Volume 1

Latvia University of Agriculture

Annual 21st International Scientific Conference

Research for Rural Development 2015

Latvia University of Agriculture

RESEARCH FOR RURAL DEVELOPMENT 2015

Annual 21st International Scientific Conference Proceedings

Volume 1

Jelgava 2015

LATVIA UNIVERSITY OF AGRICULTURE

ONLINE ISSN 2255-923X
ISSN 1691-4031

RESEARCH FOR RURAL DEVELOPMENT 2015
http://www2.llu.lv/research_conf/proceedings.htm

Volume No 1 2015

The ethic statements of the conference „Research for Rural Development 2015" are based on COPE's Best Practice Guidelines: http://www2.llu.lv/research_conf/proceedings.htm

Approved and indexed:The Proceedings of previous Annual International Scientific Conferences "Research for Rural Development" published by Latvia University of Agriculture since 1994 and has been approved and indexed in to databases: AGRIS; CAB ABSTRACTS; CABI full text; EBSCO Academic Search Complete; Thomson Reuters Web of Science; Thomson Reuters SCOPUS.

Editorial office: Latvia University of Agriculture, Lielā ielā 2, Jelgava, LV -3001, Latvia
Phone: + 371 630 05685; e-mail: Ausma.Markevica@llu.lv

Printed and bound in „Drukātava"
Supported by:

LATVIA UNIVERSITY OF AGRICULTURE

ONLINE ISSN 2255-923X
ISSN 1691-4031

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http://www2.llu.lv/research_conf/proceedings.htm

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FOREWORD

The four independent reviewers estimated each paper and recommended 89 articles for publishing at the proceedings consisted of 2 volumes, which started life as presentations at the Annual 21st International Scientific Conference "Research for Rural Development 2015" held at the Latvia University of Agriculture, in Jelgava, on 13 to 15 May 2015.

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 185 researchers with very different backgrounds. There were 147 presentations from different universities of Lithuania, Estonia, Poland, Turkey, Greece, Slovakia, Nepal, Russia, 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 21st International Scientific Conference "Research for Rural Development 2015" (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 21st International Scientific Conference

"Research for Rural Development 2015"

Ausma Markevica
Latvia University of Agriculture

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MAPPING OF SOIL SALINITY PREDICTED BY DRAINMOD FOR DRAINED AND UNDRAINED CONDITIONS IN IRRIGATED LANDS

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Abstract
The purpose of this study was to predict and compare salt accumulation in the soil profile under drained and undrained conditions. The water management simulation model, Drainmod (Ver. 6.1) was used to determine the optimal drainage system design parameters, which will decrease soil profile salinity and provide maximum crop yields in Ankara-Bala Basin of Turkey. Soil sampling points were coordinated with the Global Positioning System (GPS). Soil, crop and site parameters were obtained as an input. The model was run for 5 years from 2005 to 2010 to simulate optimum drainage design parameters (drain depth, drain spacing) while controlling soil salinity in the root zone. Soil water conditions and soil salinity level were simulated for crop rotation of corn (*Zea mays*) and winter wheat (*Triticum*). Yield of individual crops was predicted for each growing season. The results of the simulations were analyzed to identify alternatives of subsurface drainage system that would satisfy maximum crop productions. According to the simulation results, the drain spacing of 130 m and drain depth at 160 cm are recommended for Bala Basin. Soil salinity maps were created for undrained and drained conditions. Results showed that the soil salinity level and salinity stress can be reduced and yield increased by installing a drainage system.
Key words: soil salinity, Drainmod, drainage, mapping.

Introduction

Under most arid and semi-arid climate, as is the case with almost all Mediterranean countries, drainage improvement works are needed to alleviate waterlogging and salinity problems caused directly, or indirectly, by irrigation practices. And more often than not, subsurface drainage systems are needed to reclaim these areas for viable agricultural production. The main cause for waterlogging and soil salinization is usually water seepage from the irrigation canals that lose a lot of water through their unlined banks and beds (Ayers et al., 1987). Furthermore, frequent irrigation applications also tend to keep the water table close to the soil surface, and this combined with normal fertilizer applications causes a slow salinization of the root zone, and affects crop yields. The natural drainability of these soils, as such, cannot cope with this man-caused problem. If this phenomenon is not checked on time through the installation of interceptor drains and subsurface drainage systems, most of the farmland that was very productive at one point becomes unproductive. Then the farmers either have to change their cropping practices or, in some cases, they cannot grow any crop at all (Gupta et al., 1993).

Waterlogging problems in arid and semi-arid regions are usually associated with high salinity problems. Salinity build-up in the soil has an adverse effect on crop yield because of many factors. The processes involved are complicated, and interrelated with such factors as crop species, soil properties and salinity of irrigation water and subsurface drainage (Kandil et al., 1995). Computer simulation models are developed to describe this comprehensive system. Drainmod is one of the well known drainage simulation models used to characterize the response of the soil water regime to various combinations of surface and subsurface water management.

Bala basin opened for irrigation in 1970. Until mid-1980 the irrigation rate was not more than 50% because of the inadequate system component, field problems or uneducated farmers. In recent years, the irrigation rates are much higher than before, however, farmers meet different kind of problems at this time. The most important problem is that no efficient drainage system exists in this area. So a high water table, waterlogging and soil salinity problems get increased day by day because of the irrigation. The aim of this study was to estimate the optimum drainage system design parameters to prevent soil salinity, water logging and insufficient yield. And additionally, to show the initial and simulated salinity results to the decision makers by creating soil salinity maps.

Materials and Methods

Experimental sites are located in Ankara Bala - the Central Anatolia region of Turkey. The average annual rainfall is about 350 mm and annual pan evaporation is 1255 mm for the region. The field (39°25'N, 33°23'E) experiment was carried out on 1475 ha plot area. Irrigation waters are diverted from Kesikkopru Dam Lake on the Kızılırmak River. Irrigation water quality is high saline and non-alkaline. Forty one soil sampling points were coordinated with GPS on the basin. Soil, crop and irrigation inputs were obtained from those points as an input for the model. Sampling points and geographic conditions are shown in Figure 1.

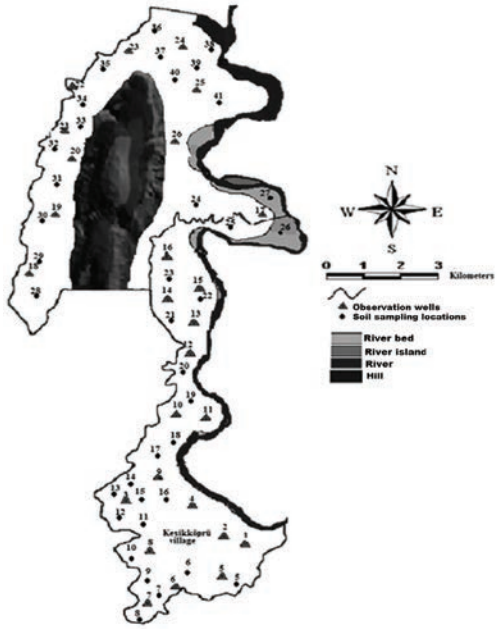

Figure 1. Sampling points in the experiment area.

Table 1

Soil textures and hydraulic conductivity of the field site

Soil depth, cm	K (cm h^{-1})	Texture
0-23	0.59	C
23-54	0.33	C
54-102	0.54	C
102-140	0.20	C
140-180	0.45	C

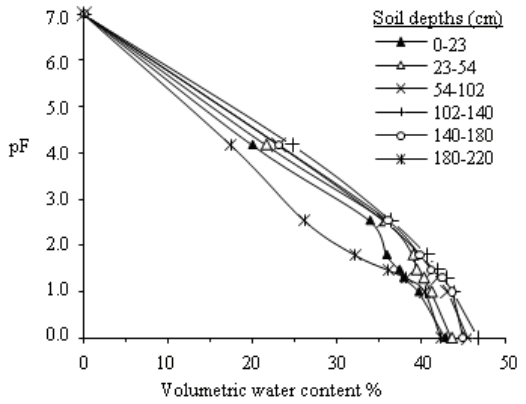

Figure 2. Soil water characteristics (pF curves).

Drainmod was used to simulate the drainage system design effects on soil profile salinity and crop yields of wheat (*Triticum)* and corn (*Zea mays)*. Simulation results were combined with Geographic Information System (GIS) and created the soil profile salinity (for different depths) maps.

Drainmod, which was developed by R.W. Skaggs, is a water management model based on a water balance for a section of soil on a unit surface area that extends from the impermeable layer to the surface and is located midway between adjacent drains. The model can be used to predict the water table depth, subsurface drainage, evapotranspiration and surface runoff as affected by the various drainage, weather and soil property data. The research project was carried out to calibrate and validate the model in the same catchment (Kale, 2004).

The model requires soil, weather, crop, salinity and irrigation inputs. Soil inputs are initial soil water content, soil water content versus pressure head (pF curve), lateral conductivity of each soil layer. Disturbed and undisturbed soil samples were taken in the field for laboratory analysis. Soil water characteristic was determined by active standard method (D6836-02) on soil cores. The lateral conductivity was found by Auger Hole method (Van Beers, 1958) in the field. Impermeable layer was measured at 4 m. Soil texture and hydraulic conductivity are given in Table 1.

The soil water characteristic data for the predominant soil type were determined on soil cores using pressure plate tests, which allowed a calculation of the volumetric water content at suction pressures of 10, 20, 33, 63, 346, and 1500 kPa (pF curve in Figure 2).

Weather inputs are daily maximum and minimum air temperature, hourly rainfall amounts, Potential evapotranspiration (PET). Climate data were obtained from Bala meteorology station. PET was calculated by Penman-Montheid method. PET data were used directly in the model.

Model simulation can be run with a relative yield input data set or without specifying a relative yield input data set, and crop inputs are rooting depths, planting delays, excess soil water stress, deficient soil water stress and salinity stress.

While more research is needed to determine crop parameters, they are directly available for some crops, such as corn (Evans and Skaggs, 1993) and can be estimated, based on data in the literature FAO, Irrigation and Drainage Paper 33 (Doorenbos, 1979) for others. For this region the wheat planting dates are generally 15-20 October, harvesting dates 15-20 July, planting dates of corn are 10-15 May and harvesting dates 15-20 October. According to the planting and harvesting date, two periods are specified in the model - a spring and fall period - for calculating trafficable conditions in the field. The equation (1) was used for computing crop relative yields. .

$$YR = Y/Y_o = YR_p * YR_w * YR_d * YR_s \quad (1)$$

Where YR is the relative yield, Y is the yield for a given year, Yo is the optimum long term average yield,

YR_p is the relative yield that would be obtained if only reduction due to planting date delay is considered, YR_w is the relative yield if only reductions due to excessive soil water conditions are considered, YR_d is the relative crop yield if the only reductions are due to deficient soil water and YR_s is the relative crop yield if the only reductions are due to soil salinity.

An excessive accumulation of salts in the soil profile causes a decline in productivity. Some plants can survive in a salt affected soil, but many are affected to varying extent depending on their tolerance to salinity. Even the same crop has different tolerance levels of salinity for its different growing stages. E.V. Mass and Hoffman G.J. (1977) indicate that each increase in soil salinity (salinity was expressed in terms of the electrical conductivity of the saturated paste) in excess of the concentrations that initially begin to affect yield will cause a proportional decrease in yield. They have proposed equation (2) to express this effect.

$$YRs = 100 - b^* (EC_e - a) \quad (2)$$

Where RY is the relative crop yield (%), ECe is the salinity of the soil saturated extract (dS m^{-1}), a is the salinity threshold value for the crop representing the maximum ECe at which a 100% yield can be obtained (dS m^{-1}) and b is the yield decrement per unit of salinity, or % yield loss per unit of salinity (EC_e) between the threshold value (a) and the EC_e value representing the 100% yield decrement. The threshold value depends on the crop tolerance to salinity. The coefficients a and b for corn and wheat were 1.7 dS m^{-1} and 12 dS m^{-1} and 6.0 dS m^{-1} ve 7.1dS m^{-1}, respectively (Maas and Hoffman, 1977).

The order of crops grown in the rotation was corn and winter wheat. The simulations were performed for 5 years (from 2005 to 2010). An effective rooting depth as a function of time is used in Drainmod to define the zone from which water can be removed to meet the ET demand. The effective root depths for crops were measured in the growing season. Relative yield to stresses due to planting delay, excessive and deficient soil water conditions along with stress-day factors were taken from R.O.Evans et al., (1990), R.O.Evans and R.W.Skaggs, (1992), R.W.Skaggs, (1982) and R.M.Seymour (1986).

Initial soil salinity, irrigation water salinity, dispersion coefficient and crop salt tolerance parameters are required as salinity input in the model. Dispersivity has been derived using the S.P.Neuman (1990) equation (3).

$$\alpha_L = 0.0175\ L^{1.46} \quad (3)$$

Where a_L is dispersivity and L is the field scale. Dispersivity was calculated as 4.13 cm.

The timing of irrigation requires the day and month when the irrigation is initiated along with the irrigation interval. The starting and ending hours of irrigation are also specified. The total irrigation water requirement for corn is 590-600 mm in the Bala basin. According to the carried out irrigation projects on this area, the irrigation scheduling for corn and wheat are given in Table 2.

Table 2

Irrigation date and applied irrigation water amount (mm)

Crop	Irrigation date	Irrigation water
Corn	23 June	43.4
	15 July	171.6
	6 August	132.0
	28 August	132.0
	19 September	113.1
Wheat	25 October	88
	15 April	88 (fine textured soils)
	20 May	179
	15 June	79

In this study, the model was used to compare the soil salinity changes in soil profile with the installed drainage system (predicted) and without drainage system in the field. Generally farmers are using 100-150 mm more water than required. Excess water applications are assumed as leaching water. So excess water applications are causing an increasing water table depth in the soil profile. On average 125 mm of leaching water amount was added to the irrigation water. Thus, these simulation results will show that differences between the soil salinity level on soil profile for farmer irrigation applications with and without drainage.

Soil salinities were continuously simulated for crop rotation of corn and wheat during a 5 year period. Yields of individual crops were predicted for each growing season. Simulations were made for each soil sampling points (40 points). The model was run in the years from 2005 to 2010. Simulation was made for six drain depths (from 100 cm to 200 cm with 20 cm interval) and ten drain spacing (from 40 m to 220 m with 20 m interval) using by analysis option of the model.

Results and Discussion

In the experiment site there is no big drainage problem in the winter-time due to the semi-arid climate conditions. Generally in this region evapotranspiration is high and rainfall is not enough for plant water requirement. Because of that irrigation is definitely essential for crop production. Irrigation water applications by farmers are not conscious in

the experiment site. Because of that, water table level is getting up in irrigation seasons. Depending on the irrigation without drainage, soil salinity also is increasing and accumulating in the soil profile.

The simulated wheat yield results for almost all points (except sampling point number 10 where the soil salinity level was much higher than the threshold value so the yield was very low without enough leaching water) showed that crop yields were not affected by salinity stress.

Results for corn relative yields indicate that soil salinity level in the root zone caused a 10-40% drop in yield. Soil salinity level of sampling point numbers 10, 28 and 41 was still high for corn production after installed drainage systems with an existing condition. Corn yields were less than 30% for those points. Stress due to excessive and deficit soil water conditions did not limit yields too much. Relative corn yield results are showed in Figure 3.

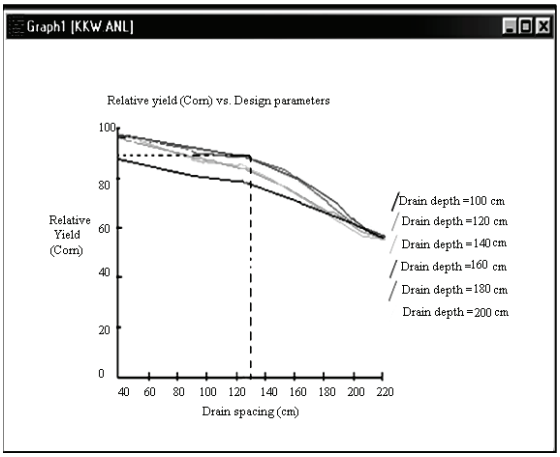

Figure 3. Relative corn yield results.

According to simulation results; any reduction was not observed on wheat yield for different drainage

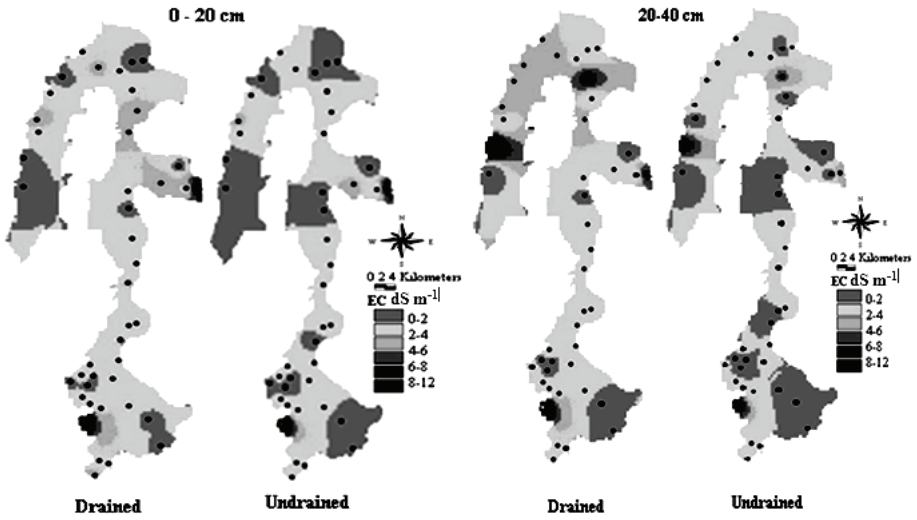

Figure 4. Soil salinity level for 0-20 cm and 20-40 cm soil depths with and without the drainage system.

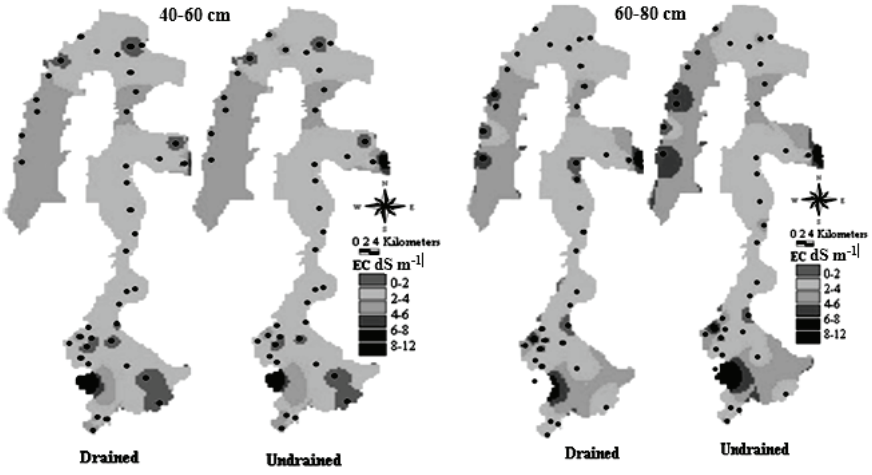

Figure 5. Soil salinity level for 40-60 cm and 60-80 cm soil depths with and without the drainage system.

design parameters. Because of that only corn yield results were taken into consideration for evaluation. Optimum drain depth of160 cm and optimum drain spacing 130 m were accepted for Bala Basin, according to corn yield results. Those drainage design parameters provide 90% corn yield and 100% wheat yields.

At the end of the study, the soil salinity simulation results of optimum drainage system design were compared with the field soil salinity values. Simulated and measured data were used to create salinity situation maps of the basin with the drainage system and without it. Created measured soil salinity and simulated soil profile salinity maps for 0-20 cm and 20-40 cm soil depths are presented in Figure 4 while those between 40-60 cm and 60-80 cm soil depths are shown in Figure 5.

Results showed that soil salinity diversity changed after installed drainage system. Soil salinity level decreased at almost all depths. Drainage systems were effective on soil salinity decreases, especially at 0-20 cm and 20-40 cm soil depths.

Conclusions

The results of the simulations were analyzed to identify the effects of subsurface drainage system that would satisfy maximum crop production. According to the simulation results, the drain spacing of 130 m and drain depth at 160 cm are recommended for Bala Basin. Results showed that soil salinity level and salinity stress can be reduced and yield increased by installing drainage systems. Results of simulations presented herein clearly demonstrate the interdependence of drainage requirement and soil salinity. This supports the often stated proposition that drainage, irrigation and salinity for arid lands should be considered a component of water management system and that design of each component should depend on the others.

According to the model predictions, if the current conditions remain without a drainage system in this basin salinity will be very a important factor for limiting crop productivity. Created maps provide a visual tool for evaluating the potential impact of salinity on soil profile, thereby providing knowledge to make management decisions with the aim of minimizing environmental impact without reducing future agricultural sustainability. The greatest attention must be given to reduction of salt loading either through the installation of drainage systems or changes in irrigation systems and management strategy.

Acknowledgements; Related research studies were conducted at the Research Station of Soil, Fertilizer and Water Resources Central Research Institute and funded by the Institute and Ministry of Agriculture.

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COMMON OAT (*AVENA SATIVA* L.) HUSK CONTENT DEPENDING ON GENOTYPE AND GRAIN SIZE

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Abstract
Oat (*Avena sativa* L.) is one of the small grain crops produced in temperate climate zone. Common oat has been studied most often due to its multifunctional characteristics and nutritional profile. The main function of the oat husk is to protect grain from harmful conditions during harvesting and storage time. Oat grain size uniformity is an important parameter to the oat milling industry. The aim of this study was to compare the husk content of common oat cultivars grown in Latvia and to obtain its changes at different grain size fractions during three growing seasons. The field trial was carried out at the State Stende Cereal Breeding Institute from 2012 to 2014. Ten husked oat genotypes were studied. Oat samples were fractioned into size fractions and samples of each fraction dehulled by hand. Results showed that significant ($p<0.05$) differences in the husk content were observed among genotypes, growing season and different grain size fractions. Significantly lower husk content was for genotype 'Arta' in all growing seasons. For majority of used genotypes increasing grain size the husk content decreased.
Key words: oat, husk content, grain size, environmental conditions.

Introduction

Oat *(Avena sativa* L.) has lately been one of the frequently studied small grain crops produced in temperate climate zone and distinct among the cereals due to its multifunctional characteristics and nutritional profile. Prime origin oats were grown for medical purposes, but nowadays it is mostly used both for human and animal nutrition because it is a nutritious source of protein, carbohydrate, fiber, vitamins and minerals (Biel et al., 2009). The husk content of husked oat amounts to an average of 20 – 30%, depending on genotype and is made of 30 – 35% crude fibre (cellulose), 30 – 35% pentosans, 10 – 15% lignin and average 15% of protein along with ash and silicic acid (Doehlert et al., 2001). The main function of oat husk is to protect grain from harmful conditions during harvesting and storage time, while naked oats have more mechanical damage during harvesting. The husk content is mostly dependent on environmental factors.

Oat grain size uniformity is an important parameter to the oat milling industry because the processing of oats for human food generally involves size separation of grains into different streams before dehulling. Oat spikelet may contain one, two, tree, or more grains, and the main grain is always larger than others. Larger oat grains can be dehulled at slower rotor speed than smaller oat grains; it is because an oat grain with a larger mass will possess more energy of inertia when impacting the walls of the impact dehuller than smaller oat grains at the same rotor speed. So it is better if oat cultivar is characterized by larger grain fraction or more of the same size grains (Doehlert et al., 2004; Doehlert et al., 2006).

There are several studies about the husk content and its interactions with the test weight, and it was mentioned there that both groats percentage and husk content are closely related to the grain size (Doehlert et al., 2004a). Smaller grains have lower husk percentage than larger grains (Doehlert et al., 2004). C.D. Doehlert et al. (2004) have used the grain size fractions – >2.58 mm, 2.38 – 2.58 mm, 1.98 – 2.38 mm and <1.98 mm. By mass distribution small grain size fraction (1.98 – 2.38 mm) took the greatest part (47.9%). The husk content for these fractions has decreased by decreasing grain size. Oat breeders through hybridization and selection have improved the yielding ability potential of oat varieties and lower husk content as well.

The aim of this study was to compare the husk content of several common oat genotypes grown in Latvia and to obtain its changes at different grain size fractions during three growing seasons.

Materials and Methods

The field trials were carried out at State Stende Cereal Breeding Institute (State Stende CBI) using 10 oat genotypes (factor A) - (int. al. 5 Latvian origin genotypes: standard genotype 'Laima', 'Stendes Darta', 'Stendes Liva', 'Arta', '33122'; and 5 foreign oat genotypes: 'Pergamon', 'Freja', 'Scorpion', 'Kirovec', 'Vendela') from 2012 to 2014 (factor C). All agro-technical operations were carried out at optimal terms according to the weather conditions during the vegetation period and depending on the plant development phases. Seed rate was 500 germinable seeds per 1 m^2. Before the cultivation of the soil a complex mineral fertilizer was applied: N – 51, P – 30, K – 42 kg ha^{-1}. Sowing and harvesting dates depended on meteorological conditions (sowing date – 28.04., 03.05., 22.04.; harvesting date – 09.08., 07.08., 22.08., according 2012, 2013, 2014). Variants were arranged in four replications with a plot size 10 m^2 in a randomized block design. The soil of the

site was sod-podzolic, its parameters are given in Table 1.

Table 1

Soil parameters and pre-crop 2012 – 2014, State Stende CBI

Parameter	2012	2013	2014
Humus content, g kg^{-1}	18	20	22
pH KCl	6.2	6.6	6.0
Phosphorus (P), mg kg^{-1}	42	39	47
Potassium (K) mg kg^{-1}	59	53	63
Pre-crop	barley	barley	potatoes

Meteorological conditions in their mean daily temperature and precipitation amount of studied years differed from each other and long term average as well and are shown in Figure 1. The temperature and atmospheric precipitations provided a perfect oat field germination in 2013. Precipitations exceeding long term average and sufficient mean daily temperatures in May and June provided good conditions for germination and tillering. But during the same period lower mean daily temperatures and high precipitations in 2014 and 2012 slowed the oat growing and flowering, consequently affecting the pollination. The low sum of precipitation and mean daily temperature close to long term average in July and August of 2013 in July and August ripened the oat grains and gave excellent yield, while in 2014 the mean daily temperature was higher than long term average and with the lack of precipitation in July caused stress for oat plants. The harvesting in 2012 and 2014 were delayed approximately by a week because of heavy rainfalls at the first two decades of August.

