theta_{max}$', 'C', 'H' – constant signal generator blocks for coefficient K and thermal time constant T calculation. Digital displays are used to visualize the input and output values: - P_1 – electrical losses, W; T – thermal time constant, min; θ – stator end windings steady-state temperature, °C. 'Scope' for visualization of stator winding temperature transient heating process - θ = f(t). Simulation results of the shaft side end winding heating process under no-load fan cooled and naturally cooled conditions are shown in Figure 5.

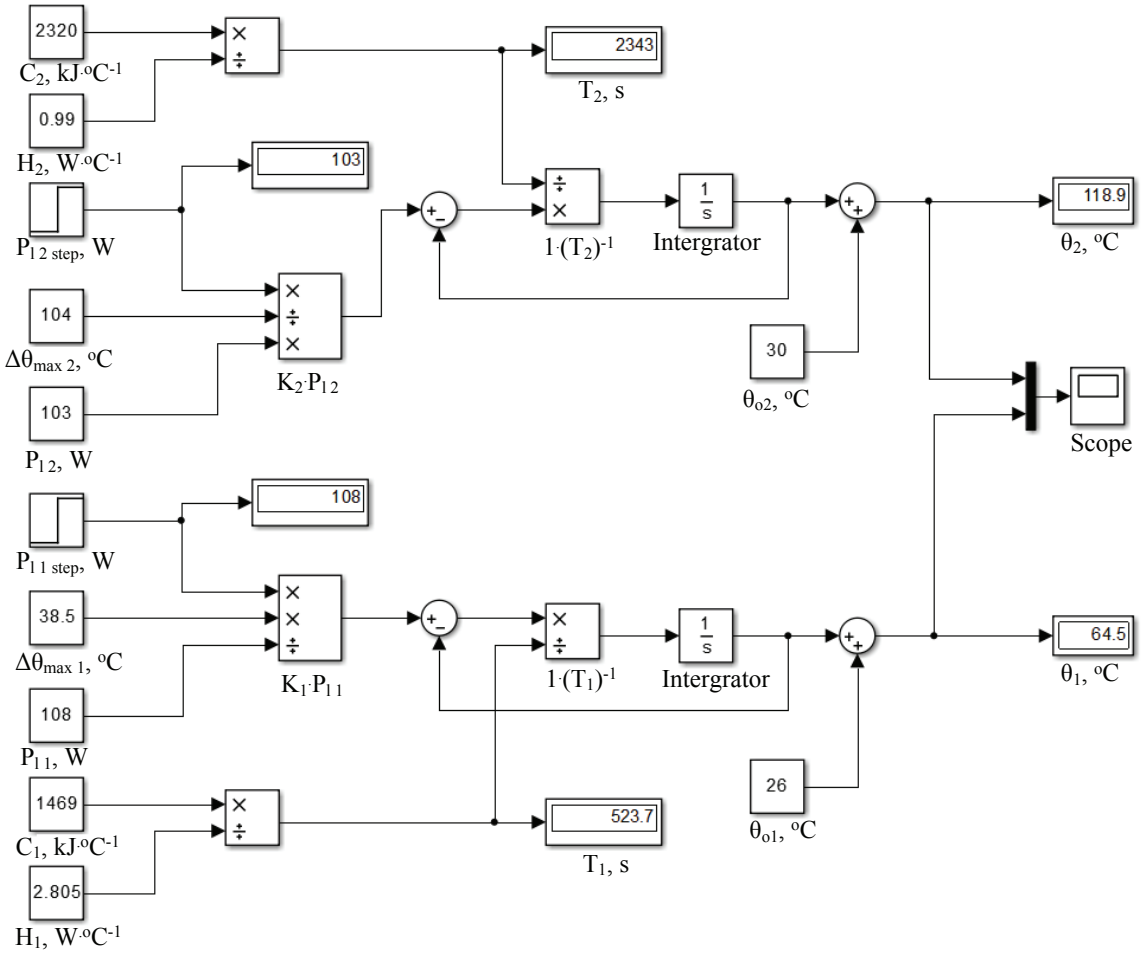

Figure 4. Simulation block diagram of heating process of IM end windings.