Grain size fractions were determined by separator machine SORTIMAT. A cleaned sample of 100 g was weighed on a balance with accuracy of up to 0.01 g and then placed onto the top sieve. The sieving period was set from 3 min, recommended by producers. Sieves with diameter 2.8, 2.5 and 2.2 mm were used. With a weighed batch of 100 g the percentage proportion was then obtained by weighing the individual fractions. The husk content was determined by four samples (factor B) of 5 g of each genotype's unfractionated sample and size fraction (>2.5mm, 2.5-2.2mm, <2.2mm), separating manually husk from grain and weighed, calculating the percentage of husks.

The obtained results were statistically processed by MS Excel program package using the methods of descriptive statistics; arithmetic mean value and standard deviation 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 oat grain size uniformity is an important parameter to the oat milling industry, as well as husk content, which should be separated from grain during the dehulling process. Grain size is nonuniform because of the multifloret habit of oat spikelet, which can contain one, two, three or more grains. The largest called primary grain, whose size depends on the number of grains in spikelet. Doehlert et al. (2008) have mentioned that primary grains in triple grain spikelet are significantly larger than primary grains in double grain spikelet. Distribution of grain size uniformity of our study is represented in Figure 2. Differences in grain size were significant ($p<0.05$)

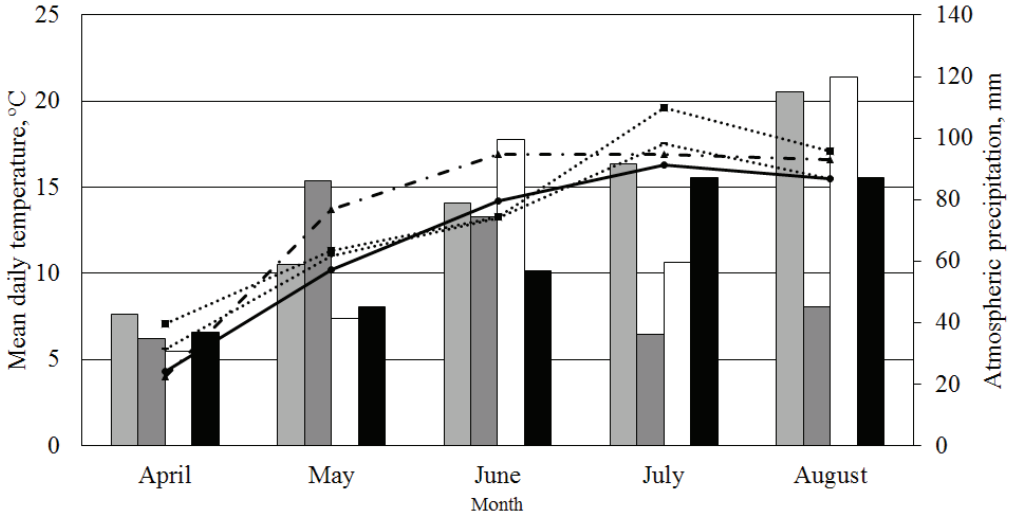

Figure 1. Meteorological conditions during experimental years:

■ 2012, ■ 2013, □ 2014 ■ LTA, *LTA – Long term average value of temperature
2012, 2013, 2014 Long term average value of precipitation.

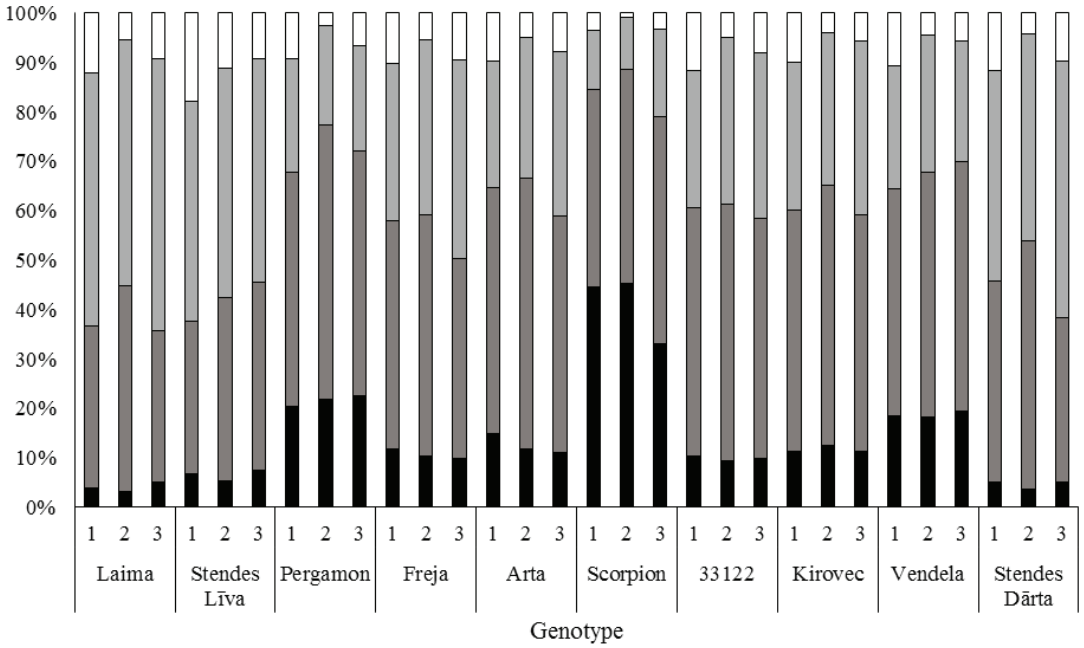

Figure 2. Grain size uniformity (%) of oat genotypes at State Stende CBI, 2012 – 2014: □ < 2.2 mm, ■ 2.2 – 2.5 mm, ■ 2.5-2.8 mm, ■ > 2.8 mm, 1 – 2012, 2 – 2013, 3 – 2014

among the tested cultivars. Grains in the spikelet are evolving gradually and for genotypes, which have a tendency to form tertiary grains in spikelet (usually smaller in size), smaller grains are more, for example, genotype 'Stendes Līva', where the small grain fraction (<2.2 mm) varies from 9.4% to 17.8% opposite genotype 'Scorpion', where smaller grain fraction occupies only from 0.9% to 3.6%. Following our results comparing with information in other studies (Doehlert et al., 2004; Doehlert et al., 2008) we can assume that the genotype 'Scorpion' has a tendency to make triple grain spikelet (primary grains are larger and more in amount). The influence of growing season on grain size distribution was significant ($p>0.05$) at smaller grain fractions (2.5-2.2, <2.2 mm); for larger grain fractions (>2.8, 2.8-2.5 mm) the growing season influence was not significant. It is found in literature that in hot and dry conditions at grain filling process the content of smaller grains increases (Dolferus et al., 2011).

Groat percentage and husk percentage are a very important quality characteristics of oat. It provides information on the economic value of the sample of oats for milling and information on the digestibility

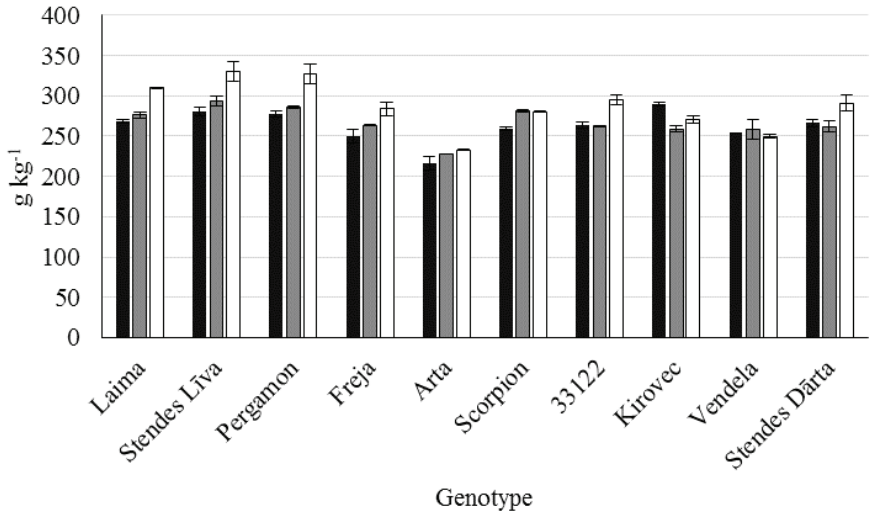

Figure 3. Husk content (g kg^{-1}) of unfractionated sample at State Stende CBI, 2012 – 2014: ■ 2012, ■ 2013, □ 2014,

Table 2

Husk content for selected genotypes at different grain sizes and unfractionated sample at State Stende CBI, mean ± sd[1]

Cultivar (A)	Sample (B)	Husk content (g kg^{-1})		
		2012 (C)	2013 (C)	2014 (C)
Laima	>2.5 mm	249.5	271.4	284.6
	2.5 – 2.2 mm	245.9	255.6	276.8
	<2.2 mm	416.9	505.2	601.2
	Unfractionated sample	**268.0**	**276.3**	**310.1**
Stendes Līva	>2.5 mm	255.6	280.2	289.3
	2.5 – 2.2 mm	254.1	254.5	315.0
	<2.2 mm	399.3	503.5	601.1
	Unfractionated sample	**280.4**	**293.3**	**330.4**
Pergamon	>2.5 mm	250.0	276.6	263.7
	2.5 – 2.2 mm	280.9	306.1	424.2
	<2.2 mm	472.6	417.4	708.2
	Unfractionated sample	**277.3**	**286.0**	**327.4**
Freja	>2.5 mm	241.9	255.3	255.1
	2.5 – 2.2 mm	234.5	255.6	268.4
	<2.2 mm	342.7	410.7	501.1
	Unfractionated sample	**249.7**	**263.7**	**284.0**
Arta	>2.5 mm	221.0	229.5	233.1
	2.5 – 2.2 mm	206.1	220.8	231.8
	<2.2 mm	217.2	243.1	234.0
	Unfractionated sample	**216.9**	**237.8**	**248.1**
Scorpion	>2.5 mm	255.3	278.7	276.5
	2.5 – 2.2 mm	265.3	299.1	280.4
	<2.2 mm	336.3	320.8	380.6
	Unfractionated sample	**259.4**	**281.2**	**280.5**
33122	>2.5 mm	250.5	246.2	263.5
	2.5 – 2.2 mm	255.8	260.2	290.5
	<2.2 mm	349.6	470.3	547.7
	Unfractionated sample	**263.6**	**261.8**	**295.8**
Kirovec	>2.5 mm	246.9	259.9	256.1
	2.5 – 2.2 mm	246.7	247.6	271.0
	<2.2 mm	324.3	335.9	424.2
	Unfractionated sample	**290.0**	**259.0**	**270.7**
Vendela	>2.5 mm	235.6	227.1	225.8
	2.5 – 2.2 mm	237.6	277.1	266.0
	<2.2 mm	405.2	614.4	480.8
	Unfractionated sample	**253.8**	**258.8**	**249.3**
Stendes Dārta	>2.5 mm	249.5	253.0	266.3
	2.5 – 2.2 mm	251.4	249.0	273.6
	<2.2 mm	383.0	499.3	479.2
	Unfractionated sample	**266.1**	**261.9**	**290.7**
LSD0.05 A = 1.40	LSD0.05 B = 0.76	LSD0.05 C = 0.76		
LSD0.05 AB= 2.42, LSD0.05 AC= 2.42, LSD0.05 BC= 1.32				

of oats to be fed to animals. The husk of oat grain protects the groat and in unfavorable conditions its content can increase (Bleidere et al., 2014). The husk content mentioned for husked oats in literature varies from 405 to 210 g kg^{-1} of grain mass (Doehlert et al., 2004; Peltonen-Sinio et al., 2004; Zute et al., 2010). The husk content of the unfractionated sample in our study varied from 217 to 330 g kg^{-1} depending on the cultivar and growing season (Figure 3). Significant ($p<0.05$) differences were observed among genotypes and growing season as well. P. Pieltonen-Sainio and J. Peltonen (1993) observed only significant ($p<0.05$) differences among growing seasons, but differences among 29 genotypes were not significant. Literature shows that the husk content has a positive correlation with mean daily temperature in May and less amount of husks can be observed in years when rainfalls in July are higher comparing with long term average (Zute at al., 2010). Comparing with our study nearly all genotypes characterized by a lower husk content in 2012, when atmospheric precipitations were the highest from studied seasons and higher than the long term average value. Genotype 'Kirovec' showed the highest husk content in 2012, opposite to the information found in literature, but maybe this genotype was not influenced by atmospheric precipitations in July, but some other obstacles during vegetation season. Latvian origin genotype 'Arta' is characterized by the lowest (below 250 g kg^{-1}) husk content during all growing seasons. Genotype 'Arta' is early maturing oat and such oats are characterized by lower husk content (Bleidere et al., 2014, Doehlert et al., 1999).

There are several studies about groats percentage (opposite husk content) and its interactions with test weight, which were not studied this time, but researchers have mentioned that groats percentage and husk content as well are closely related to grain size (Doehlert et al., 2004a). In this study the husk content of different grain fractions was observed and represented in Table 2.

In our study smaller grain fractions were characterized by higher husk content. Husk content of smallest grain fraction (<2.2 mm) varied from 217.2 to 708.2 g kg^{-1} while for largest (>2.5 mm) it was from 221.0 to 289.3 g kg^{-1}. Our results differ from those found in the literature. C.D. Doehlert et al. (2004) have noticed that smaller grains have lower husk percentage than larger grains. As we used cleaned randomized grain sample for separation there could be a situation when tertiary grains, which are smaller in size, are not filled, containing only husk, but is still connected with secondary grain and is not separated by primary processing. Significant differences in husk content were observed among growing seasons and grain size fractions as well. Also the differences among tested genotypes were significant ($p<0.05$). For genotype 'Arta' the husk content was the lowest in each grain size fraction. As we mentioned before, the husk content of early maturing oat genotypes is the lowest.

Conclusions

The results of this study indicated that husk content among 10 husked oat cultivars varied from 216.9 to 330.4 g kg^{-1} and was significantly influenced by genotype, growing season and grain size fractions (>2.5 mm, 2.5-2.2. mm, <2.2 mm). Smaller grains have significantly ($p<0.05$) higher husk content comparing with larger grains. The lowest husk content was determined for genotype 'Arta', which is the early maturing genotype.

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IMPACT OF AGROECOLOGICAL CONDITIONS ON THE HAGBERG FALLING NUMBER OF WINTER WHEAT GRAIN

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Abstract
Grain quality adversely affects price and consumer acceptance of finished products. Hagberg falling number (hereinafter falling number) is one of the most important grain quality indices of winter wheat (*Triticum aestivum* L.), especially in humid climate countries (Northern Europe). Field experiments with winter wheat cultivars 'Bussard' and 'Zentos' were conducted at the Latvia University of Agriculture, Study and Research farm 'Peterlauki' during a three year period (from 2009/2010 to 2011/2012). The aim of this investigation was to clarify variation of the falling number (FN) depending on cultivar, weather conditions and different rates of nitrogen (N) fertilizer applied (N 60, N 90, N 120, N 150) on fresh and stored grain (60, 120 and 360 days). During the investigation period, wheat 'Zentos' grain was characterised by a higher falling number compared with 'Bussard' grain. The falling number values for fresh grain for both wheat varieties studied were high: averagely 301 s for 'Bussard' and 359 s for 'Zentos', and reached demands set for grain suitable for bread baking. Differences in the falling number values were noted when freshly harvested winter wheat grain was compared with grain stored for 60 or 120 days and 360 days. During storage (60 – 360 days), α-amylase activity in winter wheat grain reduced and the FN increased on average up from 94 to 110 s. The falling number significantly depended on weather conditions, grain storage period and nitrogen fertilizer. The higher was the falling number of freshly harvested grain, the higher its increase during grain storage of compared to freshly harvested grain with a lower falling number value.
Key words*:* cultivars, nitrogen fertilizer, weather conditions, grain storage.

Introduction

Wheat (*Triticum spp.*) production according to quality food requirements is a relevant problem for wheat growers, grain handlers, millers and bakers. This paper addresses one particular wheat grain quality attribute, the Hagberg falling number, an important test of bread-making quality in wheat because of its negative association with α-amylase activity. Excessive α-amylase activity leads to the production of sticky dough that is difficult to process, and loaves that are poorly structured and discoloured (Mustatea et al., 2006).

The Hagberg falling number (FN) is an indicator of α-amylase activity and a measurement of how far the break-down of starch has progressed in the kernel through enzymatic activities. A high falling number indicates minimal activity, whereas a low falling number indicates more substantial enzyme activity (Hruskova et al., 2004). Alpha-amylase activity depends on weather conditions, especially precipitation (Triboi-Blondel, 2001; Mašauskiene and Cesevičiene, 2005; Skudra and Linina, 2011). E.Johansson (2002) found that the weather conditions during grain ripening of wheat affected the FN in 1998 and 1999 in Sweden. Under rainy conditions, the grains of wheat germinate in the ear either before or at harvest – ripeness, known as sprouting in the ear (Kettlewell, 1999; Ruza et al., 2002; Kondhare et al., 2015). Pre–harvest sprouting or sprouting during storage at a high temperature and humidity increases the level of alpha-amylase enzyme. Sprouted wheat has a low FN and lower values of other quality elements (Ingver et al., 2002; Lan et al., 2005; Krupnova and Svistunov, 2014). Alpha-amylase degrades starch and in excessive amount renders flour unfit for baking (Gooding et al., 1997).

The falling number value depends on the cultivar genetic characteristics (Raza et al., 2010; Liniņa and Ruža, 2012). Analyzing data over 20 years, M.J.Gooding et al. (1997) found that different wheat cultivars planted in the middle of the 1970s had a lower FN value than those cultivars planted in the 1990s, although the climatic conditions and nitrogen fertilizer level were similar. E.Johansson (2002), D.Kunkulberga and co-authours (2007), Ž.Liatukas and his colleagues (2012) also confirmed that the falling number of different cultivars may vary in the same growing conditions.

Nitrogen (N) fertilizer influenced the falling number. Grain of winter wheat without nitrogen fertilizer tended to have a lower falling number (Knapowski and Ralcewics, 2004; Clarke et al., 2004). According to T.Knapowski and M.Ralcewics (2004), the application of N 120 kg ha^{-1} increased the falling number in winter wheat grains significantly, as compared with the control (N0) and the object treated with N 80 kg ha^{-1}. D.R. Kindred and co-authors (2005) have found, that while increased nitrogen leads to lodging and associated sprouting in the ear, applying nitrogen tends to decrease the FN. In the absence of lodging, however, nitrogen application often increases the FN, but this effect varies with year, cultivar and site (Teesalu and Leedu, 2001). B.A.Stewart and G.V.Dyke (1993) and also J.Wang with others (2008) found that nitrogen fertilizer rate may be affected in two ways: increase and decrease the FN of wheat

grain. The results obtained in Lithuania (Basinskiene et al., 2011) showed that organically grown wheat had a lower α-amylase activity in comparison with conventionally grown wheat (N 120). However, nitrogen fertilizer influence is lower than the effect of cultivar and climatic conditions (Smith and Gooding, 1996). In general, the FN depends significantly on the interaction between cultivar and the environmental conditions at the corresponding location (Farrell and Kettlewell, 2008).

Changes of the falling number in grain storage period have been reported by several authors. Some scientists have concluded that α-amylase activity decreased over the wheat grain storage duration (Jafri, 2010; Raza et al., 2012; Ruska and Timar, 2012). Other researchers (Lan et al., 2005) found a distinct negative relationship between the activity of α–amylase and the falling number value. Similarly, O.M.Lukow and P.B.E.McVetty (1991), also J. Gonzalez-Torralba and his colleagues (2013) found that the FN values increased significantly during wheat grain storage. M.Hruskova and D.Mackova (2002) observed that the FN increased by 28 s (from 206 to 234 s) in wheat flour over the storage duration of three months. Many other studies conducted by various researchers have shown different results. The conclusion reached by the Hungarian researcher Z.Mezei and co-authors (2007) suggest that the falling number value did not change significantly when the grain of winter wheat was stored for 129 days.

This research is a continuation of previous work (Liniņa and Ruža, 2012) in which we investigated the quality of winter wheat freshly harvested grain (in 2010 and 2011) and during grain storage. The aim of this investigation was to clarify variation in the falling number value depending on cultivar, different rates of nitrogen fertilizer applied, weather conditions and grain storage period. In Latvia, changes in the falling number value during the grain storage were studied for the first time.

Materials and Methods

Field experiments in 2009/2010, 2010/2011 and 2011/2012 were conducted at the Latvia University of Agriculture, Study and Research farm 'Peterlauki' (56° 30.658′ North latitude and 23° 41.580′ East longitude), Endoprotocalcic Chromic Stagnic Luvisol (Clayic Cutanic Hypereutric), silty clay loam/clay, organic mater 20 – 31 g kg^{-1}, pH KCl – 6.6 – 7.0 and medium phosphorus and potassium content easily utilized by plants. Winter wheat (*Triticum aestivum* L.) cultivars 'Bussard' and 'Zentos' (Germany) were sown after black fallow in four replications (rate of 400 germinating seeds per m^2). The sowing was carried out in the second ten-day period of September. These cultivars were grown in four replications with a plot size of 36 m^2, field layout – randomised. Before sowing plots were fertilized with phosphorus and potassium fertilizers at the rate: P 31 kg ha^{-1} and K 108 kg ha^{-1}. Nitrogen (N) fertilizer was applied in spring after renewal of vegetative growth. Nitrogen top–dressing rates were as follows: N 60, N 90, N 120 and N 150.Winter wheat in all investigation years was harvested at optimal time when the growth stage GS 88-91 was reached. On 4 August winter wheat was harvested in 2010, on 5 August in 2011 and in 2012 on 3 August. Sampling procedure for grain quality evaluation was performed according to the standard ICC 101/1 for obtaining average sample. The grain with a moisture content exceeding 14% was dried. Freshly harvested grain of each cultivar and nitrogen top dressing treatment were put into separate cotton bag. Grain was sampled four times: fresh and stored grain – 60, 120 and 360 days after harvest. The grain was stored in a storage house in which the indoor temperature depended on the outdoor temperature and relative air humidity was 52 – 75%. Initially placed for storage, the grain moisture content was 13.5 – 13.8% for 'Bussard' and 13.0 – 13.8% for 'Zentos'. During storage, the grain moisture content changed but never exceeded 14%. Respiration processes became more

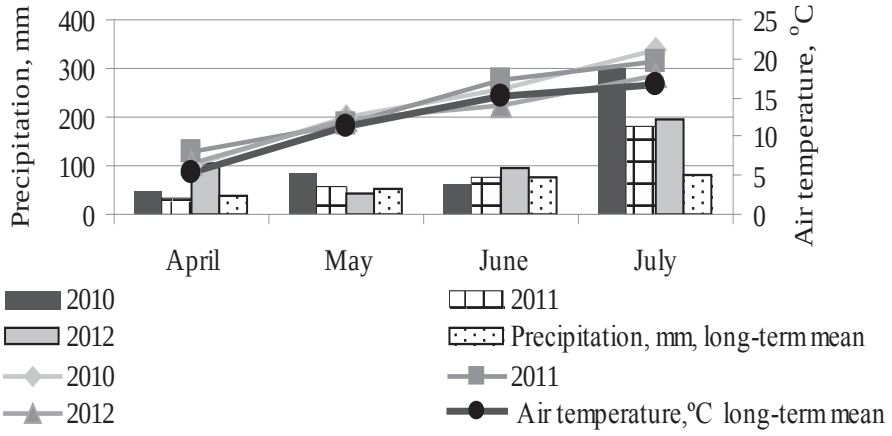


Figure 1. Meteorological conditions in investigation years.

intensive in grain with the moisture content above 14.6%.

Winter wheat sown in 2009, 2010 and 2011 overwintered successfully. In 2010, 2011 and 2012 the air temperature in spring (Figure 1) was close to the long-term average. It promoted plant growth and development. The mean May temperature was close to the long-term mean. The mean daily temperature in June of 2010 and 2011 was higher than the long-term mean, while in 2012 by 1.1 °C lower. Temperatures in grain filling period (July), which is the most decisive for grain quality formation, were 4.7 °C higher in 2010, 3.0 °C higher in 2011 and 2.8 °C higher in 2012 compared to long-term average.

Precipitation in April 2010 and 2011 was close to long-term average, but in 2012 by two times more than long-term mean data. May in 2010 was wet; in 2011 and 2012 precipitation was close to the long-term mean data for this month. Precipitation in June 2010 and 2011 was close to long-term mean; but in 2012 more than long-term mean data. July in 2010, 2011 and 2012 was very rainy; it two to three times exceeded the long-term average data.

Wheat grains were analyzed at the Latvia University of Agriculture in Grain and Seed Research laboratory. A 300 g grain samples were milled to wholemeal flour using 'Perten Laboratory Mill 3100' (Sweden) with 0.8 mm sieve. The Hagberg falling number – α-amylase activity – was measured by the Hagberg-Perten method using a Perten Instruments (Sweden) 'Falling number 1500' was assessed to LVS EN ISO 3093 using 7 g of flour adjusted for moisture content to 15%.

Experimental data evaluation was done using two–factor analysis of variance (ANOVA), the test of statistically significant differences at Fiscer's criterion and impact factors influence (η^2), probability <0.05% were used for the analyses of mean differences. Mean, standard error of the mean, coefficients of variation were determined. Data analyses were done with MS Excel.

Results and Discussion

Falling number is an indication of degree of soundness of wheat in terms of freedom from sprouting (Karaoglu et al., 2010) which causes the production and activation of α–amylase inside the wheat kernel which, in turn, has a very drastic affect on the dough and bread making process. According to the requirements of 'Dobeles Dzirnavnieks' ('Dobele's Miller') (Dobeles Dzirnavnieks, 2012) a grain processing company, wheat grain could be classified by the falling number into five classes. The first class (Elite) and second A class are set with the falling number value above 280 s, the third class with 270 s, respectively, fourth class with 250 s, and the fifth class above 220 s.

The falling number of grain was different for both cultivars. During investigation period, wheat 'Zentos' grain was characterised by a higher falling number compared with 'Bussard' grain. Average data show that the falling number for cultivar 'Zentos' was 395 ± 7.0 s, range min – max 300 – 503 s, for 'Bussard' – 346 ± 9.2 s and 221 – 490 s, respectively. The coefficient of variation of trait was stable for 'Zentos' 12%, while 18% for 'Bussard'.

Falling number of fully ripe winter wheat grains

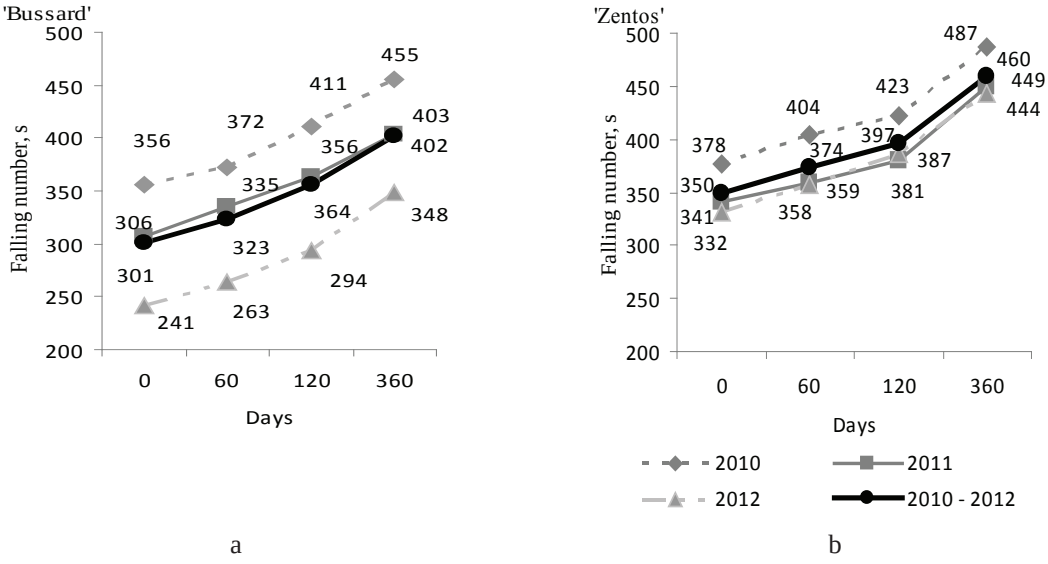
The falling number values for fresh grain for both wheat varieties studied were high (Figure 2): averagely 301 s for 'Bussard' and 350 s for 'Zentos' and reached the standards (Elite) of grain suitable for bread baking exceeding 280 s. The falling number significantly varied depending on the cultivar. High falling number values indicate low α–amylase activity (Lunn et al., 2001b). The falling number was not similar in every year. Weather conditions in investigation years influenced grain α–amylase activity. A higher falling number test for both wheat cultivars was observed in 2010 and 2011 compared to the harvest year of 2012. E.Johansson (2002) found that the higher the sum of active temperatures in summer, the higher the increase in the falling number value in wheat grain. Our results confirmed this conclusion: a higher sum of active temperatures (+10 °C) from the stem elongation (GS 30) until the harvest was in 2010 (1418 °C) and in 2011 (1407 °C) resulted in a higher FN value compared with 2012 (1252 °C). Especially low falling number (241 s) was in 'Bussard' grain – only in the fifth quality class.

The falling number is affected by precipitation during grain maturation. High rainfall in grain maturation period results in higher α–amylase activity and lower falling number (Johansson, 2002). Cereal maturation and harvesting can occur during rainfall period, which can often be a reason for lower grain quality and even grain sprouting in ears (Lunn et al., 2002; Kunkulberga et al., 2007, Skudra and Linina, 2011; Kondhare et al., 2015). Cereal grain is of the highest quality during growth period between wax maturity and full maturity. During this period cereal yield forming is already finished and, in case of unfavourable weather conditions, grains can start sprouting, which would result in a reduced falling number. Under very wet harvesting conditions wheat reaches the limit when α–amylase activity is considered to be too high (Kettlewell, 1999; Lunn et al., 2001a; Gooding et al., 2003). Grain sprouting in the ears may also affect the dormancy period duration. Grain dormancy period is depending on the weather

conditions in grain formation and ripening phase (Lan et al., 2005). In our investigation, the rainfall in July 2010, 2011 and 2012 during the grain maturation was high: 298, 179 and 197 mm, respectively, that two to three times exceeded the long-term average data (81.7 mm). In 2010 and 2011 rainy weather in July did not affect the FN of wheat grain because rain alternated with hot (average air temperatures were respectively 21.2 °C and 19.5 °C and sometimes exceeding 30 °C) and sunny weather and grain rapidly dried up. Harvesting of the winter wheat cultivars should not be delayed. Weather conditions in July 2012 were colder, therefore, the FN in wheat grain was lower, however corresponded to the demands set for grain suitable for bread baking > 220 s.

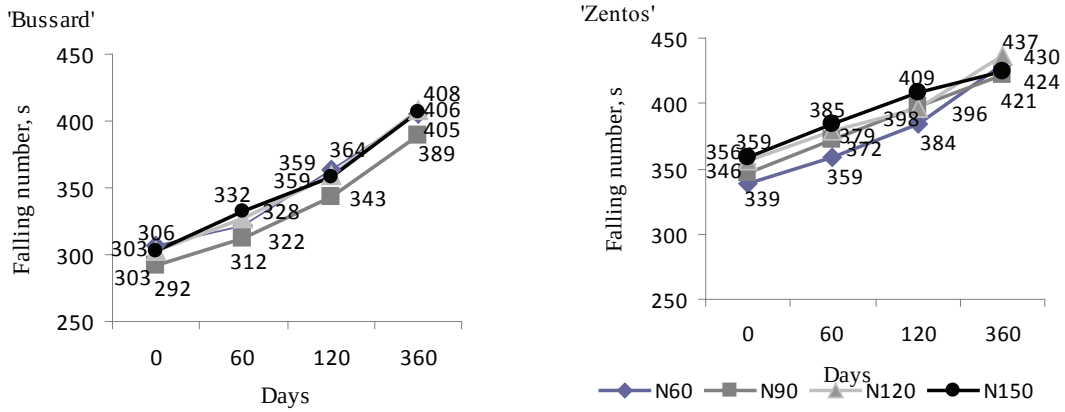
The falling number of wheat fertilized with higher rates of nitrogen was higher than when lower rates were used (Cesevičiene and Mašauskiene; 2007). In our experiment, the average data show that for wheat 'Zentos' grain the falling number was significantly higher with higher nitrogen rates N 120 and N 150 compared with crops that received N 60 (Figure 3). Nitrogen fertilizer rate significantly increases the falling number and it is consistent with the conclusions made by T.Teesalu and E.Leedu (2001), T.Knapowski and M.Ralcewics, (2004) and S.Stankovski et al., (2004), who have reported that the falling number is dependent on the rates of nitrogen fertilizer.

According to Fisher's criteria, the investigation year, nitrogen fertilizer and year×nitrogen fertilizer



a b

Figure 2. Winter wheat cultivars 'Bussard'(a) and 'Zentos' (b) grain falling number (s) during grain storage (days), depending on investigation years.



a b

Figure 3. Winter wheat cultivars 'Bussar'(a) and 'Zentos' (b) grain falling number (s) during grain storage (days), depending on nitrogen fertilizer.

interaction had a significant ($p<0.05$) impact on the both cultivars grain falling number.

In cultivar 'Bussard' grain this tendency was not observed. B.Varga and co-authors in Croatia (2003) also found that some cultivars failed to significantly improve the falling number under more intensive nitrogen fertilization.

The analysis of variance for two cultivars in 3 experiment years suggest that winter wheat 'Bussard' and 'Zentos' grain falling number (impact factors influence – η^2, probability < 0.05%) recpectively by 87% and 65% depended on weather conditions (year), year×nitrogen fertilizer interaction was also remarkable – 10% and 19%, while the influence of nitrogen fertilizer was 1% and 10% respectively. Similar results obtained in England (Smith, Gooding, 1996), suggest that the falling number by 67% depends on the rainfall in grain ripening period, and the temperatures from June to winter wheat harvest. Influence of the year was most remarkable also in the investigation with 15 winter wheat cultivars in the years 2004 – 2007 in Estonia (Koppel and Ingver, 2008).

Changes falling number during winter wheat grain storage

The activity of α-amylase reduced during the grain storage (Figure 2) and FN increased. Averaged data show that the falling number for cultivar 'Bussard' for 60 days stored wheat grain was 16 – 22 s higher if compared to freshly harvested grain, 71 – 86 s higher for 120 days stored grain, while after 360 days of grain storage it increased 97 – 101 s. For 'Zentos' grain we observed a similar tendency: after 60 days stored wheat grain was 18 – 27 s higher, 40 – 55 s higher for 120 days stored grain, after one year of grain storage it increased for 109 – 112 s. Winter wheat grains 'Zentos' before storage had a higher falling number, it grew by 12 s more, in comparison with the variety of 'Bussard' grains.

Similar results were found by H. Kibar (2015) who stored wheat grain for 6 months and reported that the FN increase was 65 s (from 230 to 295 s), while M.M.Karaoglu with co-authors (2010) stored wheat grain for 9 months and concluded that the FN was 86 s higher compared to freshly harvested grain. A.M.Buchanon and E.M. Nicholas (1980) reported that the FN increase was 100 s (from 250 to 350 s) after a year of wheat grain storage. The same tendency was also observed in trials performed by M.Hruskova and D.Machova (2002) and J.Cesevičiene (2007). The reason for the increase in the falling number, as indicated in references, could be a reduction in the activity of pericarp α-amylase which occurs during storage (Lunn et al., 2001a; Ruska and Timar, 2012), while M.M.Karaogly with co-authors (2010) reported that the increase in the falling number may be attributed to degradation of amylase enzyme and the variation of starch gelatinization properties during storage period.

More rapid increase in the falling number value was found when the grain was stored for 120 to 360 days. Similar results were reported by J.Cesevičiene (2007), who stored wheat grain for one year. Biochemical changes in grain were going on during storage (Rehman and Shah, 1999). Winter wheat grains 'Zentos' before storage had a higher falling number, after one year grain storage it grew by 12 s more, in comparison with the variety of 'Bussard' grains. Our experimental results support the conclusion made by J.Cesevičiene (2007) that the higher the falling number of freshly harvested grain, the higher its increase during grain storage compared to freshly harvested grain with a lower falling number value.

Variation in the falling number values during grain storage was related to nitrogen fertilizer rates used. There was a tendency, - if given a higher fertilizer rate (N 120 and N 150), the falling number in grain stored for 360 days increased more, than when given lower nitrogen rates (N 60 and N 90), compared with freshly harvested grain. After a year of grain storage, for cultivar 'Zentos' grain the FN increase averaged 75 s if given fertilizer rates N 60 and N 90, at N 120 and N 150 – 113 s, the difference was less for 'Bussard' grain – respectively 98 and 105 s.

The analysis of variance for each cultivar in every experimental year suggest that storage time and nitrogen fertilizer had a significant ($p < 0.05$) impact on the both cultivars grain falling number, while storage time×nitrogen fertilizer interraction this indicator affected significantly only in the harvest year of 2012. In the harvest years of 2010, 2011 and 2012, for cultivar 'Bussard' grain the falling number depended on 77%, 75 and 78% on grain storage time, respectively; on nitrogen fertilizer – 21%, 21 and 20%, while grain storage time×nitrogen fertilizer interraction impact factor (η^2) was significant only in 2012 and it was low – 1%. For 'Zentos' grain the falling number by 94% (2010), 95% (2011) and 74% (2012) depended on grain storage time but the influence of nitrogen fertilizer was, respectively – 2%, 3% and 24%, while storage time×nitrogen fertilizer impact factor was significant only in the harvest year of 2012 – 2%.

Conclusions

1. During three trial years, the falling number of the studied winter wheat grain showed a low activity of α-amylase and corresponded to demands of grain suitable for bread baking. Wheat 'Zentos' had averagely higher falling number values compared to wheat 'Bussard'.

2. The falling number depended on precipitation, the sum of active temperatures and cultivar. Nitrogen fertilizer influence on the falling number was smaller.
3. Differences in the falling number values were noted when freshly harvested winter wheat grain was compared with grain stored for 60 or 120 days and 360 days. Within 360 days of storage, α-amylase activity in winter wheat grain reduced and the increase in the falling number values on average was 94 – 110 s. The higher was the falling number of freshly harvested grain, the higher its increase during grain storage, compared to freshly harvested grain with a lower falling number value.

Acknowledgement

The research was supported by the State research programme 'Agricultural Resources for Sustainable Production of Qualitative and Healthy Foods in Latvia' Project No. 1 *Sustainable use of soil resources and abatement of fertilisation risks (SOIL).*

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THE EFFECT OF PLANTING DENSITY ON POTATO (*SOLANUM TUBEROSUM* L.) MINITUBER NUMBER, WEIGHT AND MULTIPLICATION RATE

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Abstract
The study was aimed to investigate the effect of potato (*Solanum tuberosum* L.*) in vitro* plantlets planting density under greenhouse conditions on obtained minitubers number per unit area, multiplication rate and their weight distribution. Three cultivars of different maturity ('Monta' – early maturity, 'Prelma' – medium early and 'Mandaga' – medium late maturity) were used for the study. *In vitro* plantlets were planted in a greenhouse of State Priekuli Plant Breeding Institute, Latvia in 2014 at four planting densities (PDs) 63 plants per m^2, 95 plants per m^2, 142 plants per m^2 and 184 plants per m^2 respectively. Fertilized peat was used as a substratum. Significant effect of planting density ($p<0.001$) and cultivar ($p<0.01$) was found on analyzed yield parameters. Increased planting densities resulted in increased minitubers number per m^2 (from 272 minutubers m^{-2} at PD 63 plants m^{-2} to 414 minutubers m^{-2} at PD 184 plants m^{-2}), decreased multiplication rate (4.3 to 2.7 minitubers per planted plant) and mean fresh weight of minitubers (from 20.26. g to 12.11 g). The highest increase of minitubers number per m^2 was observed within size (weight) range 3 to 5 g. Minitubers number per m^2 increase within bigger size ranges (5 to 10 g, 10 to 20 g) was less pronounced. Slight insignificant ($p=0.330$) decrease of minitubers number >20 g was observed in relation of planting density increase (112 minitubers m^{-2} at PD 95 plants m^{-2} to 84 minitubers m^{-2} at PD 184 plants m^{-2}).
Key words: *Solanum tuberosum*, potato, minitubers, planting density, tuber size distribution.

Introduction

Initial potato seed stock material (known as breeder's seed in Latvia), which is free of tuber-borne pathogens, especially virus diseases, is crucial in any potato seed production system. Traditional systems involving clonal selection for obtaining of healthy seed stocks could take more than 10 years to obtain seed material at satisfactory amounts. Therefore, nowadays most of seed production systems worldwide involve healthy *in vitro* plants mass-propagation at initial stage of seed production with subsequent minitubers production, which is called rapid multiplication. This system was involved more than three decades ago both worldwide and in Latvia.

Rapid multiplication of seed stock material allows obtaining of healthy initial seed material at big amounts thus minimizing field generations, as well as fastening seed production of new cultivars.

Potato *in vitro* plants can be planted both in field (Tadesse et al., 2001; Särekanno et al., 2010) or in greenhouse conditions. Growing of minitubers in solid substrates in greenhouses is still the most common and robust minitubers growing method (Struik and Wiersema, 1999), although soil-less production systems are very popular (Lommen, 2007).

The aim of seed stock multiplication is to produce as many minitubers of adequate size as possible. It has been reported that one potato plant produces 2 to 5 minitubers on average (Struik, 2007); however, larger amount of minitubers from one plant can be also obtained (Roy et al. 1995).

As reviewed by I. Dimante and Z. Gaile (2014), several factors such as soil type, substratum layer, fertilizing protocols, extra lightening etc., and their combination can affect progeny minitubers yield parameters.

Manipulation with the *in vitro* plants planting density could be considered as one of the most popular ways to manage minitubers number per area unit (Roy et al., 1995; Lommen and Struik, 1992; Veeken and Lommen, 2009; Jin et al., 2013). Multiplication rate usually changes conversely if to compare with minitubers number per area unit change (Veeken and Lommen, 2009). The solution of this issue greatly depends on what is considered as the most efficient approach by a producer – increased multiplication rate or bigger numbers of minitubers per area unit.

Low mean fresh weights of minitubers can affect their field performance. Therefore, it should not be the main goal itself to obtain many minitubers at any size.

Some authors mention that too small minitubers have larger losses during the storage (Lommen, 1993) and smaller yield when planted in field (Lommen and Struik, 1995; Karafyllidis et al., 1997; Barry et al., 2001). As various authors use various minitubers sizes for their field performance experiments, it is not clearly stated which minitubers size (by dimensions or by weight) can be considered as big enough. Wiersema et.al., (1987) state that minitubers bigger than >5 g are sufficiently large for good field performance; nevertheless, experiments with smaller minitubers have shown adequate field performance as well (Lommen et al., 1995).

Assumptions based on our previous experience, allow us state that in this study we consider minitubers size (weight) of 3 g as a threshold which could be

appropriate and commercially applicable. However, all grown minitubers have been counted and weighted to see the proportion of acceptable fraction.

The purpose of this study was to investigate minitubers multiplication rate (number of minitubers per planted plantlet) and final number of minitubers per area unit in relation with potato *in vitro* plantlets planting density. Additionally, minitubers size distribution both across various size ranges and in cumulative stratum was in the scope of this study.

Materials and Methods

The experiment was carried out at State Priekuli Plant Breeding Institute (SPPBI, latitude 57°31′ N, longitude 25°34′ E), Latvia in 2014 with plantlets of cultivars (CVs) bred at SPPBI 'Monta' (early maturity), 'Prelma' (medium early) and 'Mandaga' (medium late maturity) at four planting densities (PDs).

In vitro propagation of plantlets

Only virus indexed plantlets with no virus diseases detected were subjected to further micropropagation. *In vitro* plants were propagated routinely at Potato tissue culture laboratory of State Priekuli Plant Breeding Institute. Single node cuttings were sectioned and placed in test tubes (1 cutting per tube) on fresh MS medium (Murashige and Skoog, 1962), supplemented with 30 g L^{-1} regular sugar from supermarket and 6 g L^{-1} food grade agar. Subculturing of microplants was performed once every 4 weeks. The temperature in growth room was 20–26 °C; photoperiod was 16/8 h day and night respectively.

Planting of in vitro plantlets and crop husbandry practises in a greenhouse

In vitro plantlets of three CVs were planted in a greenhouse at four PDs – 63 plants m^{-2}, 95 plants m^{-2}, 142 plants m^{-2} and 184 plants m^{-2} respectively.

Plastic boxes with permeable sides and bottom were used for planting of *in vitro* plantlets. Inner dimensions of the boxes were 0.55 m × 0.35 m × 0.20 m (length × width × height). Fertilized peat with pH adjusted to 5.3 was placed in boxes at 0.13 m height. Peat contained macronutrients at following rates kg per m^3: N 0.30; P 0.24, K 0.24; Ca 0.37; S 0.18 Mg 0.05 kg. Following micronutrients were added at such rates g per m^{-3} as follows: B 3.6; Mo 2.4; Mn 1.9; Cu 1.8; Fe 1.1 and Zn 0.48 g. The peat was entirely moistened with water before planting.

Desired planting densities were obtained by modifying the procedure, described by A. Veeken and W. Lommen, 2009. Thirty five holes per box were pressed in the peat in rectangular order (5 rows with 7 holes each). Each hole was 8 cm deep and 2 cm in diameter. *In vitro* plantlets of 10 cm length were planted into pressed holes, obtaining the maximum density by planting plantlets into each of 35 holes. The density of 142 plants m^{-2} was obtained by planting 27 plants per box (planting into the second and the fourth row was reduced to three holes at equal distance between plants). For the density of 95 plants m^{-2} the first, third and fifth row were planted with four plants, the second and the fourth row – with 3 plants. Eighteen plants per box were planted in this case. The planting density of 63 plants m^{-2} (12 plants per box) was obtained by planting plantlets only in the first, third and fifth rows and reducing planting to four holes per row. With decreasing of the PD, distance between plants at each separate row remained constant within the same PD. This approach contributed to uniform planting densities and uniform distances between planted plants over CVs and replications.

Plants were watered by hand five times per week during the first four weeks of the growth. Later watering was reduced to three times per week.

Foliar fertilizer applications were used three times per growing season starting at the sixth week after planting and following once every ten days. One litre of media used for applications contained 1.34 g KH_2PO_4, 1.34 g KNO_3, 1.34 g $Ca(NO_3)_2$, 0.7 g $MgSO_4$.

Planting was conducted on 23 April 2014, haulms were removed by hands and minitubers of CVs 'Monta' and 'Prelma' were harvested 78 to 79 days after planting (DAP) and 90 DAP for CV 'Mandaga'.

The environmental conditions in greenhouse were poorly controlled. Regardless of extensive ventilation, the air temperature reached more than 30 °C on some days.

Experimental design and statistical analysis

The split-plot design with 3 replications (blocks) was used in this experiment with cultivars assigned as main plots and planting density assigned as sub-plots. Cultivars were randomized within each block, planting densities were randomized within each main plot (cultivar).

Each sub-plot was surrounded by boxes with plants of the same cultivar and the same planting density in order to avoid side effects as well as competition between different CVs or PDs.

Data on minitubers number per planted plant (multiplication rate), minitubers number per m^2, mean weight of minitubers as well as minitubers size distribution were collected, calculated and subjected to analysis.

The obtained data was analyzed using the SPSS program, version 17.0. Significance level used for the separation of means was α=0.05. The analysis of variance was performed to evaluate the effects of treatments and Least Significant Difference test (LSD) was used to separate the significant treatment

means. Relationship between dependent variables was examined by Pearson's correlation coefficient, when applicable.

Results and Discussion

Number of minitubers

Planting density of *in vitro* plants and cultivar had significant effect on such minitubers yield parameters as multiplication rate (mean number of minitubers per planted plant), number of minitubers per m^2 and mean minitubers weight (Table 1). This finding confirms results obtained by A. Veeken and W. Lommen (2009). No significant interaction effect was found between CV and PD on these parameters. A. Veeken and W. Lommen (2009) figured out an interaction between CV and PD in case of such yield parameters as minitubers number per m^2 and mean minitubers fresh weight.

Partition sum of squares showed that main factors (together PD and CV) explained more than 50% of the variance in all analyzed yield parameters, PD being as dominant factor determining yield

Table 1

Number of minitubers per planted plant, per m^2 and mean fresh weight analysis at different planting densities of *in vitro* derived plants

Planting density, plants m^{-2}	Multiplication rate (mean number of minitubers per planted plant)				Mean number of minitubers per m^2				Mean weight of minitubers, g			
	>0 g	SE	>3 g	SE	>0 g	SE	>3 g	SE	>0 g	SE	>3 g	SE
means within cultivars												
'Monta'												
63	5.4^{b}	0.57	**4.7^{c}**	0.32	342^{a}	35.8	**293^{a}**	19.8	13.96	1.135	**15.88**	1.072
95	4.4^{b}	0.26	**3.9^{b}**	0.25	416^{a}	24.3	**368^{b}**	23.7	13.47	2.369	**14.81**	2.345
142	2.9^{a}	0.29	**2.5^{a}**	0.21	414^{a}	41.9	**354^{ab}**	30.4	14.21	0.963	**16.21**	0.784
184	3.2^{a}	0.15	**2.6^{a}**	0.08	591^{b}	28.1	**481^{c}**	15.3	10.94	1.606	**12.97**	1.629
$LSD_{0.05}$	1.2	×	**0.8**	×	108	×	**75**	×	NS	×	**NS**	×
'Prelma'												
63	4.1^{b}	0.12	**3.9^{c}**	0.13	256^{a}	7.6	**247^{a}**	8.0	21.31^{c}	1.039	**22.08**	1.133
95	3.3^{ab}	0.27	**3.0^{b}**	0.26	309^{ab}	25.3	**288^{ab}**	24.7	18.87^{bc}	1.359	**20.13**	1.643
142	2.9^{a}	0.56	**2.4^{ab}**	0.31	409^{bc}	79.3	**340^{ab}**	44.5	13.51^{ab}	2.965	**15.38**	3.002
184	2.5^{a}	0.11	**2.1^{a}**	0.14	451^{c}	19.8	**377^{b}**	26.2	12.62^{a}	1.427	**14.74**	1.361
$LSD_{0.05}$	1.0	×	**0.7**	×	140	×	**97**	×	6.05	×	**NS**	×
'Mandaga'												
63	3.5	0.84	**3.2**	0.70	218^{a}	53.1	**204^{a}**	44.3	25.51^{c}	4.18	**26.51^{c}**	3.543
95	3.2	0.23	**3.1**	0.32	305^{ab}	21.9	**290^{ab}**	29.9	21.15^{bc}	1.953	**22.32^{bc}**	2.425
142	2.6	0.17	**2.4**	0.16	363^{bc}	24.1	**335^{bc}**	22.4	14.28^{ab}	0.801	**15.30^{ab}**	0.890
184	2.4	0.03	**2.1**	0.02	432^{c}	6.1	**384^{c}**	3.0	12.77^{a}	0.553	**14.12^{a}**	0.686
$LSD_{0.05}$	NS	×	**NS**	×	102	×	**95**	×	7.69	×	**7.24**	×
means over cultivars												
63	4.3^{c}	0.42	**3.9^{c}**	0.30	272^{a}	26.2	**248^{a}**	19.2	20.26^{b}	2.122	**21.49^{b}**	1.904
95	3.6^{b}	0.23	**3.3^{b}**	0.20	343^{b}	21.7	**315^{b}**	18.7	17.83^{b}	1.495	**19.08^{b}**	1.550
142	2.8^{a}	0.20	**2.4^{a}**	0.12	395^{b}	28.0	**343^{b}**	17.1	14.00^{a}	0.938	**15.63^{a}**	0.943
184	2.7^{a}	0.15	**2.3^{a}**	0.10	491^{c}	27.1	**414^{c}**	18.9	12.11^{a}	0.705	**13.94^{a}**	0.695
$LSD_{0.05}$	0.6	×	**0.5**	×	61	×	**46**	×	3.31	×	**3.24**	×
P value for effects of factors												
PD	***	×	***	×	***	×	***	×	***	×	***	×
CV	**	×	**	×	**	×	**	×	**	×	**	×
PD × CV	NS	×	NS	×	NS	×	NS	×	NS	×	NS	×
partition of sum of squares (main factors), η^2, %												
PD	44	×	60	×	54	×	56	×	38	×	37	×
CV	21	×	12	×	19	×	16	×	18	×	16	×

>0 g=total number of minitubers, >3 g=minitubers bigger than 3 g, SE=standard error, PD=planting density, CV=cultivar, PD×CV=interaction planting density × cultivar

** – effect of the factor significant at $p<0.01$; *** – effect of the factor significant at $p<0.001$; NS – not significant ($p\geq0.05$).