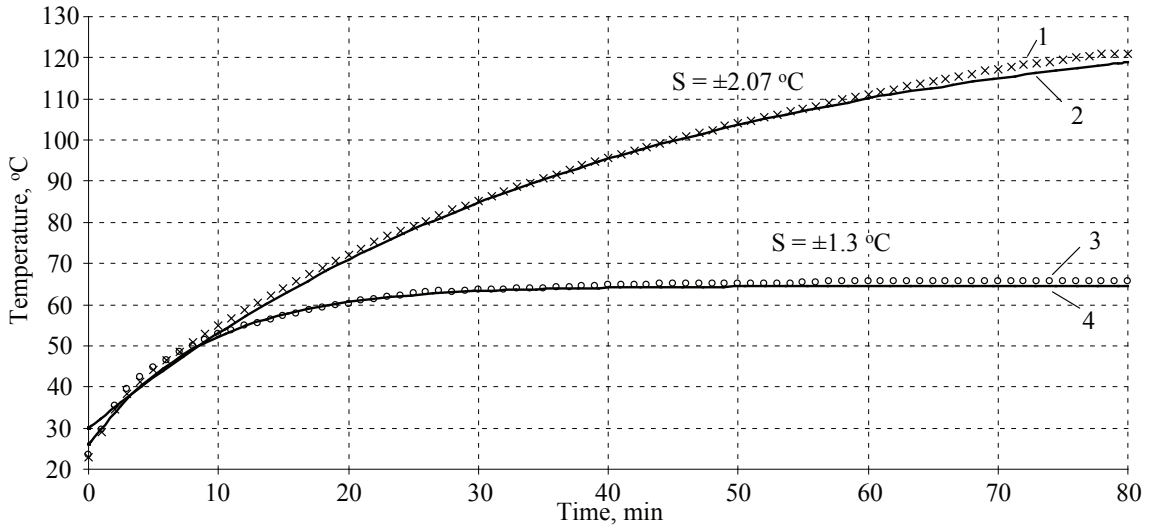

Figure 5. Simulation results of heating process of IM shaft side end windings under no-load fan cooled conditions: 1 – heating process test results under no-load naturally cooled conditions; 2 – heating process simulation results under no-load naturally cooled conditions; 3 – heating process test results under no-load fan cooled conditions; 4 – heating process simulation results under no-load fan cooled conditions.

The standard error for simulations results of no-load fan cooling conditions is S = ± 1.3 °C and under no-load naturally cooled conditions - S = ± 2.07 °C. Simulation of no-load naturally cooled conditions show that the steady-state temperature reaches 132 °C in 240 min. The results show that the virtual model is adequate and can be used to simulate the heating process and get thermal parameters under conditions that cannot be achieved experimentally due to safety reasons.

Conclusions

1. The results of the experimental tests that under no-load fan cooled conditions the stator end winding maximum temperature is θ_{max} = 65 °C, but under no-load naturally cooled conditions the winding temperature reaches the limit value of 120 °C for thermal class B in 78 min. Therefore, forced ventilation plays an essential role in the induction motor cooling process.
2. During the heating process under naturally cooled stator the current decreases by 6% and overload protection relays will not trip if the induction motor windings are overheating. Therefore, a protection device that monitors temperature of the windings directly has to be used to ensure adequate protection of IM in places under variable cooling and environment conditions.
3. The experimental data and simulation results show that windings heating process may be represented as the first order inertial model described by differential equation with constant thermal parameters for each operation conditions. Simulation results show, that the standard error under no-load fan cooled conditions is S = ± 1.3 °C and under naturally cooled conditions S = ± 2.07 °C, that testifies simulation adequacy to experimental data.
4. Simulation results under no-load naturally cooled conditions show that the steady-state temperature of the end windings is θ_{max} = 132 °C, which cannot be achieved experimentally due to safety reasons. It proves that a virtual model can be used to increase the range of investigation of IM heating under operations conditions that cannot be done experimentally.

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Volume 1