Values labelled with a similar letter are not statistically significantly different ($p\geq0.05$)

parameters variance. PD determined even 60% of multiplication rate variance and 57% of mean minitubers number per m^2 variance within minitubers size (weight) range >3 g.

The effect of increased PD showed a significant increase of progeny minitubers number per m^2, but it led to reduced multiplication rates (number of minitubers per planted plant) simultaneously. Similar tendencies were obtained by W. Lommen and P. Struik (1992) and R. Roy et al. (1995).

Considering minitubers size >3 g, the highest increase of minitubers number per area was found between PD 63 and 95 plants m^{-2} (1.3 fold increase). PD change from 142 to 184 plants m^{-2} increased minitubers number significantly ($p<0.01$) as well. Regardless of 1.5 fold increase of planted plants between PD 95 and 142, obtained minitubers number per m^2 did not increase significantly ($p=0.203$). The results published by W. Lommen and P. Struik (1992) showed phenomena that gradual increase of PD did not necessarily mean simultaneous increase of minitubers at significant rates between any used PD, whereas the results obtained by A. Veeken and W. Lommen (2009) confirmed significant increase of minitubers number per m^2 coincidentally with gradual increase of PD.

Increase of minitubers number per m^2 was more evident (1.7 fold increase of tubers >3 g) than simultaneous multiplication rate decrease (1.4 fold decrease within tuber size range >3 g) between two marginal PDs. Furthermore, at two highest densities minitubers number per plant remained at the lowest level and did not change significantly. This finding could contribute to optimization of PD with respect to multiplication rate retain. Similar trend was observed by W. Lommen and P. Struik (1992) at the highest planting densities. In our study the change of multiplication rate and minitubers number per m^2 showed similar trends both in size ranges >0 g and >3 g. However, increase of minitubers number in size range >0 g was slightly bigger (2 fold increase) than was in size range >3 g (1.7 fold increase) which was mainly due to increase of <3 g minitubers number (Figure 1).

The effect of cultivar on all analyzed yield parameters was significant. As it can be figured out from the Table 1, cultivar 'Monta' had higher multiplication rate and mean minitubers number per m^2 than cultivars 'Prelma' and 'Mandaga' both in size range >0 g and >3 g.

However, the largest increase of minitubers number per m^2 was observed for cultivar 'Mandaga' (1.9 fold increase between PD 63 and 184 of minitubers >3 g), this effect was especially evident between PD 63 and 95, where minitubers number >3 g had 1.4 fold increase. At the same time multiplication rate showed an insignificant decrease ($p>0.05$).

Mean minitubers weight and minitubers size distribution

Higher PDs resulted in decreased mean minitubers fresh weight (Table 1) similarly to findings of other researchers (Roy et al., 1995; Veeken and Lommen 2009; Jin et al., 2013).

Higher PDs resulted in higher minitubers number per m^2 (Table 1). Based on this relation, minitubers number per m^2 and mean minitubers weight was subjected to correlation analysis.

Statistically significant negative correlation was found between mean minitubers number >0 g per m^2 and mean minitubers weight >0 g ($r = -0.767$, $p<0.001$) as well as between mean minitubers number >3 g per m^2 and mean minitubers weight >3 g ($r = -0.708$. $p<0.01$). This finding conforms to the study of Jin et al. (2013), which has stated that minitubers size distribution depends on the total number of minitubers produced per unit area and the mean minitubers weight.

Nevertheless, mean minitubers weight over cultivars did not reduce significantly between PD of 142 and 184 plants m^{-2} in both size ranges >0 g ($p=0.251$) and >3 g ($p=0.293$) (Table 1). Furthermore, only cultivar 'Mandaga' had significant ($p<0.01$) mean minitubers weight decrease within minitubers >3 g between the smallest and the largest PDs. Within size range >0 g, mean tuber weight decrease was observed for cultivars 'Prelma" and 'Mandaga'.

At the two lowest PDs minitubers of size range >20 g were produced at the largest amounts per m^2 if compared to other size ranges. However, the difference of tuber number of this particular size range between PDs was not significant ($p=0.330$). The largest total amount of minitubers at two highest PDs was obtained within the size range 10 to 20 g (Figure 1). The smallest total amount of tubers was obtained within the size ranges <3 g and 3-5 g at all PDs.

Significant ($p<0.01$) increase of minituber number within the size range <3 g, 3–5 g, 5–10 g, and 10–20 g was determined by the PD (Figure 1). Despite smaller absolute values, the largest proportional increase between PDs was observed within the size range 3–5 g, when a 4.5 fold increase was found between the smallest and the largest PD. The major proportion of minitubers number increase within this particular size range was found between PD 63 and 93 plants per m^2. Within next minitubers size ranges a tuber number increase in relation with PD was less pronounced, reaching two times increase in the range 5–10 g and 1.7 times increase in class 10–20 g between the smallest and the largest PD. In the size range >20 g minitubers number change was not significant ($p=0.330$) between PDs, even a slight decrease was observed. R. Roy et al. (1995) and A. Veeken and W.

Lommen (2009) observed a similar tendency when the minitubers number increase within size ranges was significantly determined by PD of up to particular minitubers weight.

Another important yield parameter is cumulative number of obtained minitubers, which is a total number of tubers over the certain size.

Significant ($p<0.001$) increase of minitubers >3 g and >5 g was determined by PD (Figure 2). Regardless of significant ($p<0.001$) minitubers number increase in the size range 10–20 g, no significant ($p=0.166$) changes were observed in cumulative stratum within minitubers bigger than >10 g. This phenomena can be explained by the fact that mentioned cumulative size range accumulated minitubers of >20 g weight which showed insignificant ($p=0.330$) changes with a tendency of minitubers number decrease with PD increase. Nevertheless, at the highest PD, tubers

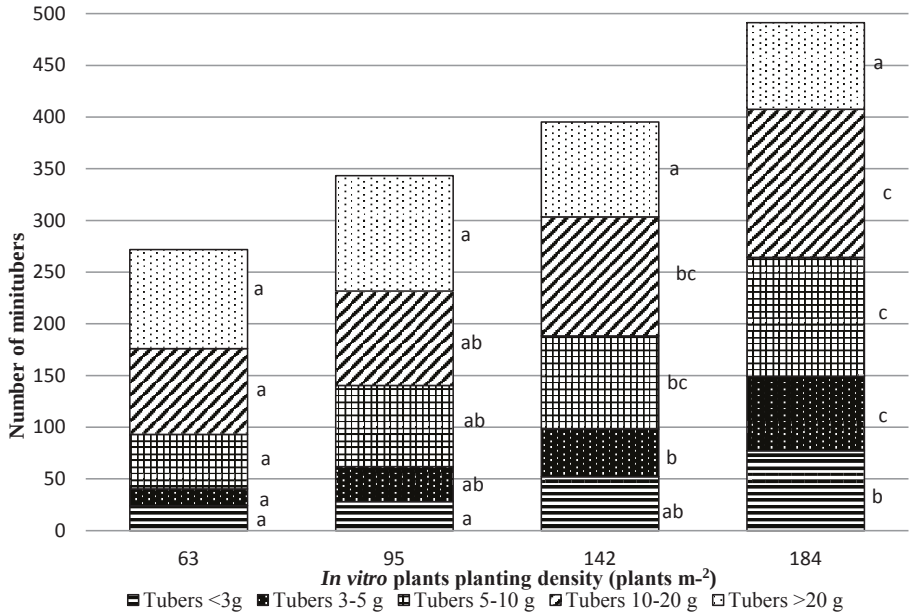

Figure 1. Minitubers weight distribution depending on *in vitro* plants planting density. Number of minitubers of the same size range with the same letter is not significantly different between planting densities ($p\geq0.05$).

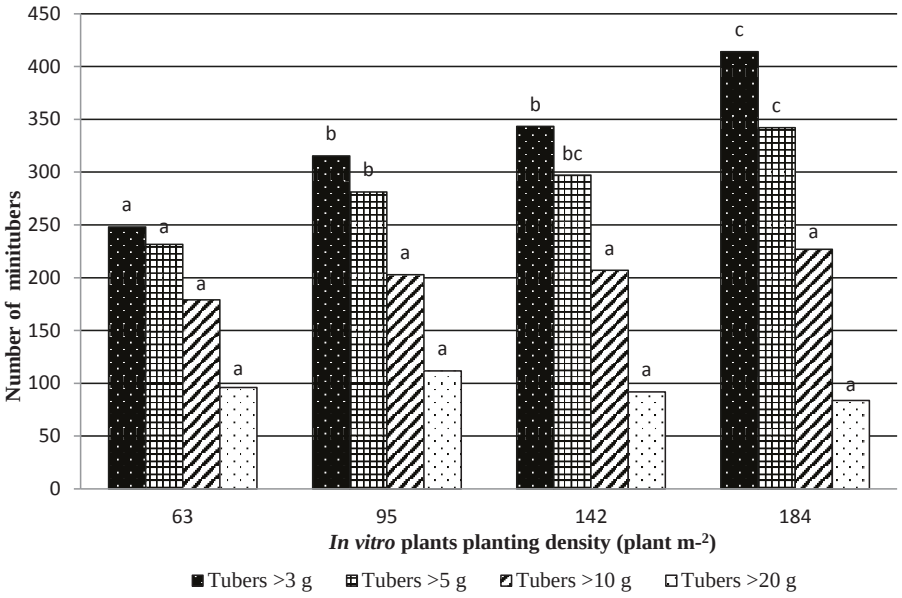

Figure 2. The cumulative number of minitubers depending on *in vitro* plants planting density. Number of minitubers of the same cumulative size range with the same letter is not significantly different between planting densities ($p\geq0.05$).

>10 g were still almost half of all produced tubers (46%).

Regarding the proportion change to total number of tubers per m^2, the total number of tuber >3 g decreased from 91% to 84% between the marginal PDs (data not shown). This tendency was caused by the respective increase in tuber number of size <3 g. The same trend (a slight decrease in percentage stratum) was observed in all cumulative size ranges.

Practical considerations

Minitubers number per area unit and the tuber number per planted plant of desirable size are two main yield parameters. It depends on a producer which of the two parameters is accepted as the major one. In case when a minitubers' grower is *in vitro* plants producer at the same time, minitubers number per area unit could become the most important parameter. The desirable minimum minitubers size depends mostly on quality of storage conditions and on minitubers field performance during subsequent field generation. Smaller minitubers size can require more careful field practices, which is not always affordable.

The number of planted plants per m^2 increased more considerably than minitubers number m^{-2} increase (2.9 fold and 1.7 fold (>3 g) to 1.8 (>0 g) fold increase between the lowest and the highest PD respectively; Table 1). Nevertheless, a decrease of multiplication rate was less evident. This could let us assume that increased PD can contribute to optimization of minitubers production at limited greenhouse space. Nevertheless, more data must be obtained during the repetitive experiments in order to see which PD could be considered as the optimal one.

Conclusions

1. The number of obtained minitubers per m^2 per planted plant (multiplication rate) and mean tuber weight was significantly determined by cultivar ($p<0.001$) and planting density ($p<0.001$).
2. Increased planting densities led to reduced multiplication rate, reduced mean tuber weight and increased minitubers number per m^2.
3. An increase of tuber number per m^2 was significantly ($p<0.01$) determined by planting density within size ranges <3 g, 3–5 g, 5–10 g, and 10–20 g of up to the size range >20 g where a slightly insignificant ($p=0.330$) decrease was observed. The same trend was observed in tuber number cumulative stratum up to the size range >10 g. However, even at the highest PD harvested minitubers of size >10 g were still almost half of all produced tubers (46%).

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THE EFFECT OF DOUBLE INOCULATION ON THE BROAD BEANS (*VICIA FABA* L.) YIELD QUALITY

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Abstract
Legumes (*Leguminosae*) are one of the most widely grown crops in the world after cereals (*Poaceae*). They are not only an important source of protein in food and feed, but also a significant component of different agrosystems. The N_2 fixation by legumes is of great importance in nutrient management and sustainable economy of nitrogen. Legume productivity largely depends on a successful formation of symbiosis between the plant and soil microorganisms. The most important among those are rhizobia and mycorrhiza fungi. The field experiment was carried out at the Institute of Soil and Plant Sciences, Latvia University of Agriculture in 2014 to evaluate the influence of double inoculation using *Rhizobium leguminosarum* and mycorrhiza fungi preparation on yield formation of broad bean *V. faba* L. var. *major* Harz 'Bartek'. The bean seeds were treated with rhizobia and/or mycorrhiza fungi before sowing. Seeds were treated with rhizobia by soaking in bacteria suspension for 30 minutes directly before sowing. Mycorrhiza fungi preparation was added in soil under seeds before sowing. Plant height, fresh and dry weight and the weight of nodules were measured at the beginning of broad bean flowering (BBCH 60-61). *Rhizobium leguminosarum* response to double inoculation differed between the strains. *Rhizobium leguminosarum* strain RL407, isolated from *Vicia faba*, was shown to be the most appropriate strain used for inoculation of broad bean seeds. Bean seed double inoculation increases the protein content significantly comparing to single inoculation using mycorrhiza fungi preparation.
Key words: legumes, *Rhizobium leguminosarum*, mycorrhiza fungi, nodulation, protein.

Introduction

Legumes (*Leguminosae*) are economically important crop, the growing areas of which increase yearly not only in Latvia. The sowing area of legumes during the period from 2007 to 2013 increased approximately four times. Beans (*Vicia faba* L.) in these volumes averaged about a quarter (Central Statistical Bureau of Latvia). Legumes are a high-quality, protein-rich animal feed and wholesome source of nutrition for people; moreover, they are a valuable crop in different crop rotation systems. Legumes are able to fix atmospheric nitrogen up to 250 kg ha^{-1}, thus reducing the load on the agro fertilizer.

In order to develop sustainable farming systems with reduced inputs of manufactured fertilizers, more attention should be given to the use of microorganisms that could have beneficial effects on crop production. Symbiotic N_2 fixation by legumes and the potential N_2 transfer to other non-legume plants has great importance in nutrient management and sustainable economy of nitrogen (Høgh-Jensen and Schjoerring, 1994; Chalk et al., 2006). In the *Rhizobium*-legume symbiosis, the process of N_2 fixation depends not only on the physiological state of the host plant (Mabrouk and Belhadj, 2012; Franzini et al., 2013), but is limited by a competitive and persistent rhizobial strain and soil microorganism association activity too (Goel et al., 2001; Barea et al., 2005).

For the development of effective symbiotic system it is necessary to ensure the host and rhizobia not only with carbon and nitrogen, but also with other mineral elements and water. The uptake of mineral elements can be improved through mycorrhizal fungi. Therefore, a growing interest in the world is given to mycorrhiza fungi and rhizobia symbiotic association, which contributes not only to the supply of the plant with certain mineral elements, but also improves plant stress tolerance. Symbiotic associations' effectiveness is affected by each of the association components and interaction between the soil and climatic conditions (Xavier and Germida, 2002; Chalk et al., 2006; Jia and Gray, 2008).

Soil arbuscular mycorrhiza fungi (AMF) colonize roots of host plants and promote plant growth, which is generally attributed to the improved uptake of nutrients with particular emphasis on P nutrition (Franzini et al., 2010; Farzaneh et al., 2011). However, other studies indicate that AMF inoculation increases dry matter accumulation and nutrient uptake compared with indigenous AMF communities (Pearson et al., 1993; Chalk et al., 2006; Farzaneh et al., 2011). R. Porcel et al. (2003) observed that the inoculation of soybean plants with AMF enhances protein accumulation as well as increases the nodule activity. Different legume species have shown the development of symbiotic associations with both phosphorus–acquiring arbuscular mycorrhizal fungi and nitrogen fixing rhizobia. Suitable symbiotic association provides legume with essential mineral elements for good protein accumulation in the seeds (Xavier and Germida, 2002; Scheublin et al., 2004; Scheublin and van der Heijden, 2006).

Research confirms the importance of the formation of the tripartite symbiosis, but there is no clear mechanism known to ensure that. It has been shown that the response of legume host to *Rhizobium* can be

Table

Microorganisms used in the experimental variants

Label of variants	Treatment
K	control without treatment
RL23	inoculated with *Rhizobium leguminosarum* strain RL23
RL23M	inoculated with *Rhizobium leguminosarum* strain RL23 and mycorrhiza fungi
RL407	inoculated with *Rhizobium leguminosarum* strain RL407
RL407M	inoculated with *Rhizobium leguminosarum* strain RL407 and mycorrhiza fungi
M	inoculated with mycorrhiza fungi
KN	used addition mineral nitrogen fertilizers, without microorganism preparations

modified by the AMF species involved in the tripartite association. It is not clear whether the presence of AMF influences nodule formation and functioning (Scheublin and van der Heijden, 2006). Formation of the tripartite symbiotic association can be determined and regulated by a series of biochemical signals. A.K. Goel et al. (2001) found out that some rhizobium strains can produce bacteriocin. I. Sampedro et al. (2007) pointed out the significance of xylogenase activity, which characterizes microsymbiont ability to penetrate plant root cell wall. Another, even more important biochemical signal between legume and rhizosphere microorganisms are flavonoids. They can be released from plant cells in response to biotic and abiotic signals in the rizosphere and exudation from the root changes during the symbiosis (Maj et al., 2010; Abdel-Lateif et al., 2012; Hassan and Mathesius, 2012).

The aim of the experiment was to evaluate the influence of double inoculation with *Rhizobium leguminosarum* and mycorrhiza fungi preparation on yield formation of broad bean *V. faba* L. var. *major* Harz.

Materials and Methods

Experiments were carried out at the Institute of Soil and Plant Sciences (56º39′ N, 23º45′ E), Latvia University of Agriculture in 2014. Field experiment was conducted in a loamy sand soil characterized by ph kcl 7.6, ec ms cm^{-1} 0.73 and mineral element content in 1m hcl solution (mg l^{-1}): n-78, p-523, k-170, ca-161450, mg-3850, s-65, fe-1920, mn-170, zn-10.5, cu-6.5, mo-0.08, b-0.4.

The field experiment was carried out in four replications. Size of each experimental plot was 1 m^2. Plants were sown in rows by hand. Distance between rows was 30 cm and 10 cm between seeds. Experimental plots were separated by one meter buffer zones.

Broad bean *Vicia faba* L. var. *major* Harz 'Bartek' was grown in the experiments. Seeds were treated with rhizobia – soaked in bacteria suspension for 30 minutes directly before sowing. Mycorrhiza fungi preparation was added in soil under seeds before sowing. The experimental variants are summarized in Table.

Rhizobium leguminosarum strains were obtained from the Collection of Rhiziobia of Latvia University of Agriculture. Strains RL23, isolated from *Pisum* sp., and RL407, isolated from *Vicia faba*, were used in the experiment. All used strains are streptomycin resistant. Suspensions for seed inoculation contain 10^6 to 10^8 cells per mL.

Mycorrhiza fungi inoculum was obtained from company Symbiom Ltd. in the frame of EU 7th Research Framework Programme of the European Union project 613781, EUROLEGUME (Enhancing of legumes growing in Europe through sustainable cropping for protein supply for food and feed). Mycorrhiza inoculums contain a mixture of at least three species of mycorrhiza fungi.

Meteorological conditions during all vegetation period of 2014 were characterized by larger precipitation than average (123-220% from long term data); however, at the beginning of the flowering stage water deficit in the soils was observed. After warm beginning, the end of June was relatively cool. Temperature on average was 0.9 °C lower in comparison with the long term average. July was warm and dry. Temperature on average was 2.7 °C higher in comparison with the long term average but precipitation was only 74% of the long term average. August was warm, but with higher precipitation in comparison with July. Precipitation was 178% of the long term average[1].

The plant height, fresh and dry weight and the weight of nodules were measured at the beginning of broad bean flowering (BBCH 60-61). The bean yield parameters – 100 bean weight and protein content of the seeds was determined at the end of vegetation period. Protein content of the bean seeds was determined using mature, dry seeds. For protein analyses the average sample of replicates was used. Protein content was determined in two replicas by

[1] www.meteo.lv

Kjeldal method (LVS EN ISO 5983-2:2009) in the Analytical laboratory for agronomic research, Latvia University of Agriculture.

All statistical analyses were done using Excel (Microsoft Corporations, Redmond, Washington, USA).

Results and Discussion

Efficient symbiotic relationships can have a positive impact on the formation of leguminous plants and crop production. The obtained results show that inoculation of bean seed with symbiotic microorganisms influences plant shoot and root growth (Fig. 1.). The results show different effects of double inoculation of bean seeds. *Rhizobium leguminosarum* strain RL407 significantly stimulated the growth of shoot in the variants with double inoculation. However, no significant differences between variants inoculated with RL23 were observed.

The activity of symbiotic microorganisms in the plant root and rhizosphere influenced the growth intensity of roots. Bean shoot and root ratio ranged between 2.4 and 2.9. The lowest ratio was obtained in the variant with *Rhizobium* strain RL407 but the highest one with strain RL23 and double inoculated variant RL407M. Plant shoot and root ratio in the control variant was 2.7. Broad beans with double inoculation were characterized by more extensive root system. The average root weight in the variants RL23M and RL407M in comparison with control were about 28 and 30% higher, respectively. Single inoculation using only *Rhizobium leguminosarum* or mycorrhiza fungi increased the average root weight from 12 to 16%. These results are consistent with data in literature where different responses of legume inoculation are reported (Xavier and Germida, 2002; Barea et al., 2005). L.J.C. Xavier and J.J. Germida (2002) concluded that the influence of different strains of rhizobia on shoot and root dry weight of lentil (*Lens culinaris*) were altered by mycorrhiza fungi.

Effective symbiosis between legume and rhizobia is characterised by the number and size of nodule on plant roots. As pointed out by T.R. Scheublin and M.G.A. van der Heijden (2006), it is not yet clear whether the presence of mycorrhiza fungi influences the nodule functioning. In our experiment the nodule formation was observed at the beginning of flowering (BBCH – 60-61) (Fig. 2). Some nodules were found on the control plant roots too, suggesting that experimental soil contains indigenous rhizobia. These bacteria can compete with experimental rhizobia strains. Goel et al. (2001) found out that some rhizobium strains can produce bacteriocin which inhibits the growth of homologous *Rhizobium* strains.

The weight and size of nodule depends on specific activity of rhizobia. The most efficient bacterium stimulates the formation of nodule which provides legumes with nitrogen, so the plants can develop a greater weight. The various numbers of nodules on experimental plant roots may indicate the competitiveness of *Rhizobium* strains. Results showed significant differences ($p = 0.018$) between the average

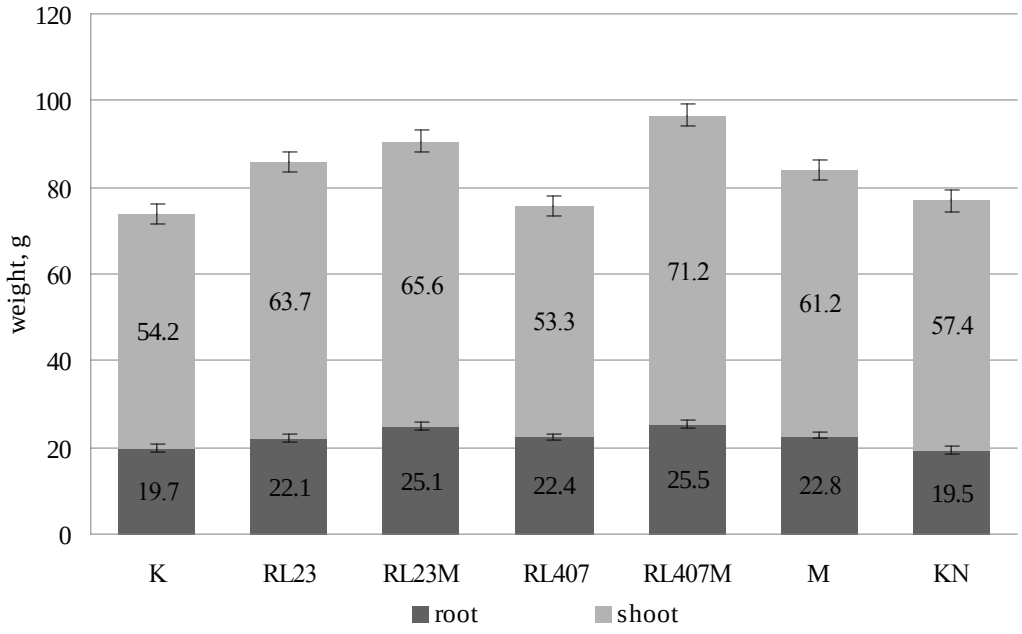

Figure 1. Average shoot and root weight at the flowering stage (BBCH – 60-61) of broad beans: K – control, RL23 and RL407 – *Rhizobium leguminosarum* strains, M – mycorrhiza preparation, KN – with additional nitrogen, without microorganism preparations.

number of nodules compared to control (K). Results show that both mycorrhiza alone and an additional N fertilizer have contributed to the indigenous rhizobia activity, as nodule is significantly heavier than in controls.

The effect of double inoculation on broad bean yield was analysed by seed weight and protein content in the seeds. An increase of 100 bean weight was detected only in the variant with double inoculation with *Rhizobium leguminosarum* strain RL407 and mycorrhiza fungi (Fig. 3.). A significant difference was observed between single inoculation variants with RL407 and double inoculated RL407M. For all other variants no significant differences of 100 bean weight were detected.

Protein accumulation in the seeds depends not only on plant biosynthetic activity but can be affected by microbial symbionts. A significant increase ($p>0.005$) of protein content was observed in all treatments comparing with control (Fig. 4). The highest protein content in the seeds was determined in the double inoculated plant (R23M and R407M).

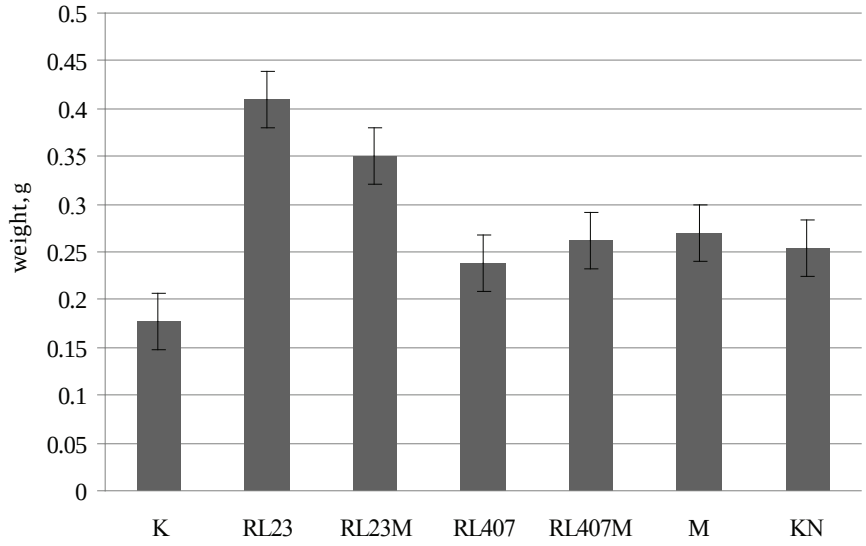

Figure 2. Weight of 10 nodules from broad beans root at the beginning of flowering stage (BBCH – 60-61) of broad beans: K – control, RL23 and RL407 – *Rhizobium leguminosarum* strains, M – mycorrhiza preparation, KN – with additional nitrogen, without microorganism preparations.

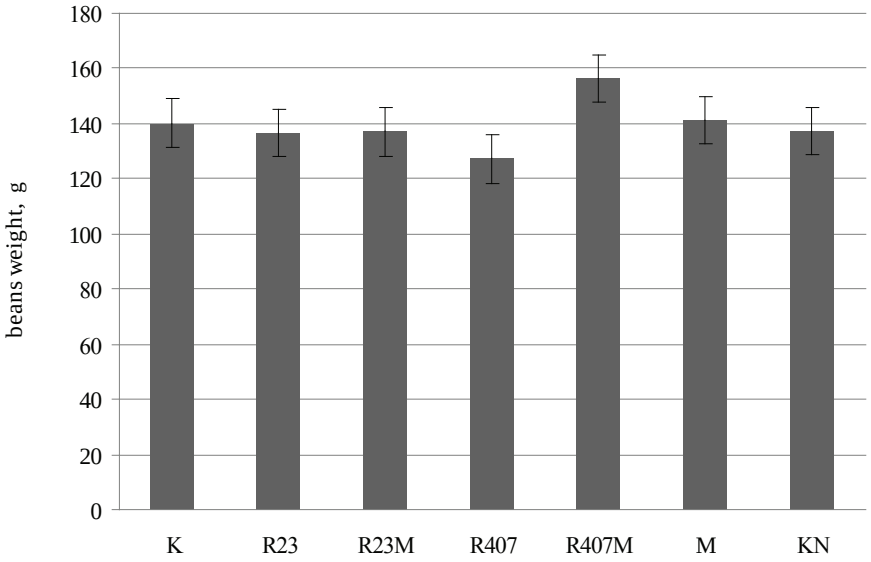

Figure 3. 100 bean weight (g), depending on the treatment: K – control, RL23 and RL407 – *Rhizobium leguminosarum* strains, M – mycorrhiza preparation, KN – with additional nitrogen, without microorganism preparations.

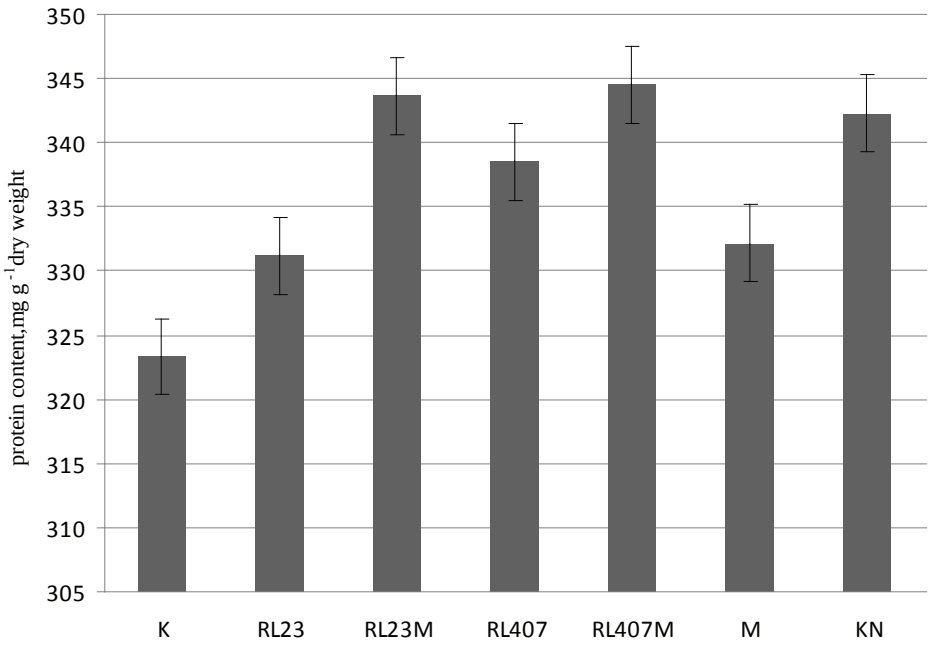

Figure 4. Protein content in bean seeds, mg g^{-1} dry weight, depending on the treatment: K – control, RL23 and RL407 – *Rhizobium leguminosarum* strains, M – mycorrhiza preparation, KN – with additional nitrogen, without microorganism preparations.

Single inoculation with mycorrhiza fungi (M), in comparison with double inoculation (RL23M and RL407M), did not increase the protein content in seeds. Inoculation of seeds with single microsimbiont gave the increase of protein content in broad been seeds from 7.9 to 15.2 mg g^{-1}. Double inoculation increases protein content in seeds on average by 20.3-21.2 mg g^{-1} in comparison with control. The obtained results are consistent with some literature data. P.M. Chalk et al. (2006) pointed that inoculation with rhizobia and mycorrhiza fungi has beneficial effects on legume dry matter or grain yield. P.M. Chalk et al. (2006), Y. Jia and V.M. Gray (2008), T.R. Scheublin and M.G.A. van der Heijden (2006) explained this effect as follows – that mycorrhiza fungi support legume plant with phosphorus uptake and another immobile nutrients, including microelements.

Conclusions

Bean seed double inoculation with *Rhizobium leguminosarum* and mycorrhiza fungi preparation increases the protein content significantly comparing to single inoculation using mycorrhiza fungi preparation.

Rhizobium leguminosarum strain RL407, isolated from *Vicia faba,* is more appropriate for inoculation of broad bean seeds, while stimulates protein accumulation more than strain RL23.

The selectivity of mycorrhiza fungi to Rhizobia strains was observed. More suitable for triple symbiosis was the combination of broad beans-mycorrhiza fungi – *Rhizobium* strain RL407.

Acknowledgement

This research is supported by the 7th Research Framework Programme of the European Union project 613781, EUROLEGUME (Enhancing of legumes growing in Europe through sustainable cropping for protein supply for food and feed).

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HEAT OF WINTER CEREAL CROPS

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Abstract
Heat is one of the most important types of energy at northern latitudes. In 2013 the total consumption of renewable energy resources (RER) in Latvia was 68 PJ. The heating systems can function on plant or other organic material, for example, wood chips or agricultural residues. By using local biomass resources it is possible to reduce the pollution of atmosphere caused by greenhouse gas emissions. Different variety of winter wheat (*Triticum aestivum*), triticale (*Triticosecale*) and rye (*Secale cereale*) were used in the research. The following aspects were determined during the research: dry matter yield, chemical composition and the higher heating value of grains and straw. The evaluation of grains and straw of winter cereals showed that the higher heating value (MJ kg^{-1}) was acquired from the straw of winter cereals, whereas the grains had the highest dry matter yield, thus the grains of winter cereals had the highest heating yield from one hectare (GJ ha^{-1}).
Key words: winter cereals, grain, straw, chemical composition, heating value.

Introduction

Heat is acquired from coal, gas, wood as well as from plant biomass – straw, grass, grains. The natural renewal process of fossil energy resources (coal, gas) takes a very long time whereas the plant biomass that accumulates solar energy, renews itself every year. Photosynthesis results in the production of structural and non-structural carbohydrates in the plant tissues. The components of biomass include cellulose, hemicelluloses, lignin, lipids, proteins, simple sugars, starches, water, hydrocarbon ash, and other compounds (Jenkins et al., 1998).

According to the data of Central Statistical Bureau (CSB), in 2013 the total consumption of renewable energy resources (RER) in Latvia was 68 PJ. In comparison to year 2012 the RER consumption had decreased by 2.5%. It is related to the decrease of energy production in hydroelectric power stations. According to the data of CSB, the main types of RER in Latvia are firewood and hydro resources, in 2013 reaching 34.2% of the total energy consumption. Wind energy, biogas, biofuel, straw and other types of biomass were used to a smaller extent.

However, according to different scenarios the share of oil and natural gas in heating will not diminish. The use of biomass in heating systems makes up only a small part of the total amount of different resources (Barkāns, 2001). In order to produce heat, plant or other organic matter, for example, wood chips and agricultural residues can be used. Fossil fuels (gas, oil products, coal) can be replaced by local biomass resources. By using local biomass resources it is possible to reduce the pollution of atmosphere caused by greenhouse gas emissions, the consumer does not suffer from the fluctuating prices of fossil fuels and new jobs are created or the old ones are maintained in order to ensure the growing, processing and transportation of the raw materials (Biomass Heating .., 2005). Grains that are not suitable for food or fodder can be used in heating. They can be a high-value heating fuel. For the heating purposes a high-quality biomass needs: high yield; low ash content; low humidity level; high heating value; high bulk density (Fuel supply handbook .., 2010). Grains are like nature-made pellets. Straw can be used in heating either by being transformed into pellets or pressed into bales or coils.

The purpose of the study was to evaluate the suitability of winter cereals for heating, taking into account their yield, chemical composition and heating value.

Materials and Methods

The research was carried out in State Stende Cereal Breeding Institute in years 2009/2010 – 2011/2012. The trial fields were made in loam soil, Stagnic Retisol (Loamic) (World reference base for soil resources 2014) with the following characteristics: pH KCL 5.3–5.8, content of organic matter 19 – 24 g kg^{-1}, content of P_2O_5 readily available for plants 83 – 229 mg kg^{-1}, K_2O content 134 – 181 mg kg^{-1}. There were three rye varieties (‘Matador’, ‘Placido’, ‘Dankowskie Nowe’), three triticale varieties (‘SW Valentino’, ‘Dinaro’, line 0002-26) and three winter wheat varieties (‘Mulan’, ‘Skalmeje’ and line 99-115) examined during the research. The field experiments were placed randomly in four replications. The grains were harvested by using a harvester, the grain yield (t ha^{-1}) was determined at 100% purity and 14% humidity after the grains were dried. As regards the straw yield, the samples were gathered from an area of 0.250 m^2 in each replication. The correlation between grains and straw was determined in the laboratory. The straw yield was calculated by using the acquired grain yield and the correlation between the amount of straw and grains.

The following quality indicators were determined from the acquired samples in the Laboratory of Grain Technology and Agrochemistry of State Stende Cereal Breeding Institute: humidity content (according to LVL EN ISO 721:2010), fiber (according to ISO 5498), crude ash (according to LVS 276:2000), N (according to LVS 277:2000), P (according to ISO 6492), K (according to LVS EN ISO 6969), Ca (according to LVS EN ISO 6869). The higher heating value of straw (kJ kg^{-1}) (according to ISO 1928) was determined in the Laboratory for Testing of Wood Chemistry Products at the Latvian State Institute of Wood Chemistry by using oxygen bomb calorimeter "Parr 1341" to manufacturing USA. The lower heating value is calculated by using the following formula (1):

$$Q_z = Q_{as} - 2454\ (W + 9H), \text{ where} \quad (1)$$

Q_z – lower heating value of the fuel weight, kJ kg^{-1}
Q_{as} – higher heating value of the fuel weight, kJ kg^{-1},
2454 – amount of heat necessary for evaporation of water at 20 °C, kJ kg^{-1},
9 – multiplier, as 1 part of hydrogen combines itself with 8 parts of oxygen,
W – humidity content in fuel, %,
H – hydrogen content in fuel, %
Dispersion analysis was used for the mathematical procession of data.

Results and Discussion

Dry matter yield of grains and straw. The dry matter yield from grains and straw of winter cereals was calculated in order to make the acquired data comparable. The average dry matter yield of winter cereals during the three years of research ranged from 7.71 to 8.38 t ha^{-1}. The species and variety of winter cereals as well as meteorological conditions had a significant ($p<0.05$) meaning for the yield formation in the individual years. The dry matter yield of winter wheat grains (7.97–8.36 t ha^{-1}) did not differ significantly among the examined winter wheat variety. As regards triticale during the three years of trial the highest dry matter yield was acquired from the variety 'Dinaro' – 8.08 t ha^{-1}. The most productive winter rye variety was 'Placido', that during two years of trial provided an average of 8.38 t ha^{-1} (Table 1). The dry matter yield of all cereal species was significantly affected by the meteorological conditions during the cultivation years.

The average dry matter yield that was acquired from straw of winter cereals was lower than the dry matter yield of grains – 6.39–6.81 t ha^{-1} (Table 2). From all the winter wheat varieties the highest dry matter yield was acquired from the straw of line 99–115 (7.63 t ha^{-1}), that had the lowest grain dry matter yield. Similar pattern was observed with rye variety where the highest dry matter yield was acquired from the variety 'Dankowskie Nowe' (7.38 t ha^{-1}).

Table 1

Grain dry matter yield, ash, lower and higher heating values, gross heating yield and energy values (2010 – 2012)

Species, variety	Dry matter yield, t ha^{-1}	Ash, g kg^{-1}	Bulk density, g L^{-1}	Lower heating values, MJ kg^{-1}	Higher heating value, MJ kg^{-1}	Higher heating value, GJ ha^{-1}	Energy values, MWh ha^{-1}
Winter wheat							
Skalmeje	8.17	16.0	769.0	14.10	15.99	130.47	36.29
99-115	7.97	16.4	772.8	13.92	15.79	142.33	34.96
Mulan	8.36	16.4	767.3	14.08	15.94	128.43	37.01
Average	8.16	**16.2**	**769.7**	14.03	15.90	133.74	36.08
$LSD_{0.05}$	0.46	0.7	19.3	0.39	0.39	14.43	-
Triticale							
SW Valentino	7.89	19.2	695.1	13.91	16.17	148.94	34.54
Dinaro	**8.08**	18.3	690.6	13.89	16.26	152.61	35.35
0002-26	7.62	18.2	696.7	14.22	**16.54**	146.49	33.99
Average	7.86	**18.6**	**694.1**	14.00	16.32	149.34	34.62
$LSD_{0.05}$	0.47	1.2	7.8	0.37	0.27	20.13	-
Rye							
Placido	**8.38**	15.4	747.1	14.14	15.99	155.83	37.20
Matador	7.41	15.7	745.3	14.14	16.02	137.60	32.98
Dankowskie Nowe	7.34	**16.4**	743.7	14.11	15.97	136.06	32.55
Average	7.71	**15.8**	**745.4**	14.13	15.99	143.16	34.24
$LSD_{0.05}$	0.51	0.6	4.9	0.42	0.37	24.31	-

Table 2

Straw dry matter yield, ash, lower and higher heating values, gross heating yield and energy values (2010 – 2012)

Species, variety	Dry matter yield, t ha^{-1}	Ash, g kg^{-1}	Lower heating values, MJ kg^{-1}	Higher heating value, MJ kg^{-1}	Higher heating yield, GJ ha^{-1}	Energy values, MWh ha^{-1}
Winter wheat						
Skalmeje	5.82	54.6	15.08	16.68	108.42	26.96
99-115	**7.63**	**46.8**	15.02	16.80	**142.33**	35.62
Mulan	6.75	**46.1**	15.40	17.05	128.43	31.93
Average	6.74	**49.1**	15.17	16.84	126.39	31.50
LDS $_{0.05}$	1.54	1.3	0.77	0.80	11.73	-
Triticale						
SW Valentino	6.51	**51.8**	14.99	16.64	124.16	30.12
Dinaro	5.92	49.5	14.75	16.39	108.97	27.19
0002-26	6.76	37.0	15.76	17.39	135.80	32.64
Average	6.39	**46.1**	15.17	16.80	122.98	29.98
LDS $_{0.05}$	1.02	1.3	1.18	1.15	55.13	-
Rye						
Placido	6.36	**49.3**	16.00	17.64	124.74	31.18
Matador	6.69	44.5	15.66	17.30	133.72	32.14
Dankowskie Nowe	**7.38**	44.5	15.65	17.29	145.73	35.45
Average	6.81	**46.1**	15.77	17.41	134.73	32.92

Chemical composition of grains and straw of winter cereals. Ash content in the biomass depends on its chemical composition. The amount of ash and the deposit formation in the heating boilers depend on the chemical elements K, P, Si, Na, S, Cl, Ca, Mg, Fe in the grains (Beidermann et al., 2005). Ash content in the grains of examined winter cereals was 15.4 – 19.2 g kg^{-1}. This amount is similar to the amount for winter cereals analysed in the scientific literature (Lewandowski et al., 2003, Beidermann et al., 2005; Wachendorf, 2008). The ash content of straw was 37.0 – 54.6 g kg^{-1}, which was significantly ($p<0.05$) higher than that of grains. As regards the highest ash content in grains, the highest level was observed in triticale grains: average 18.6 g kg^{-1} (Table 1). According to scientific literature, the ash content of wood biomass is 0.4 – 0.8% (4 – 8 g kg^{-1}) (Obernberg et al., 2004), however, the biomass of willows and poplars contains 1.71 – 2.7 % (17.1 – 27.0 g kg^{-1}) of ash (Jenkins et al., 1998). The ash content of coal is 5 – 20% (50 - 200 g kg^{-1}) depending on its quality (Biomass Energy...s.g.). The European Union member states have adopted standards for production of pellets for heating. One of the indicators is ash content in fuel. It has to be in the range from 0.7 to 1.5% (7 – 15.0 g kg^{-1}) (Garcia-Maraver et al., 2011). In the trial it was observed that the ash content of grains slightly exceeds the EU standards for pellets. The ash content of straw is significantly higher than envisaged in the standards and that encumbers the utilisation of heating boilers. The ash content differed significantly from one examined species to the other and depended on the chemical composition of the material (Table 2).

The quality of the fuel is affected by the chemical composition of the biomass. Biomass proteins contain nitrogen. During combustion it completely transforms into gaseous state. The researchers have observed that the amount of nitrogen correlates with the formation of nitrogen oxide (NO_x). (Wachendorf, 2008; Shcolz et al., 2002). Trials showed that the grains of winter cereals contained from 19.1 to 21.9 g kg^{-1} of nitrogen, but the amount of nitrogen in the straw of winter cereals was significantly lower, namely, 5.4 – 6.4 g kg^{-1} (Table 3). A significantly higher amount of nitrogen in the grains of winter cereals can be explained by higher levels of additional nitrogen fertilisation, that has also been described in the research of other authors (Shcolz et al., 2002). According to the standards of Austria, wooden briquettes and pellets must contain no more than 0.3% (3 g kg^{-1}) of nitrogen. Amount of nitrogen in straw pellets and bark briquettes must not exceed 0.6% (6 g kg^{-1}) (Obernberger et al., 2004). The research data show that the amount of nitrogen in grains of winter cereals is higher, but in straw it is close to the above mentioned standard.

The amount of potassium in biomass has an impact on the corrosion of heating boilers and lowers the melting temperature of ashes which causes slag formation (Shcolz et al., 2002). During the research it was observed that the grains of winter cereals contained from 4.06 to 5.04 g kg^{-1} of potassium, whereas the straw had a higher potassium content,

Table 3

Chemical composition of winter cereal grain and straw, g kg^{-1} (2010 – 2012)

Species	Nitrogen, g kg^{-1}		Phosphorus, g kg^{-1}		Potassium, g kg^{-1}		Calcium, g kg^{-1}		Magnesium, g kg^{-1}	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
Winter wheat	21.9	6.0	2.62	**0.6**	**4.06**	**7.55**	0.47	**1.93**	0.96	0.60
Triticale	19.2	**5.4**	2.92	0.7	5.04	8.22	0.40	2.06	0.91	**0.57**
Winter rye	**19.1**	6.4	**2.57**	1.0	4.10	10.70	**0.36**	2.16	**0.73**	0.67
LDS $_{0.05}$	1.3	0.7	0.22	0.23	0.16	1.14	0.09	0.18	0.18	0.19

namely, 7.55 – 10.7 g kg^{-1} (Table 3). The potassium content in dry matter of forestry residues, fast-growing willows is lower than the one observed in the samples of cereals grains and straw (Beidermann et al., 2005).

No negative impact of phosphorus on the emission during the combustion has been observed, however, a high amount of phosphorus affects the melting temperature of ashes (Shcolz et al., 2002). The winter cereal grains contained 2.57 – 2.92 g kg^{-1} of phosphorus, while the winter cereal straw had 0.6 – 1.0 g kg^{-1} (Table 3). The wood contains less phosphorus than the grains of winter cereals (Wachendorf, 2008). It has to be noted that by fertilising the fields with ashes, potassium and phosphorus are returned to the soil.

Calcium and magnesium raises the melting temperature of ashes (Beidermann, et al., 2005). The winter cereal grains contained a smaller amount of calcium (0.36 – 0.47 g kg^{-1}), but the calcium content in straw ranged from 1.93 to 2.16 g kg^{-1}. The amount of magnesium in grains was 0.96 g kg^{-1}, but in straw up to 0.67 g kg^{-1} (Table 3). Amount of calcium and magnesium in wood is smaller than in straw and grains of winter cereals (Wachendorf, 2008).

An important indicator in the choice of fuel is fuel density or bulk density, as it affects the transportation and storage of the fuel. According to the standards elaborated in Sweden, the density of SS 187120 pellets for heating has to be from 600 kg m^3 (Obernberg et al., 2004, Garcia-Maraver et al., 2011). Density-wise the grain are similar to coal, their density is approximately 700 – 850 kg m^3 (Biomass Energy...s.g.). According to the research data, the bulk density of winter cereal grains ranges from 690.6 to 772.8 kg m^3. Looking at different species, winter wheat had the highest bulk density of grains, it was followed by rye and triticale (Table 1). No significant differences among the species were observed. The density of straw was not determined during the research.

Heating value is one of the most important indicators of heating quality. Higher heating value is energy amount per unit mass or volume released on complete combustion (Fuel supply handbook .., 2010). According to the data collected during the three years of trial, triticale grains had a significantly ($p<0$) higher heating value (MJ kg^{-1}) – 16.32 MJ kg^{-1}. The higher heating value of straw of the observed species, however, ranged from 16.81 to 17.41 MJ kg^{-1}, with no significant differences among species (Table 2). Similar data have been mentioned in the scientific literature (Wachendorf, 2008).

No significan substantial diferences in the higher heating value were observed among the investigated species. According to the data of European Association of Biomass Industry (European Biomass, s.g.), the higher heating value of coal ranges from 20 to 30 MJ kg^{-1}, that of wood – from 18 to 19 MJ kg^{-1}, and that of agricultural residues – from 15 to 17 MJ kg^{-1}. According to the data of Austrian researchers, the higher heating value of cereal grains was 18.61 MJ kg^{-1} (Friedel et al., 2005). Research data let us observe that, taking into account the heating value, the grains and straw of winter cereals are just as valuable fuel material as wood and other agricultural residues. Data of this research show a slightly smaller higher heating value than the one observed in the research of other authors.

Grains and straw contain a certain amount of humidity that affects their lower heating value. The lower heating value is amount of heat contained by a fuel unit and released when drying or burning the raw material. Thus, raw materials with a higher humidity content diminish the lower heating value (Fuel Supply Handbook, 2010). On average, the humidity of grains examined during the resarch was 10 – 11%, but the humidity of straw was 7 – 9%. Depending on the intended use the grains were dried after harvest, but the humidity content of straw was affected by the weather conditions during the harvest. The grain and straw samples of the examined species and variety did not differ significantly as regards the lower heating value. The average lower heating value of winter cereal grains for variety examined in the research was 13.89 – 14.14 MJ kg^{-1}, while the lower heating value of straw was 14.75 – 16.00 MJ kg^{-1}.

Energy value. An important indicator for heating isthe energy value. The energy acquired from the

grains of winter cereals ranged from 34.24 to 36.08 MWh ha^{-1} (Table 1). According to the scientific literature, similar results were acquired in Germany (Nagel, 2000.). The energy acquired from the straw of winter cereals was slightly lower than that of grains, namely, 29.98 – 32.92 MWh ha^{-1} (Table 2). It was observed that neither for grains nor for the straw of winter cereals the energy value was affected by the choice of species or variety.

Conclusions

1. Having examined the grains and straw of winter cereals for their higher heating value (MJ kg^{-1}), the highest amounts were acquired from the **straw** of winter cereals, while the grains had a higher dry matter yield than straw, thus, the **grains** of winter cereals had a higher heating yield from one hectare (GJ ha^{-1}). From all the species the highest heating value both from mass unit and hectare (MJ kg^{-1}, GJ ha^{-1}) was acquired from **triticale** grains. As regards straw, the best results were acquired from the **rye**.
2. The highest energy value from one hectare was acquired from the winter cereal **grains**, it was slightly lower for the **straw** of the winter cereals which can be explained by the fact that straw has lower dry matter yield.
3. As regards the chemical composition of winter cereals, **rye** provided the most suitable grains for heating. The rye grains had the lowest ash, nitrogen, potassium and magnesium content. There were no significant differences in ash content of straw among species while, as regards the chemical composition, **wheat** and **triticale** straw were most suitable for heating.

Acknowledgements

The research was supported by the ESF project „Support to the implementation of Doctoral studies in the Latvia University of Agriculture", Agreement No. 2009/0180/1DP/1.1.2.1.2/IPIA/VIAA/017.

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THE INFLUENCE OF VARIETY ON THE YIELD AND CONTENT OF PROTEIN AND NUTRIENTS OF PEAS (*PISUM SATIVUM*)

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Abstract
Pisum sativum L. (field or garden pea), is widely cultivated in Europe. The purpose of this investigation was to see whether pea varieties differ in their yield and content of protein and nutrients. Another aim was to select the best varieties suitable for breeding or production. A field experiment with different varieties of peas ('Bruno', 'Capella', 'Clara', 'Mehis' and 'Vitra') was carried out at the Estonian Crop Research Institute in 2014. Yields (t ha^{-1}) were not statistically different. Crude protein content (g kg^{-1} in dry matter) was lowest in 'Clara'; all other varieties had a higher content of protein, within much the same range. The lowest N content was found in 'Clara', followed by 'Capella' and 'Vitra', 'Mehis' (44) and the highest in 'Bruno' (45). The lowest P content was found in 'Clara', followed by 'Capella' and 'Vitra'; the highest was found in 'Bruno' and 'Mehis'. The lowest K content was found in 'Mehis', then 'Clara', followed by 'Capella' and 'Vitra' and the highest in 'Bruno'. The lowest Ca content was found in 'Clara', followed by 'Capella' and 'Mehis', 'Vitra', and highest in 'Bruno'. The lowest Mg content was found in 'Capella', followed by 'Clara' and the highest in the other varieties 'Bruno', 'Mehis' and 'Vitra'. Thus, choice of the right variety for pea cultivation is very important, but depends on the local agro-climatic conditions. This investigation has been developed with the help of the project EUROLEGUME, funded from the European Union Seventh Framework Programme for Research, Technological Development and Demonstration under the grant agreement no. 613781.
Key words: nutrients, pea, protein, variety, yield.

Introduction

Pisum sativum L., field or garden pea, is widely cultivated in Europe (Brežna et al., 2006). It is an herbaceous annual crop in the *Fabaceae* (formerly *Leguminosae*) family. Pea originates from the Mediterranean basin and the Near East, but is now widely grown for its seedpod or legume (a simple dry fruit containing several seeds and splitting along its seams on two sides). Pea is an important human food crop. Green pea production worldwide in 2011 was 17 Mt (FAOSTAT, 2013) and pea is grown on over 6.7 million hectares worldwide (Kittson, 2008). Dry peas are the most widely grown legume crop in the European Union (EU) (Aiking et al., 2006). Peas are widely consumed due to their high nutritional value; they contain fibre, protein, vitamins (folate and vitamin C), minerals (iron, magnesium, phosphorus and zinc), and lutein (a yellow carotenoid pigment that benefits vision). Dry weight is high in protein and carbohydrates (mostly sugars) (Issako, 1989).

The protein content of field peas is determined by plant genetics, strongly influenced by growing conditions. Field pea contains on average 230 g kg^{-1} protein. Field pea is a very good protein crop alongside soybean and faba bean (Narits, 2008).

Surveys carried out by the FAO, the European Commission and agricultural authorities of the EU, suggest that by increasing the cultivation of protein crops in the EU, it is possible to achieve a considerable reduction of imported protein crops while increasing the quality of agricultural products and revenue of the producers. A possible increase in conventional oilseed and protein seed acreage could replace 10-20% of EU imports of soybeans and soybean meal. Replacement of soybean meal by locally grown high nutritional quality protein sources in feed and the development of new feed products are challenging objectives.

Seed and biomass yields of legumes vary widely, influenced by habitat quality, weather conditions during the growing season and the yielding ability of available cultivars (Jeuffroy and Ney, 1997; Poggio et al., 2005).

Genotype has the most significant influence on the variability but Europe has abundant genetic resources of different peas in its gene banks, research institutions and farms. The ECPGR (The European Cooperative Programme for Plant Genetic Resources) Pisum Database documents 32,503 accessions of peas. However, a large number of local genotypes grown on farms and propagated by farmers are not included in these databases.

Mineral nutrients perform several functions; they participate in various metabolic processes in the plant, such as protein, nucleic acid and cell wall syntheses, maintenance of osmotic concentration of cell sap, electron transport systems, enzymatic activity, are a component of the chlorophyll molecule, and major constituents of macromolecules, co-enzymes and nitrogen-fixing (Weisany et al., 2013).

Mineral nutrients can influence nitrogen fixation in legumes; for example, the presence of mineral nitrogen in the soil inhibits both nodule formation and nitrogenase activity. The deficiency of phosphorous supply and availability remains a severe limitation on nitrogen fixation and symbiotic interactions. Calcium plays a key role in symbiotic interactions at

the molecular level (Weisany et al., 2013). Legumes also contain minerals such as magnesium, which is important for normal cardiac function (Kostyra, 1996).

The purpose of this investigation was to see, whether pea varieties differ in yield and their content of protein and mineral nutrients, and thus whether some varieties might be better than others.

Materials and Methods

A field experiment with different varieties of peas was carried out at the Estonian Crop Research Institute in 2014 at N 58°769' E 26°400'. The varieties were: 'Bruno', 'Capella', 'Clara', 'Mehis' and 'Vitra'. 'Capella' and 'Clara' are Swedish varieties, 'Bruno' and 'Vitra' are Latvian varieties, 'Mehis' is an Estonian variety. In our experiment the leafy varieties were 'Mehis' and 'Vitra' and semi-leafless varieties were 'Bruno', 'Capella' and 'Clara'. A completely randomized experiment design was used in 4 replications. Plot size was 10 m^2. Soil humus content was 3.15% and pH was 5.76. Soil type was soddy-calcareous podzolic soil in Estonian system (Astover, 2005), soil texture - sandy-clay. The preceding crop was winter rye. Conventional cropping system was used with ploughing in autumn 2013, and cultivation twice before sowing. Seed was sown on 28 April 2014 at a rate of 120 seeds per m^2 for all varieties and a depth of 4 cm. Plant spacing was 12.5 × 6.7 cm.

Fertilization was done with Yara Mila 7-12-25 (300 kg ha^{-1}) and weeds were controlled by Activus 330 (pendimethalin 330 g L^{-1}) EC 1.5 l ha^{-1} + Basagran 480 (bentazon 480 g L^{-1}) 1.5 l ha^{-1}, on 21 may 2014. No control measures against insects and diseases were applied. Disease damage on peas pods, pod spot (*Ascoshyta pisi*) and pulses rust (*Uromices ssp.*) was assessed at the plant development stage 71-79 (Strauß et al., 1994). Pod spot on 'Mehis', 'Bruno' and 'Vitra' was at a very low level, and on 'Clara' and 'Capella' at a low level. Pulses rust was absent on 'Clara', 'Capella' and 'Mehis', at a very low level on 'Bruno' and at a low level on 'Vitra'.

The weather during 2014 is shown in Table 1, and was characterized by a cold spring. The temperature at the end of June was 3-4 °C lower than normal, but July was near average with a mean temperature around 18 °C. Precipitation exceeded the average in June although it was quite dry in July; nevertheless plants grew well.

Peas were harvested between 6-12 August 2014, dried and the yield data (determined at moisture content of 14-15%) recorded for each plot and finally calculated for t ha^{-1}. Samples were analysed for their content of protein, nitrogen, phosphorus, potassium, calcium and magnesium. Determination of protein content was by the Kjeldahl method (EVS-EN-ISO 10520:200), for phosphorus in a Kjeldahl Digest by Fiastar 5000 (AN 5242; Stannous Chloride method, ISO/FDIS 15681), for potassium by the Flame Photometric Method (956.01), for calcium by the o-Cresolphthalein Complexone method (ISO 3696, in Kjeldahl Digest by Fiastar 5000) and for magnesium by Fiastar 5000 (ASTN90/92; Titan Yellow method). Analyses of variance were carried out on the data obtained using the programme Excel. Signs used: *** $p<0.001$; ** $p= 0.001 – 0.01$; * $p= 0.01 – 0.05$; NS not significant, $p>0.05$. On figures, on columns are marked bars, which are the bars of standard deviations.

Table 1

Weather conditions of field pea vegetation period in 2014 and long term weather averages

Period	Average of air temperature, °C*				Summary of precipitation, mm*	
	In decade	Long term average	Max	Min	In decade	Long term average
21-30 April 2014	9.3	6.2	21.7	-4.7	0.0	13.2
1-10 May 2014	5.9	8.4	17.1	-5.2	18.9	12.6
11-20 May 2014	13.1	10.6	30.5	-1.6	41.2	17.5
21-31 May 2014	15.6	11.8	29.4	5.3	4.0	19.8
1-10 June 2014	16.1	13.5	27.4	3.2	76.5	14.4
11-20 June 2014	11.9	14.4	21.3	2.2	37.1	25.4
21-30 June 2014	11.3	15.5	20.0	2.2	43.5	27.9
1-10 July 2014	17.5	16.5	27.1	8.1	19.1	22.5
11-20 July 2014	18.6	17.0	25.7	8.0	3.7	26.4
21-31 July 2014	21.5	17.0	30.5	10.7	25.0	30.6
1-10 Aug 2014	20.8	16.5	29.7	9.6	2.9	30.4
11-20 Aug 2014	16.2	15.4	27.7	9.8	31.4	28.5
21-31 Aug 2014	12.8	14.1	19.7	3.7	88.7	29.9

*according to Jõgeva Meteorological Station

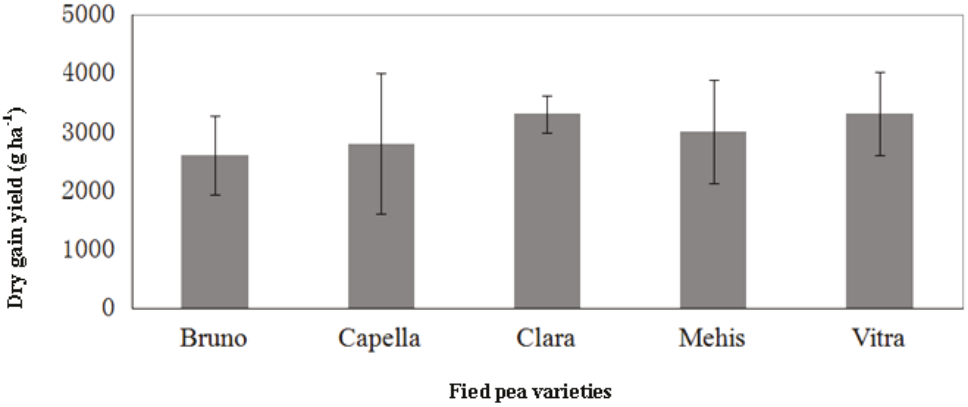

Figure 1. Dry grain yield (kg ha^{-1}) of different field pea varieties (p NS).

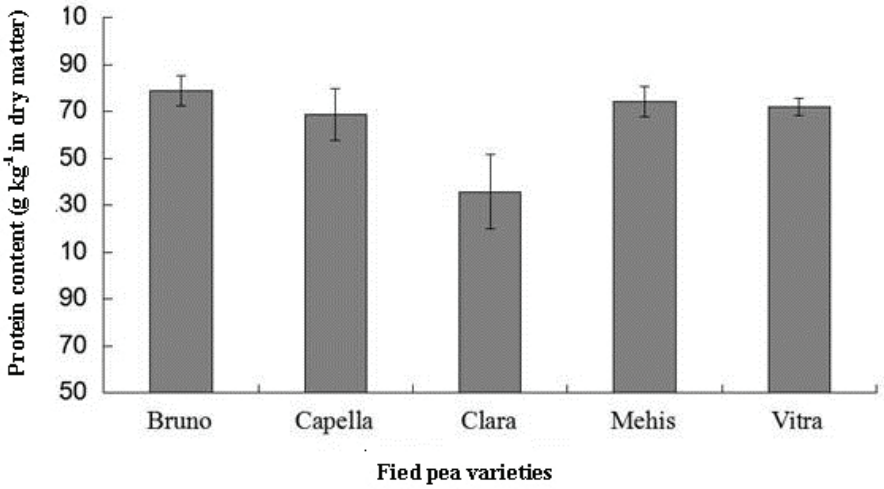

Figure 2. Average protein content (g kg^{-1} in dry matter) of different field pea varieties (p ***).

Results and Discussion

There was a tendency that the highest yield was obtained in 'Clara' and 'Vitra' (3300 kg ha^{-1}), followed by 'Mehis' (3000 kg ha^{-1}) with lowest yields in 'Capella' (2800 kg ha^{-1}) and 'Bruno' (2600 kg ha^{-1}) (Figure 1), but differences were not statistically different.

Crude protein content was the lowest in 'Clara' (236 g kg^{-1}in dry matter) and higher in all other varieties, although not ranging much from each other (269…279 g kg^{-1} in dry matter) (Figure 2).

The lowest P content was found in 'Clara' (4.7 g kg^{-1} in dry matter), followed by 'Capella' (5 g kg^{-1} in dry matter) and 'Vitra' (5.1 g kg^{-1} in dry matter), with highest in 'Bruno' (5.4 g kg^{-1} in dry matter) and 'Mehis' (5.6 g kg^{-1} in dry matter) (Figure 3).

The lowest K content was found in 'Mehis' (7.9 g kg^{-1} in dry matter), 'Clara' (8 g kg^{-1} in dry matter), followed by 'Capella' and 'Vitra' (both 8.4 g kg^{-1} in dry matter), and highest in 'Bruno' (9.9 g kg^{-1} in dry matter) (Figure 4).

The lowest Ca content was found in 'Clara' (0.4 g kg^{-1} in dry matter), followed by 'Capella' (0.5 g kg^{-1} in dry matter) and 'Mehis', 'Vitra' (both 0.6 g kg^{-1} in dry matter), and highest in 'Bruno' (0.7 g kg^{-1} in dry matter) (Figure 5).

The lowest Mg content was found in 'Capella' (1.3 g kg^{-1} in dry matter), followed by 'Clara' (1.4 g kg^{-1} in dry matter) and highest in all other varieties 'Bruno', 'Mehis' and 'Vitra' (1.5g kg^{-1}in dry matter) (Figure 6).

There was no statistical difference in yield between the pea varieties. L. Narits (2008) reported that semi-leafless varieties have a higher seed yield but this was not evident in our investigation. Probably the cold spring delayed seed emergence which reduced the yield potential. Good early growth is important for a good yield. S. Kalev and L. Narits (2004) showed that in the years when the weather conditions favoured vegetative growth leafed types gave a higher yield and better quality than semi-leafless varieties. They also noticed that in the year of unfavorable weather conditions the situation was the opposite. Similarly, A. Kotlarz et al. (2011) reported that unfavorable weather conditions may negatively influence the crop yield. Differences in climate, soil, varieties, agronomic

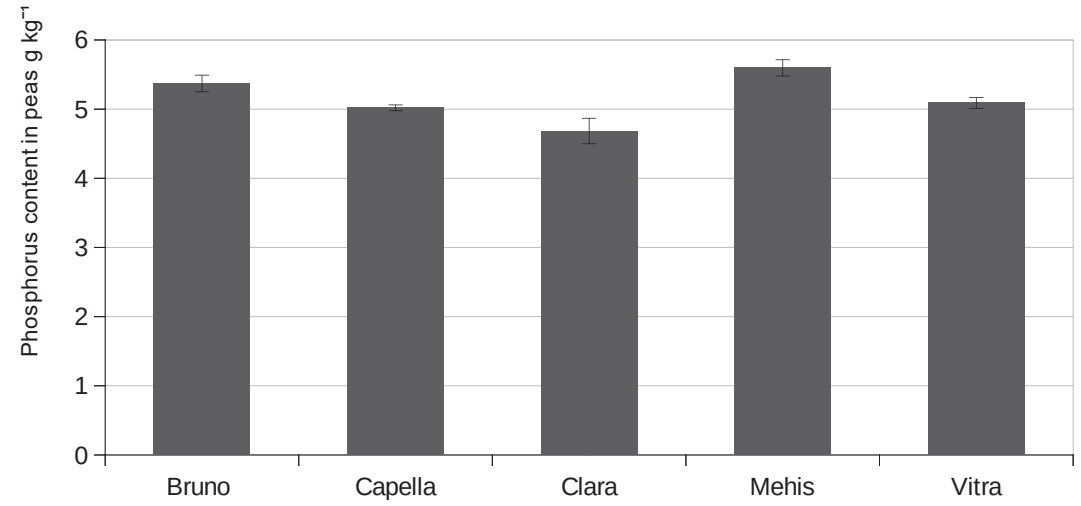

Figure 3. Average P content (g kg^{-1}in dry matter) of different field pea varieties (p ***).

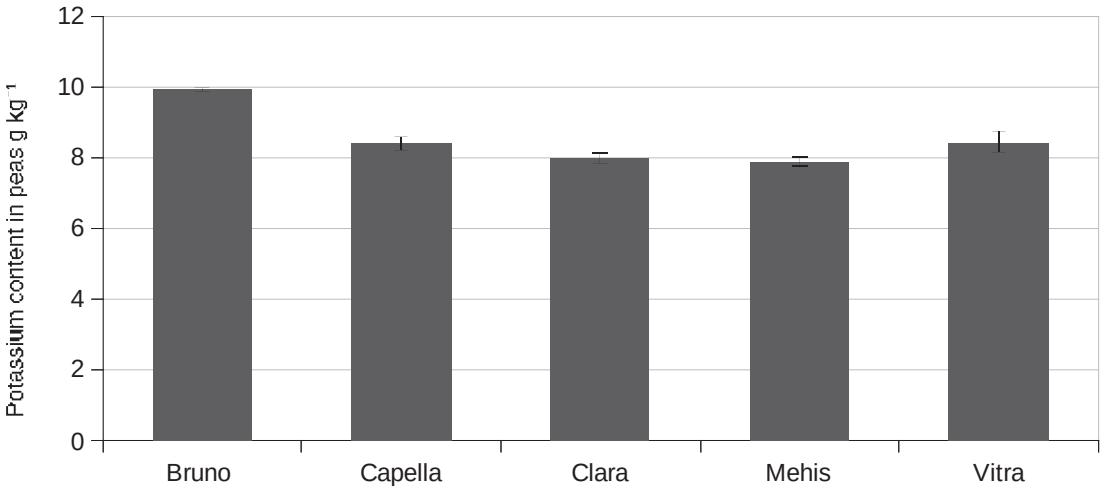

Figure 4. Average K content (g kg^{-1}in dry matter) of different field pea varieties (p ***).

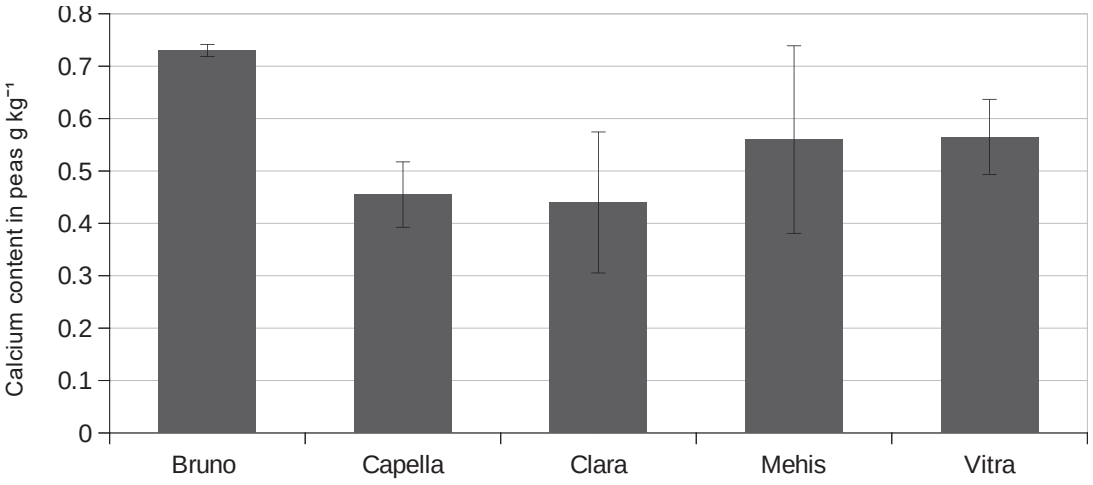

Figure 5. Average Ca content (g kg^{-1}in dry matter) of different field pea varieties (p *).

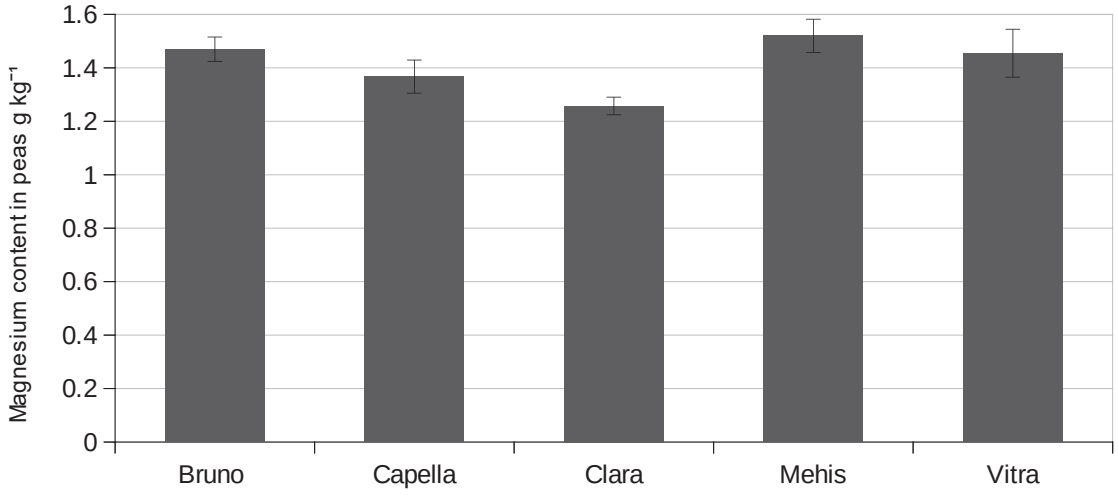

Figure 6. Average Mg content (g kg^{-1}in dry matter) of different field pea varieties (p ***).

practices may cause a different chemical composition when grown in various parts of the world.

The results obtained in this study show that variety had a significant influence on the levels of crude protein in the field pea. Similarly, A. Kotlarz et al. (2011) found that the varieties differed in protein content. L. Narits (2008) contended that the field pea contains on average 230 g kg^{-1} protein. In our experiment, even the variety Clara, which had the lowest protein content, contained 6 g kg^{-1} more than average, while all the other varieties contained 30-40 g kg^{-1} more protein. L. Narits (2008) concluded that when the field pea is grown for seed with the aim to get a high protein yield, then attention to the leaf type is important as leafy types usually have a higher protein content. In our experiment, the leafy varieties 'Mehis' and 'Vitra' also had a higher yield than the semi-leafless varieties 'Bruno' and 'Capella'. Only 'Clara', also a semi-leafless type, had quite a high protein content.

The present investigation showed that the varieties differed in nutrient content, as also shown by A. Kotlarz et al (2011). The same authors also reported that chemical content of pea seeds can vary. Genetic (variety) and environmental factors (location of cultivation area, soil characteristics, exchangeable cations, trace elements, cropping year, total rainfall, relative humidity, solarisation, temperature) are of importance (Kotlarz et al., 2011), as well as technological treatments (dehulling, cooking, soaking, germination, extrusion).

In our experiment, Ca content also varied with variety. A higher Ca-content is positive, because high Ca content reduces diseases and insect attacks, and improves transportability and storability (Olle, 2013). Moreover, a high level of Mg is desirable because it reduces the incidence of insect pests and diseases (Cakmak, 2013).

Conclusions

Choice of the right variety for pea cultivation is very important, but depends on the local agro-climatic conditions. The chemical content of pea varieties varies, but one promising variety is 'Mehis', due to the fact that 'Mehis' had the highest content of P and Mg and a middling content of Ca. A high content of Mg is desirable because Mg reduces the incidence of insect pests and diseases.

Acknowledgements

This investigation was developed with the help of project EUROLEGUME. This project has received funding from the European Union Seventh Framework Programme for Research, Technological Development and Demonstration under grant agreement no. 613781. It was also supported by institutional research funding IUT36-2 of the Estonian Ministry of Education and Research.

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EFFECT OF POST-HARVEST MOWING ON STRAWBERRY 'DARSELECT' GROWTH AND YIELD GROWN ON PLASTIC MULCH

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Abstract
The experiment was carried out at the Research Centre of Organic Farming of Estonian University of Life Sciences in 2012 and 2013. The study was aimed to find out the effect of defoliation and humic acid application on the strawberry (*Fragaria* × *ananassa* Duch.) plant growth, yield, and on the ascorbic acid, total phenolic and anthocyanin contents in 'Darselect' yield. Defoliation decreased significantly the number of leaves and inflorescences in both experimental years, while the fertilization effect was only in one experimental year. Fruit weight ranged from 19 to 42 g, being significantly decreased by defoliation in 2012, but increased in 2013. Yield was decreased due to defoliation up to 40% in 2012 and up to 51% in 2013, but humic acid application increased the yield of the defoliated plants in both experimental years. Defoliation in combination with humic acids had a significant effect on strawberry ascorbic acid content, but the effect was different for different fruit order. Tertiary fruits contained up to 46% more of total phenolics due to defoliation in both experimental years. Defoliation increased anthocyanins in primary and secondary fruits, but decreased it in tertiary fruits; while fertilizing increased the content up to 45% in defoliated plants in primary, but decreased about 13% in tertiary strawberries.
Key words: strawberry defoliation, humic acids, yield, biochemical compounds, mulch.

Introduction

Several experiments published are related to strawberry defoliation, determining the extent of leaf removal (Albregts et al., 1992; Casierra-Posada et al., 2012) or referring to different damages caused by insect/ pest incidence or accidents due to human activities (Makaraci and Flore, 2012). Partial defoliation is executed mostly manually in strawberry transplants to lower transpiration rate (Albregts et al., 1992; Casierra-Posada et al., 2012), while post-harvest defoliation as an alternative to chemical plant treatment is implemented mechanically after harvesting (Metspalu et al., 2000; Daugaard et al., 2003). More recent investigations have been aimed at determining the physical damage thresholds of strawberry leaves by using mechanical methods and understanding the plant recovery patterns better (Makaraci and Flore, 2012). Nevertheless, the mechanisms of plant reaction and recovery depend also on genotype, cultivation technologies and seasonal climatic conditions (Daugaard et al., 2003; Crespo et al., 2010; Pincemail et al., 2012; Günduz and Özdemir, 2014; Khanizadeh et al., 2014).

On the one hand, manual treatments are time consuming and demand high labor efficiency, but on the other hand, mechanical treatments can cause more plant damage. Experiments with different defoliation methods have led to more clear understanding of its relation to plant growth and development, which in turn may influence strawberry yield and quality. F. Casierra-Posada et al., (2012) have indicated the negative effect of defoliation on strawberry plant growth and yield. These kinds of impacts could be diminished by using additional fertilizing in order to improve the plant health and its recovery. M.J. Anttonen et al., (2006) claim the fertilization to be one of the most important treatments influencing fruit yield and quality. Significant effects of bio-fertilizers and growing year on strawberry yield have also been reported by M. Pešaković et al., (2013). However, besides plant growth fertilizing also affects the fruit biochemical composition. For example, U. Moor et al., (2005) have found that liquid fertilizer amendments increased the strawberry ascorbic acid content.

Strawberry defoliation has been used for different purposes and studied in many countries using various methods for the treatment. Post-harvest defoliation is a common practice in Estonia, but it is less investigated in terms of different cultivars and organic fertilizers, though being recommended in organic cultivation. The objective of this research was to determine the influence of post-harvest defoliation and humic acid application on the strawberry 'Darselect' vegetative growth, fruit weight, and the content of ascorbic acid, total phenolics and anthocyanins.

Materials and Methods

Experimental area and treatments

The experiment was carried out in 2012 and 2013 in a strawberry experimental plantation located at the Research Centre of Organic Farming of Estonian University of Life Sciences (58º21'N, 26º40'E, 68 m above sea level). Ecological fertilizer (NPK 4.5-2.5-8), produced from at least 30% malt germs was applied to the whole plantation area as a pre-establishment soil supplement. Strawberry cultivar 'Darselect' frigo plants were bought from Netherlands and planted in 2010 using 50 cm plant spacing and synthetic 0.04 mm thick black polyethylene mulch. Control plants were neither defoliated nor fertilized only watered, while two other treatments were executed. Post-harvest defoliation (D) was performed in July after

harvesting; and in combination with D, humic (H) acid application was implemented (DH). Humic acid containing substance was applied at a concentration of 50 mL of substance per 10 L of water at a rate of 0.5 L per plant, executed during flowering and fruiting and in the middle of August. The liquid humic substance contains 12% humic acid and 3% fulvic acid. The experimental design was a randomized block design with four replicates and 12 plants per replication.

Soil and weather conditions

The soil in the plantation was *Stagnic luvisol* (FAO soil classification). Soil pH KCl was 6.8 and humus content was 4.0%. The soil content of P, K and Mg was high and Ca content was sufficient (Table 1).

In 2012, the period of active temperatures (above 10 °C) started at the beginning of May and ended on the 6th October. Compared to long-term mean, the monthly mean temperatures were warmer in April, May, July and September, except in June which showed 1.3 °C lower temperature than the mean of 1971–2000 (Table 2). The sum of active temperatures in 2012 was 1967 °C. The amount of precipitation 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 7 May and ended on 23 September. The sum of active temperatures was 2263 °C which is 332 °C more than the mean of many years (1936 °C). The monthly mean temperatures were up to 3.8 °C warmer, except for April which was 0.7 °C cooler than the long term mean. The precipitation sum from 1 April to 31 October was 352 mm which was 152 mm less than in 2012, and 86 mm less than the mean (438) of 1971–2000.

Measurements and analysis

The numbers of strawberry leaves and inflorescences per plant were counted during flowering in May. The dry weight (g) of roots and crown were determined. At the end of strawberry fruit harvesting in 2013, the plants were dug out, the leaves were cut and after cleaning from soil the plant crown and the roots were dried until a constant weight was recorded. Fruits were picked according to surface color and fruit order in clusters (primary, secondary, tertiary), and stored at - 20 °C until analysis. Fruit orders were analyzed separately. All the plant growth, strawberry fruit yield and quality parameters were determined and measured in both experimental years in three replications (12 plants per replication) per treatment.

Ascorbic acid content (ASC) was determined iodometrically with the modified Tillman's method. For analysis, a mixture of meta-phosphoric and acetic acid (3% HPO3 + 8% CH3COOH) was added instantly to the pulp to avoid ascorbic acid breakdown in the air (Paim and Reis, 2000). AAC was expressed as mg 100 g^{-1} of strawberry fresh weight (FW). The content of total anthocyanins (ACC) was estimated by a pH differential method (Cheng and Breen, 1991).

Table 1

The content of soil nutrients (mg kg^{-1}), carbon and humus (%), pH and carbon content in biomass (C/ mg, DW)

Treatment	pH KCl	P mg kg^{-1}	K mg kg^{-1}	Ca mg kg^{-1}	Mg mg kg^{-1}	C %	Humus %	C in biomass C mg^{-1}
Control	6.6	256***	306***	3930***	600***	3.2	4.9	0.525
Fertilized	6.7	286***	347***	5131***	492***	3.0	5.2	0.766

The level of nutrients in the soil: high (***)

Table 2

Minimum, mean and maximum temperatures (°C) from April to September of 2012, 2013 and of long-term mean of 1971–2000

Month	2012			2013			1971–2000		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
April	-7.0	5.3	19.3	-10.6	4.0	15.6	-11.7	4.7	27.3
May	-1.6	12.0	23.7	0.5	14.9	28.4	5.7	11.1	16.6
June	1.1	13.8	24.1	7.0	18.2	28.7	9.7	15.1	20.3
July	7.5	18.3	31.6	9.1	17.9	28.7	11.9	16.9	21.9
August	2.8	15.2	25.9	5.4	17.2	32.0	11.2	15.6	20.6
September	5.2	12.4	22.5	-0.1	11.3	22.0	6.9	10.4	14.8

Data according to the Estonian Weather Service (www.ilmateenistus.ee) database.

Absorbance was measured with a Jenway 6300 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 pelargonidin-3-glucoside equivalent per 100 g of FW^{-1}. The total phenolic content (TPC) was determined with the Folin-Ciocalteau phenol reagent method, using a spectrophotometer at 765 nm. The TPC was expressed as Gallic acid equivalents in mg 100 g FW^{-1} of pulp.

All the results of the present experiment were tested by one-way analysis of variance (ANOVA). To evaluate the effect of treatment, 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

Strawberry growth and yield parameters

Defoliation (D) decreased the number of leaves and inflorescences significantly in both experimental years, while the DH affected the leaf number only in 2012 (Table 3). The considerable decrease in the number of leaves and inflorescences may refer to the negative effect of defoliation on their formation and development. Similar results were described by F. Casierra-Posada et al., (2012), who suggested that severe defoliation suppresses strawberry flower initiation, influencing leaf growth as well as the production of dry matter. After the treatments within two years, strawberry root mass was significantly increased by D, referring to high plant underground biomass production due to depressive disturbance of plant growth processes and promotion of plant recovery mechanisms. Similar mechanisms were described in experiments by A.Z. Makaraci and J.A. Flore (2012).

In the year 2012, strawberry 'Darselect' fruit weight was up to 42 g, and D decreased it significantly (Table3). In 2013, on the contrary, fruit weight was increased by D, although the values remained within range from 19 to 23 g. These results are in accordance with H. Daugaard et al., (2003) who also found that post-harvest defoliation caused a significant increase in fruit size, while F. Casierra-Posada et al., (2012) found it to be decreased.

Yield per plant was decreased due to D treatment from 679 to 403 g per plant in 2012 and up from 308 to 149 g in 2013, but humic substances had a positive effect on the defoliated plants in both experimental years. F. Casierra-Posada et al., (2012) found that defoliation decreased strawberry total yield. Significant effects of bio-fertilizers and growing year on strawberry yield have been reported by M. Pešaković et al., (2013). Nevertheless, additional humic acid application increased the yield in both investigated years, showing the positive effect even in case of high soil humus content.

Ascorbic acid content (ASC)

In 2012, strawberry ASC ranged from 72 to 124 mg 100 g^{-1} (Figure 1A). D increased the content up to 57% in primary fruits, but decreased it in secondary and tertiary fruits. In 2013, ASC varied from 71 to 107 mg 100 g^{-1} (Figure 1B). D had a decreasing effect

Table 3

The effect of defoliation and fertilization on strawberry 'Darselect' plant growth and yield in 2012 and 2013

Parameters	Treatment	2012	2013
Number of leaves	Control	51 a	63 a
	D	40 b	45 b
	DH	54 a	52 b
Number of inflorescences	Control	18 a	26 a
	D	12 b	14 b
	DH	12 b	16 b
Root mass, g*	Control	-	266 c
	D	-	404 a
	DH	-	350 b
Fruit weight, g	Control	42 a	19 c
	D	39 b	23 a
	DH	42 a	21 b
Yield per plant, g	Control	679 a	308 a
	D	403 c	149 c
	DH	478 b	279 b

Treatments: (D) – defoliation; (DH) – defoliation and humic acids application.
*Root mass was determined only in 2013 by uprooting the plants.
Statistical analysis was done according to year and parameter measured, mean values followed by the same letter are not significantly different at $p \leq 0.05$.

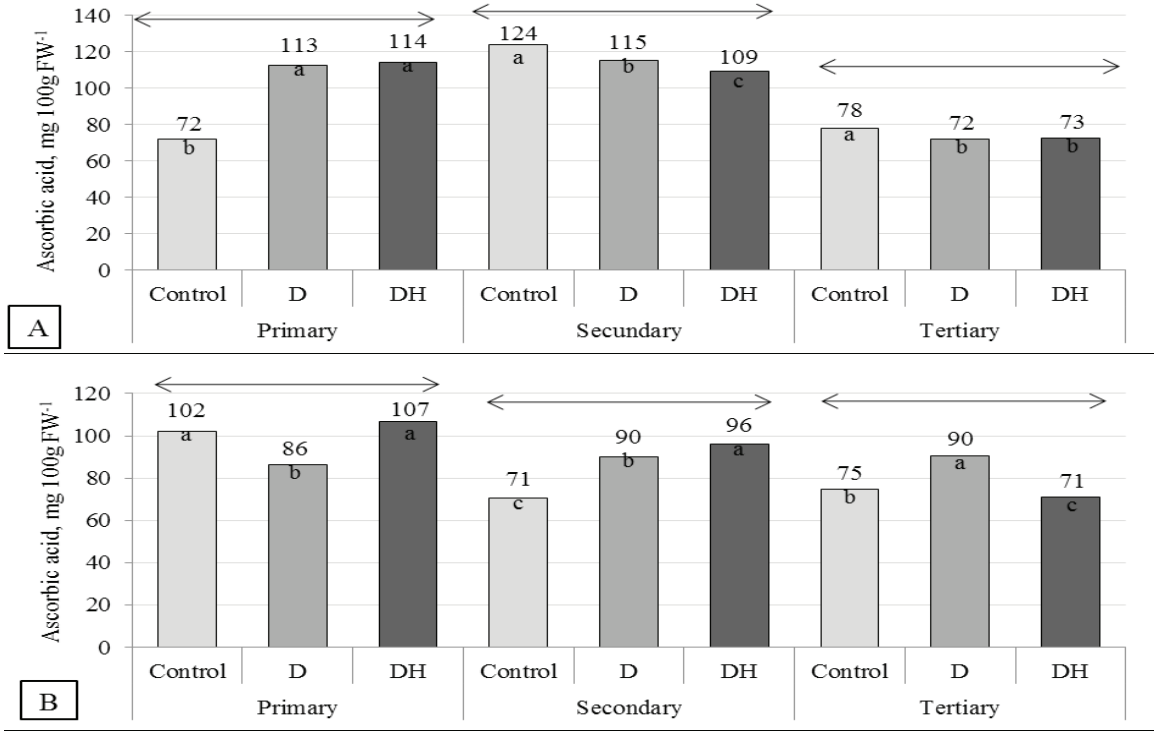

Figure 1. The effect of defoliation (D) and humic acid application (DH) on the strawberry 'Darselect' ascorbic acid content according to fruit order in 2012 (A) and 2013 (B). Different letters on figures mark significant differences at $p \leq 0.05$.

in primary, but increasing in secondary and tertiary fruits, while the influence of DH treatment was more evident in secondary fruits.

ASC values in strawberry 'Darselect' determined by J. Pincemail et al., (2012) reached up to 163 mg 100 g^{-1}, which refer to high variations of the contents depending on the growing area and weather conditions. In the present experiment, differences in ASC were apparent from one year to another. In 2012, the reason could be related to up to 4.4 ºC lower temperatures in May and June compared to year 2013 (Table 2). This is in accordance with several authors who have claimed that cooler temperatures tend to increase the content of ascorbic acid (Lee and Kader, 2000; Moor et al., 2005; Pincemail et al., 2012). Ascorbic acid content being different according to year and cultivation method indicates the fact that its content is not easily influenced by cultural practices (Moor et al., 2005; Tõnutare et al., 2009).

Total phenolic content (TPC)

TPC ranged from 155 to 236 mg 100 g^{-1} in 2012 (Figure 2A). D affected strawberry fruit TPC significantly, decreasing it in primary, but increasing it tremendously up to 46% in tertiary fruit order. DH decreased the TPC up to 14% in primary strawberry fruits. In 2013, TPC ranged from 184 to 267 mg 100 g^{-1} (Figure 2B). D had a positive effect – increasing TPC values by more than 26% in secondary and tertiary fruits. DH treatment increased the TPC up to 40% in secondary fruit order.

Significantly lower TPC in primary fruits in 2012 could be caused by dilution effect of biochemical components due to higher precipitation rate in May and June of that year. Moreover, it can be assumed that under the plastic mulch, the soil humidity could have been quite high enough during strawberry fruit ripening period. The content of phenolic compounds can be significantly affected by production systems, such as the usage of plastic mulch, but again the effects can be variable during the harvest season (Khanizadeh et al., 2014). J. Crecente-Campo et al., (2012) have indicated the decrease in phenolic acid concentrations during ripening. On the contrary, in the present experiment, significantly higher temperatures in July could have caused the increase in TPC by the time of harvesting of tertiary fruits in both years. Manyfold differences in accumulation of phenolic compounds have been determined according to fruit order by M.J. Anttonen et al., (2006). Significant variations in phenolic contents can be related to cultivar characteristics; however, the importance and influence of weather conditions cannot be underestimated (Pincemail et al., 2012; Gündüz and Özdemir, 2014).

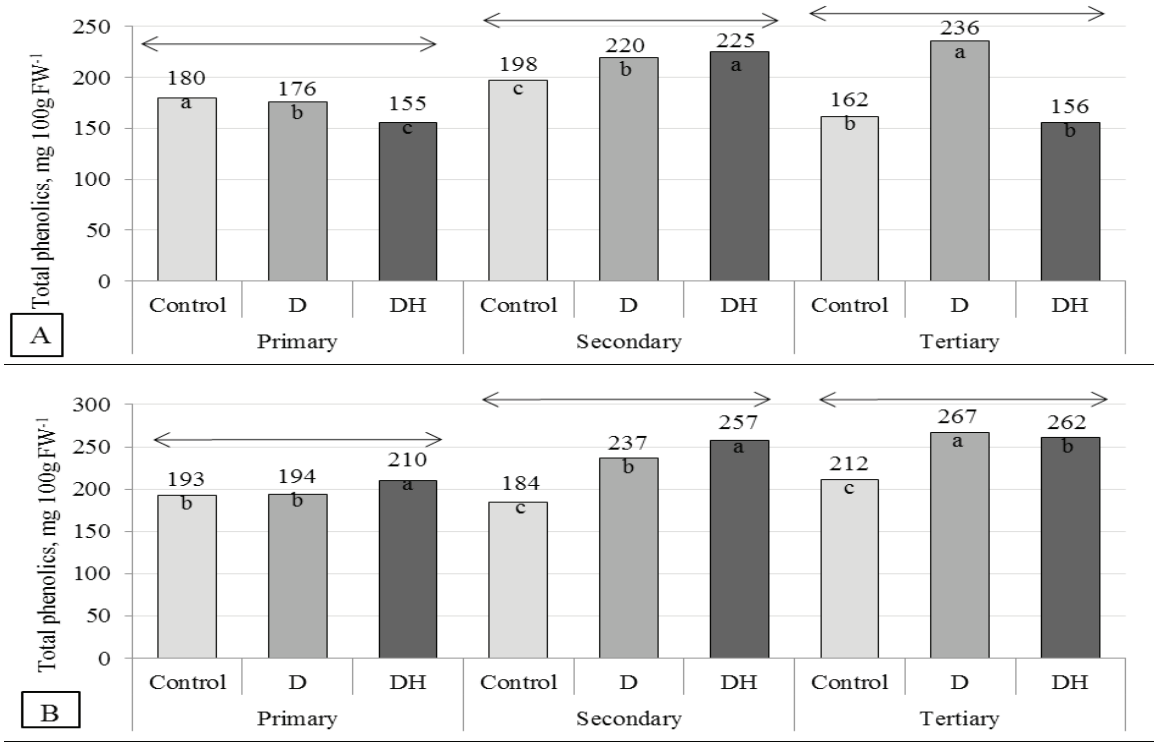

Figure 2. The effect of defoliation (D) and humic acid application (DH) on the strawberry 'Darselect' total phenolic content according to fruit order in 2012 (A) and 2013 (B). Different letters on figures mark significant differences at $p \leq 0.05$.

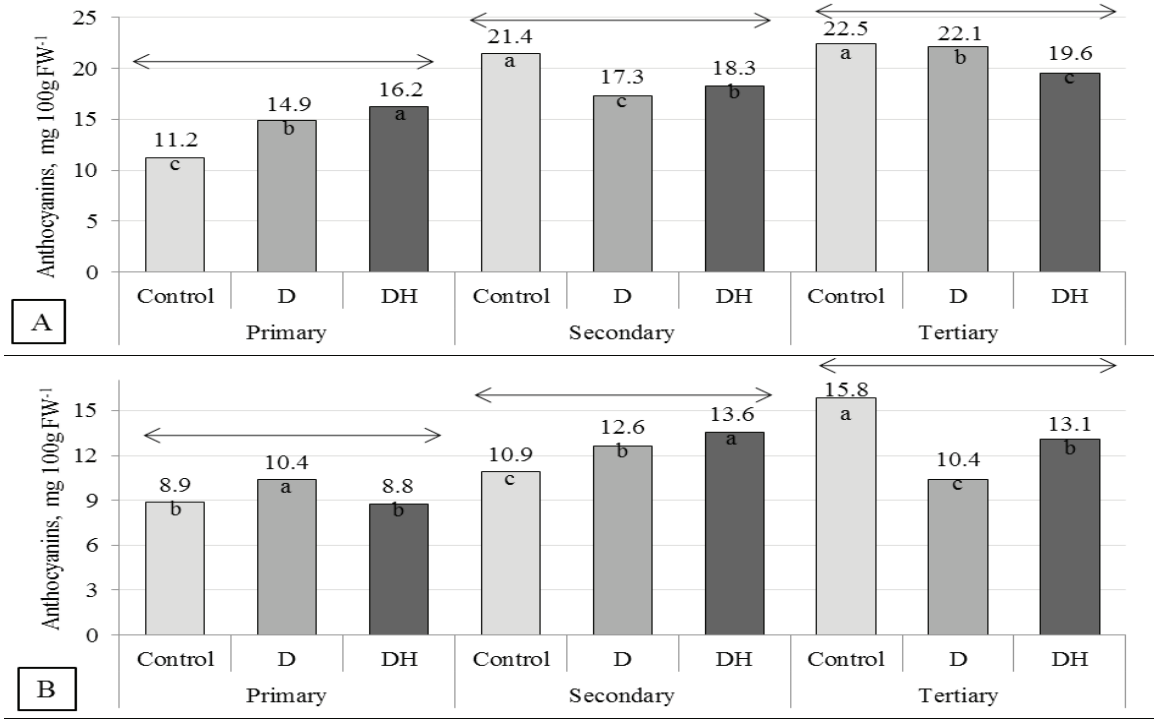

Figure 3. The effect of defoliation (D) and humic acid application (DH) on the strawberry 'Darselect' phenolic content according to fruit order in 2012 (A) and 2013 (B). Different letters on figures mark significant differences at $p \leq 0.05$.

Anthocyanins (ACC)

In 2012, ACC ranged from 11.2 to 22.5 mg 100 g^{-1} (Figure 3A). D increased the content in primary, but decreased it slightly in tertiary strawberries. DH increased the ACC up to 45% in primary fruits, but decreased the content for about 13% in tertiary strawberries. In 2013, ACC ranged from 8.8 to 15.8 mg 100 g^{-1} (Figure 3B). D had a significant effect on ACC, increasing it 14% in primary and secondary fruits, but decreased it up to 44% in tertiary fruit order. DH decreased ACC in primary fruits compared to D treatment, but increased the content in secondary fruits up to 26%.

The effect of defoliation was different according to year, but it can be presumably related to the age of plantation and variable fruit weight. U. Moor et al., (2005) have pointed out that strawberry plants growing in four- to five-year-old plantations suffer under stress conditions, and this could be one cause of increased anthocyanin production in berries. T. Tõnutare et al., (2009) have also concluded that the content of anthocyanins was decreased significantly with the aging of a plantation, although the plant age effect on strawberry quality parameters is more important than that of cultivation technology. Still, anthocyanins being a significant part of total phenolics in general, depend also on genotype and cultivation technologies (Pincemail et al., 2012; Gündüz and Özdemir, 2014). The latter indicates the fact that such interactions need to be investigated in long-term trials.

Conclusions

The results of the research revealed that the effect of defoliation and humic acid application was year dependent, and therefore, agricultural practices used for strawberry cultivation may not be of main importance. Climatic conditions can also be the major factors determining the accumulation of biochemical compounds in fruits. However, the results of the present research revealed that defoliation tends to decrease the strawberry yield, but additional fertilization with humic acids may be used in order to promote better plant growth recovery and due to the content of biochemical compounds of strawberries. Still, further research is required assessing different cultivars and the effect of defoliation and different bio-fertilizers on the vegetative growth, yield and fruit biochemical composition of strawberries in order to confirm the results obtained so far.

Acknowledgements

The research was supported by Estonian Ministry of Education and Research, project SF 0170057s09 and Estonian Ministry of Agriculture.

These studies were also supported by European Social Fund's Doctoral Studies and Internationalization Programme DoRa which is carried out by Archimedes Foundation.

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DIVIDED HARVESTING METHOD. THE IMPACT OF AGRICULTURAL TECHNOLOGY ON THE YIELD OF ENERGY HAY

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Abstract
In Estonia, the most promising perennial grass used as raw material for production of heat energy is reed canarygrass (*Phalaris arundinacea* L.). Nordic countries (Finland, Sweden) implement a technology including single harvest of the above-ground biomass from frozen soil early in spring. This technology cannot be used in Estonia as the ground does not freeze to the extent of bearing harvesting machines every year. Harvesting in spring is virtually impossible as herbage lodges excessively under the snow weight. A divided harvesting method of reed canarygrass was tested in field trials in 2010–2013 at the Estonian Crop Research Institute. Herbages were cut in July at the height of 60–65 cm, mass was dried as hay, stubble hay was left to grow and was harvested next spring before the growth started but soil had become dry. The effect of seeding rate, row spacing and nitrogen fertilizer on the dry matter yield of reed canarygrass was investigated. The amount of produced heat by trial variants was calculated. The variant with narrow spacing (15 cm), seeding rate of 8 kg ha^{-1} and usage of fertilizer N70 in the beginning of growth and N70 kg ha^{-1} after the first cut was giving the best results. Two cuts of this variant yielded on average 8.12 t ha^{-1} per year, of which the stubble hay, harvested in spring and with better combustion properties, made 64%. Energetic value of the yield was 138 GJ ha^{-1} per year.
Key words: divided harvesting method of reed canarygrass, dry matter yield, quality of yield, energetic value.

Introduction

According to the energy sustainability index of the World Energy Council (WEC), Estonia was ranked 68th among 129 member states in 2013 (World Energy Council, 2013). To direct the future of the energy industry, the Government of Estonia has initiated the drafting of the National Development Plan of the Energy Sector until 2030 (https://valitsus.ee/sites/default/files/content-editors/arengukavad/enmak_koostamise_ettepanek.pdf). The planned solutions must consider obligations resulting from the EU policy framework. The aim of the EU is to increase the percentage of renewable energy to at least 20% of total consumption by 2020 and to 27% by 2030. The increase in the share of renewable energy is necessary due to the decreased supply of fossil fuels, but also in order to enhance energy security and reduce the environmental impact of the energy industry.

There are regions in Europe that have set an objective to be entirely free of fossil fuels. This movement is most advanced in Germany, where by 2014 there were 6 regions completely independent from fossil fuels and 3 regions quite close to this level. Altogether 146 regions have set the same target in the long run, which makes more than one third of Germany's territory and includes 25 million inhabitants. Sweden and Austria have also started with the development of regions free from fossil fuels (http://www.100-ee.de). Estonia too has good preconditions for such a development, considering the availability of renewable energy resources like forests, wind and sun. Moreover, there are currently 283,000 ha, i.e. 25%, of arable land out of use in Estonia. This land could be used for energy crop production. Setting such a target has several benefits for Estonia.

1. With decreasing EU aid, maintenance cutting of arable land out of agricultural production will cease. The production of plant-based biofuel would help to prevent the fields from turning into brush and preserve them for the production of other cops in the future, when there will probably be an increased solvent demand for plant products due to the growth of world population.
2. The expansion of plant-based biofuel production will create new jobs in the countryside and reduce the migration of labour force to other countries, which has been the most topical problem in Estonia for the past decades.

In Estonia and other Baltic countries, trials have been conducted with energy field crops like tall fescue (*Festuca arundinacea* Schreb.), smooth bromegrass (*Bromus inermis* Leyss.), cock's-foot (*Dactylis glomerata* L.), reed canarygrass (*Phalaris arundinacea* L.), festulolium (×*Festulolium* Asch. et Graebn.), fodder galega (*Galega orientalis* Lam.), large-leaved lupine (*Lupinus polyphyllus* Lind.) and hemp (*Cannabis sativa* L.) (Kryževiciene, 2006, Lillak et al., 2007; Kryževiciene et al., 2008; Lauk et al., 2009; Raave et al., 2009; Rancane et al., 2014). Reed canarygrass has been most promising of these grasses in this region as well as in Finland and Sweden; this is mostly due to its high and stable dry matter yield over the years (Larsson et al., 2006; Pahkala, 2007). In Finland, large-scale growing of reed canarygrass for heat production was started in 1990 (Pahkala et al., 2008). In Estonia, this species has thus far been investigated from the point of view of fodder production. In trials carried out on peat and alluvial soil, reed canarygrass has yielded 8–12 t of dry matter per ha in a three-cut regime (Annuk, 1992).

Growing of reed canarygrass for energy production has not been studied much in Estonia. Preliminary attempts to use the technology implemented in Finland (one-cut harvest from soil frost in early spring) have essentially failed. This is due to the following reasons: 1) in Estonia, the ground does not freeze to the extent to bear harvesting machines every year; 2) during winter the plants with a height of 1.5–2.0 m lodge so much that harvesting them has been impossible (Raave et al., 2009). Similar results have been obtained in the USA (Tahir et al., 2011). Reed canarygrass is used in Estonia for heating currently only to a small extent. The implemented technology involves making one cut in July, drying the mass in the field to make hay and using it later for heating. This method has several shortcomings:

1) The combustion properties of reed canarygrass harvested in summer are not very good.
2) A lot of plant nutrients (in particular K) are removed from the field with the yield, which must be compensated with fertilization.
3) Reed canarygrass does not tolerate low cutting during the vegetation period. Reserve nutrients in the lower parts of the straw are of vital importance for its further growth, as it has only a few lower leaves near roots. Thus, a considerable part of the above-ground biomass (high stubble) remains unused as yield.
4) In the second half of summer, reed canarygrass grows an aftermath, which cannot be harvested for fuel; in autumn the humidity content of biomass is too high, in spring the aftermath is so lodged that harvesting becomes virtually impossible.

The research that was carried out at the Estonian Crop Research Institute in 2008–2013 aimed at developing a method that would allow diminishing or removing the above shortcomings from the production of reed canarygrass for fuel. This can be achieved by using a divided (in two parts) harvesting method. In the second half of July, when there are most favourable weather conditions in Estonia for hay drying, reed canarygrass is cut at the height of 60–65 cm, stubble hay is left to grow until the beginning of the next year's vegetation period and is harvested from the soil frost, or if there is no frost, later after soils have dried enough to bear machinery. The advantages of this method are:

1) The above-ground biomass of reed canarygrass from the whole vegetation period can be used for fuel. In early spring, cutting can be made as low as the harvester and the surface flatness allow. Together with stubble hay, the aftermath from the second half of summer is also harvested and can be used.
2) Stubble hay lodges less (or does not lodge at all). The upright plants dry and the soil also dries faster, which enables to start the spring harvest earlier and have a prolonged harvest period.
3) The amounts of plant nutrients removed from the field with the yield decrease.
4) Two thirds of the harvested yield has better combustion properties, because its potassium and chlorine contents decrease considerably due to translocation of mineral substances during overwintering (Samson and Mehdi, 1998). While burning reed canarygrass that has stood in the field over winter and was harvested in spring, the melting temperature of ash is higher than in case of reed canarygrass harvested in summer or autumn (Burvall, 1997).

The experiments conducted in Jõgeva investigated the effect of seeding rate, row spacing and nitrogen fertilizer on the dry matter yield of reed canarygrass and on the quality of yield while using the divided harvesting method. The amount of energy produced with the yield of trial variants was calculated.

Materials and Methods

Field trials were established in May 2008 in a field that had been bare fallow in the previous year. The trials were situated on leached soil (K_o) the agrochemical parameters of which at the time of trial establishment were as follows: pH KCL 5.8, P 27, K 67, Ca 2150, Mg 159 mg kg^{-1} and C_{org} 24 g kg^{-1}. Prior to the establishment, mineral fertilizers at the rate of P 19, K 67 kg ha^{-1} were applied to the trial plot; the complex fertilizer Scalsa (micronutrient-enriched) was used. Phosphorus-potassium fertilizers later were not applied. Altogether three trials were established.

1. In the row spacing trial, the variants were 15, 30, 45 and 60 cm, the seeding rates respectively 8, 6, 5 and 4 kg ha^{-1}. The first variant was sown with seed drill Hege 80, the rest of the variants with seed drill Hege 90-1. The fertilizer rate was N 140 kg ha^{-1} both in the year of seeding and the following year, and it was applied in two equal doses.
2. In the seeding rate trial, the variants were 4, 6, 8 and 10 kg of 100% pure live seeds (PLS) per ha, narrow spacing (15 cm) was used for sowing with seed drill Hege 80. The fertilizer rate was N 140 kg ha^{-1} both in the year of seeding and the following year, and it was applied in two equal doses.
3. The nitrogen fertilizer trial was established with the seeding rate of 8 kg ha^{-1}, sown with narrow spacing with seed drill Hege 80. The variants of fertilizer rates were both in the year of establishment and the following year as follows: N 35 + N 35; N 35 + N 35 +N 35; N 70 + N 35 and N 70 + N 70 kg ha^{-1}.

In the trials, ammonium salpeter was used as nitrogen fertilizer. In the years of maintenance, stands were fertilized twice: the first time one week after the beginning of growth and the second time

after the harvest of first growth (end of July). In a treatment with split nitrogen application in spring, the second top-dressing was scheduled to the onset of plants' culm elongation. Fertilizers were given with fertilizer spreader Hege 33. All trials were established and conducted in four replications in a randomised block design. In the year of seeding, the trial area was sprayed with the herbicide MCPA 750 for the control of broadleaf weeds, the application rate being 1.0 l ha^{-1}. At the time of spraying, the plants of reed canarygrass had 2–4 leaves. To debilitate the survived weeds, the trial area was cut once with the MF 70 motorobot at the height of 15 cm. In the variants sown with wide spacing, weeds were additionally controlled mechanically. The stand established in the year of seeding was cut and the mass was harvested at the end of the vegetation period at the height of 10 cm. In the first year of maintenance (2009), the seed yield was harvested from the trial plots, stubble hay was cut and harvested in the middle of October after the end of vegetation. The determination of biomass yields started in the third year. In the years 2010–2012 the first growth was cut in the second half of July at the stage of full maturity of seeds at the height of 60–65 cm, the mass was dried, gathered and weighed. Stubble hay was left to grow in the field and was harvested the following spring (2011–2013, respectively) before the start of the vegetation period. The yield was determined with Hege 212 harvester. Samples for the determination of moisture content and for laboratory analyses were taken both from the yield that had been dried in the field and from the one harvested in spring. The analyses of soil and plant material were performed in the accredited laboratory of the Estonian Agricultural Research Centre. The following analysis methods were used: for the determination of moisture content in biomass EVS_EN 14774-3:2009; for the determination of crude protein (CP) content according to Kjeldahl method procedure EÜ 152/2009 IIIC; for the determination of acid detergent fibre (ADF) ASN 3429; for the determination of neutral fibre ASN 3431; for the determination of ash content EVS-EN 14775:2010 and for the determination of potassium PMK-JJ-4c (ISP-OES). The analyses of stems and leaves were performed in the laboratory of the Estonian Crop Research Institute. The trials were carried out with the variety 'Pedja'. The energetic value of the biomass of reed canarygrass was calculated on the basis of data by Strasil et al., 2005.

The vegetation period of the seeding year (2008) was rather chilly and rich in precipitation (in June 157%, in August 219% of the long term average), i.e. very favourable for the germination and development of canarygrass. The temperatures and amount of precipitation of the following year were close to the long term average. The vegetation periods of 2010 and 2011 had higher temperatures than the long term average and were arid, 2012 was close to the average as to the temperature, but more rainy in June (162%) and August (146%). The winters during the period of experiments were rich in snow in Jõgeva. The measured snow depth was more than 30 cm, in the winter of 2010/2011 even exceeding 50 cm.

For statistical analysis of the trial results, the software AGROBASE 20™ was used. To determine the significance of differences between variants, LSD test was used.

Results and Discussion

Based on the trial results of three harvest years, it can be said that the row spacing affected the yields of both straw and stubble hay (Table 1). The variant with narrow spacing yielded significantly more stubble hay in three years than the variant seeded with 60 cm row space. The difference was major in the first two years of comparison, in the third year it became minor. By that time the space between rows had become almost overgrown. The yield of straw had bigger variations over the years than that of stubble hay. This is due to the fact that reed canarygrass develops a different number of generative shoots in different years. Due to the same reason, seed yield of this species also varies a lot. In our trial, straw yield was significantly higher in the variant that had been seeded with 30 cm row spacing. In total of three years, the lowest dry matter yield of 17.5 t ha^{-1}, was obtained in the variant that had been seeded with 60 cm row space; the highest dry matter yield of 20.99 t ha^{-1} was obtained in the variant seeded with 15 cm spacing. Considering the least significant difference, the differences in dry matter yields of three years of variants 15, 30 and 45 cm were not significant.

The tested seeding rates of 4, 6 and 8 kg ha^{-1} did not have a significant effect on dry matter yield harvested during three years (Table 1). In comparison with the variant with the seeding rate of 4 kg ha^{-1}, a significantly higher dry matter yield was obtained only with the variant that was seeded with 10 kg ha^{-1}. The effect of seeding rate in the trial results of dry matter yield was more evident in the first yield determinations, later the plant cover became evenly dense as a result of shooting and yield differences were not statistically significant.

According to literature, while establishing reed canarygrass fields for energy production, it is recommended to use a seeding rate of 11–16 kg ha^{-1} with the germination rate of 90% in Finland (Pahkala et al., 2005). Seed fields of reed canarygrass can be established with a reduced seeding rate (Bender et al., 2011). In our trials, which aimed at the growing of biomass for energy production, the advantage of the recommended seeding rate of 10 kg was evident,

Table 1

Yields of straw and delayed harvested stubble hay of reed canarygrass, DM t ha^{-1}

Variant	Straw	Stubble hay	Straw	Stubble hay	Straw	Stubble hay	Straw	Stubble hay	Stubble hay	DM**
	22.07.10	27.04.11	14.07.11	12.04.12	26.07.12	02.05.13	total	total	%	total
Row spacing										
15 cm	3.38	4.93	2.10	4.30	2.39	3.89	7.87	13.12	62.5	20.99
30 cm	3.86	3.76	1.94	3.13	2.83	3.82	8.63	10.70	55.4	19.33
45 cm	3.20	3.58	2.55	3.42	2.39	4.56	8.13	11.55	58.7	19.69
60 cm	3.07	3.20	2.23	3.17	2.05	3.78	7.35	10.15	58.0	17.50
$LSD_{0.05}$	0.32	0.44	0.55	0.71	0.28	0.3	0.49	0.93	0.7	2.73
Seeding rate										
4 kg ha^{-1}	3.46	5.02	2.66	4.48	2.21	3.46	8.33	12.96	60.9	21.29
6 kg ha^{-1}	3.88	4.79	2.42	3.95	2.79	3.85	9.10	12.59	58.1	21.69
8 kg ha^{-1}	3.46	5.33	2.68	4.38	2.45	3.89	8.60	13.60	61.3	22.20
10 kg ha^{-1}	3.58	6.01	2.85	4.51	2.35	3.78	8.79	14.30	61.9	23.09
$LSD_{0.05}$	0.49	0.35	0.82	0.57	0.78	0.45	0.38	0.93	1.1	1.69
Nitrogen fertilizer										
N35+N35	2.51	5.49	2.30	4.10	2.05	2.83	6.85	12.42	64.4	19.27
2×N35+N35*	3.11	5.55	2.54	3.74	2.35	2.97	7.99	12.26	6.05	20.25
N70 + N35	3.17	5.54	2.76	4.04	2.21	3.25	8.15	12.83	61.2	20.98
N70+ N70	3.58	7.81	2.96	4.16	2.28	3.57	8.82	15.54	63.8	24.36
$LSD_{0.05}$	0.34	0.79	1.36	0.49	0.27	0.33	0.41	1.18	1.06	1.19

* interval in spring 3 weeks ** dry matter

but the differences in yields caused by the reduced seeding rates were not large. When the soil is free from perennial weeds and seeding is not late, the field can be established also with 4–6 kg per hectare. In favourable moisture conditions the species has a good shooting ability, which ensures the required further densification of the stand. The use of a reduced seeding rate is justified in the case when in the year after establishment, reed canarygrass is harvested for seed, straw is dried for energy hay and stubble hay is left in the field until the following spring (Bender, 2014). The stand that has been seeded with a lower seeding rate forms more generative shoots, which is a prerequisite for high seed yield.

As expected, the rate of the nitrogen fertilizer had the greatest effect on dry matter yield. By applying nitrogen during the vegetation period in two equal doses with the total rate of N 70 kg ha^{-1}, the total dry matter yield of straw and stubble hay of reed canarygrass over the three-year period was 19.27 t ha^{-1}. With the double application rate of nitrogen (2 × 70 kg ha^{-1}), the dry matter yield of straw and stubble hay was 5.09 t ha^{-1} higher. The difference of the above variant was greatest in the first year of comparison, in the following two years the extra yield was less prominent. The divided application of nitrogen in spring (N 35 + N 35) in comparison with a single dose (N 70) did not give a significant extra yield; on the contrary, the yields remained even lower.

In Finland, where they have long-term experience with energy hay production, nitrogen is applied in the year of establishment with the rate of N 40–60 kg ha^{-1} before reed canarygrass is seeded and in the following years with the rate of N 60–90 kg ha^{-1}. Harvest for energy hay starts in the third year (just like in our trials), and the stand is utilized there for 10 years. In Finland, the average dry matter yield of energy hay that is harvested in spring is considered to be 3–8 t ha^{-1} per year (Pahkala, 2007). With the implementation of a two-cut harvest system, we have achieved the same yield level – 5.8–8.1 t of dry matter per hectare per year. In Finland these results have been obtained in production conditions; the harvest losses are considered to be 20–50% from the above-ground biomass. Our results were obtained in trial conditions in which the harvest losses were kept as low as possible. Based on our earlier trials, in Estonia it is not expedient to fertilize reed canarygrass in spring with more nitrogen than 70 kg ha^{-1}. In years rich in precipitation, the stand may lodge, resulting in great losses in dry matter yield and the divided harvesting method loses its advantage.

Different data can be found in literature regarding the energetic value of reed canarygrass's biomass used for heating fuel. The variations may be due to the time of harvesting and the fact that often it is not the energetic value of dry matter but rather that of biomass with different moisture content that is presented (Alakangas, 2012; Platace and Adamovics, 2014). In

Table 2

Energetic value of dry matter of reed canarygrass straw and stubble hay over a three-year period

Variant	Straw t ha^{-1}	Stubble hay t ha^{-1}	DM* total t ha^{-1}	Energetic value of straw, GJ ha^{-1}	Energetic value of stubble hay, GJ ha^{-1}	Total GJ ha^{-1}
Row spacing						
15 cm	7.87	13.12	20.99	133.1	225.5	358.6
30 cm	8.63	10.70	19.33	145.9	183.9	329.9
45 cm	8.13	11.55	19.69	137.5	198.5	336.0
60 cm	7.35	10.15	17.50	124.3	174.5	298.8
Seeding rate						
4 kg ha^{-1}	8.33	12.96	21.29	140.9	222.8	363.6
6 kg ha^{-1}	9.10	12.59	21.69	153.9	216.4	370.3
8 kg ha^{-1}	8.60	13.60	22.20	145.4	233.8	379.2
10 kg ha^{-1}	8.79	14.30	23.09	148.6	245.8	394.5
Nitrogen fertilizer						
N 35 +N 35	6.85	12.42	19.27	115.8	213.5	329.3
2× N 35+ N 35**	7.99	12.26	20.25	135.1	210.7	345.9
N 70 + N 35	8.15	12.83	20.98	137.8	220.5	358.4
N 70+ N 70	8.82	15.54	24.36	149.1	267.1	416.3

* dry matter ** interval in spring 3 weeks

the Czech Republic, the energetic value of dry matter of reed canarygrass harvested in early spring has been determined to be 17.80 MJ per 1 kg dry matter. But when the same fuel contains 20% moisture, the energetic value is only 14.59 MJ (Strašil, 2012). The energetic value of biomass is in positive correlation with the content of lignin, therefore grasses should be harvested at the latest possible developmental stage (Raclavska et al., 2011). As far as known, the soil properties of the growing place also affect the energetic value of reed canarygrass (Burvall, 1997) as well as the harvest time. In the Czech Republic, the energetic value of dry matter of reed canarygrass harvested in different times has been studied and it became evident that the energetic value of biomass harvested in July was 16.91 and that of biomass harvested in early spring (March) was 17.19 MJ per kg of dry matter (Stašil et al., 2005). These two figures serve as basis for the calculations of energetic value of dry matter yields harvested from our trials (Table 2).

The biggest amount of energy was produced in the variant that was seeded with narrow spacing (15 cm) with the seeding rate of 8 kg ha^{-1} and fertilized with N 70 at the beginning of growth and second time with N 70 kg ha^{-1} after the first cut. In the total of three years, the energetic value of dry matter of this variant was 416 GJ per hectare. A yearly production of energy from straw and stubble hay harvested in spring could have been in this variant on average 138 GJ ha^{-1}. In our trials, least energy was produced in the variant seeded at 60 cm row space – 299 GJ ha^{-1} in total of three years, i.e. about 100 GJ ha^{-1} per year.

In addition, the quality of dry matter of reed canarygrass and its change dynamics were investigated in the trials (Table 3). Quality indices were chosen considering both the requirements of forage production (CP, ADF, NDF) and energy production (proportion of leaves and stems, K and crude ash contents). Based on the contents of crude protein, acid detergent fibre and neutral detergent fibre, the stand cut in the autumn of seeding year can be used as fodder. There is no sense of leaving it in the field over winter, since the stand will lodge and cannot be harvested in the following spring.

The dry matter of reed canarygrass harvested in July at the stage of full seed maturity and dried in the field is not valuable as fodder – it has low crude protein content and high acid detergent and neutral detergent fibre contents. Straw can be used for energy, but requires specific measures due to relatively high potassium and crude ash contents (15.20 g kg^{-1} and 54.6 g kg^{-1} respectively). The yield of reed canarygrass harvested in spring has the best properties for energy production. During winter the contents of potassium (1.81 g kg^{-1}) and crude ash (48.7 g kg^{-1}) in the above-ground biomass decrease considerably. A significant change occurs also in the proportion of leaves and stems in favour of the latter. As stems contain less mineral substances (including potassium) than leaves (Pahkala and Pihala, 2000), the loss of leaves in winter improves the combustion properties of the biomass harvested in spring.

The moisture content of straw of reed canarygrass that was cut in July and dried in the field was 14.5% in 2010, 20.3% in 2011 and 14.4% in 2012. The moisture content of stubble hay harvested in spring was 14.3% in 2011, 14.4% in 2012 and 15.2% in 2013. In 2013 the snow cover persisted at the trial site until 11 April, which was quite unusual compared with the long term average. The surface became dry only at the beginning

Table 3

Quality indices of reed canarygrass

Harvest time	Leaves,%	Stems,%	Weeds,%	CP g kg^{-1}*	ADF g kg^{-1}**	NDF g kg^{-1}***	K g kg^{-1}	Ash g kg^{-1}
Green mass (2008) autumn	58.7	36.8	4.5	98.3	291.0	480.8	14.30	106.2
Straw 14.07.11	54.4	38.8	6.9	91.8	348.6	617.4	15.20	54.6
Stubble hay 18.07.11	30.0	62.0	8.0	58.5	414.3	641.8	9.48	51.4
Stubble hay 10.10.11	41.9	54.4	3.8	77.2	438.2	652.8	7.66	63.6
Stubble hay 12.04.12	16.9	83.1	×	×	×	×	1.81	48.7

* crude protein. Conversion factor for the calculation from N-content 6,25; ** acid detergent fiber;
*** neutral detergent fiber

of May. Cutting of stubble hay took place on 2 May, not in April as in the two previous years. By that time vegetation had already started and a small amount of leaves from young shoots got into the cut stubble hay. The maximum permitted moisture content of reed canarygrass for energy production is 20%. The material harvested in our trials met this requirement at all harvest times. This kind of biomass does not require additional drying. It can be stored without risk of self-heating. In Finland, the moisture content of reed canarygrass that has been kept in the field over winter and harvested in spring has been 10–20% (Lindh et al., 2005). In Finland a higher price is paid for energy hay with the moisture content below 14% (Kontturi and Pahkala, 2007). In our trials we did not achieve this level at any of the harvest times.

Conclusions

Based on the results of experiments conducted at the Estonian Crop Research Institute in 2008–2013 it can be said that it is possible to grow reed canarygrass for energy production in Estonia provided that the divided harvesting method of biomass is used. In the case of the divided harvesting method, reed canarygrass yields 5–8 t of dry matter per hectare, 40% of which is gathered in July and 60% in the following spring before the start of vegetation. The total energetic value of biomass produced per hectare per year is 100–140 GJ. The advantages of the divided harvesting method are as follows:

1. The biomass of reed canarygrass formed during the whole vegetation period can be used for energy production.
2. Stubble hay does not ledge under snow cover, thus harvest losses are minimized.
3. Less nutrients are removed from the field with the yield, thus it is possible to save on fertilization costs.
4. Compared to cutting once during summer, the divided harvesting method ensures that 2/3 of the fuel has better combustion properties.

The production field should be established with narrow spacing (15 cm) and with a seeding rate of 8–10 kg ha^{-1}. Nitrogen fertilizer should be applied in two doses: in spring after the start of growth with the rate of N 70 kg ha^{-1} and in July after harvest with the rate of N 70 kg ha^{-1}. Since in July the harvest takes place in the late developmental stage of plants, it is possible to combine the divided harvesting with the seed production of reed canarygrass. In this case the first growth is cut with a combine harvester, seed is threshed and straw is harvested for energy hay.

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THE CHANGE IN THE FORAGE QUALITY OF SMOOTH BROMEGRASS (*BROMUS INERMIS* L.) IN GRAZING AND NON-GRAZING PASTURES

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Abstract
This research was conducted to determine the chemical composition of smooth bromegrass in artificial pasture from the years 2010 to 2012 in Isparta Province located in the Mediterranean region of Turkey. The mixture of the pasture used were alfalfa (*Medicago sativa* L.) + sainfoin (*Onobrychis sativa* Lam.) + crested wheatgrass (*Agropyron cristatum* L.) + smooth bromegrass (*Bromus inermis* L.). Animal grazing applications were performed in the second and the third year of the study since the first year covered only the establishment of the artificial pastures. Forage samples were collected from grazing and non-grazing areas once every 15 days during the grazing seasons. The crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) contents, total digestible nutrient (TDN) and relative feed value (RFV) were determined on the smooth bromegrass forage samples. According to results, CP, TDN and RFV values decreased throughout the grazing season, while ADF and NDF contents increased in grazing and non-grazing areas. The ADF and NDF contents of smooth bromegrass in non-grazed areas were higher than the grazed areas, while CP, TDN and RFV values of grazed areas were higher than non-grazed areas. It can be concluded that the harvesting at the late stages caused a reduction in forage quality of smooth bromegrass in grazing and non-grazing areas.
Key words: *Bromus inermis*, artificial pasture, acid detergent fibre, crude protein, relative feed value, total digestible nutrient.

Introduction

Smooth bromegrass (*Bromus inermis* Leyss.) is a high-yielding grass but requires longer recovery periods than other grasses. It is best adapted to well-drained soils and is an excellent choice for drought-prone areas (Undersander et al., 1996). Because of its highly developed root system, smooth bromegrass is resistant to temperature extremes and drought. It grows best on deep, well-drained silt or clay loam but may also establish itself in sandier soils. The forage quality of smooth bromegrass is higher than that of most other cool-season grasses such as orchardgrass (*Dactylis glomerata* L.) or tall fescue (*Festuca arundinacea*); crude protein levels in smooth bromegrass often exceed 120 g kg^{-1} if it is harvested in the boot stage. However, smooth bromegrass recovers poorly from cutting because its tiller apices, or tips, are vulnerable to removal. This leads to lower yields after a first cutting and poor seasonal distribution of yield. In addition, older stands may easily become dense and sod-bound, resulting in markedly lower productivity.

Despite its disadvantages, smooth bromegrass lends itself to a variety of purposes, especially when combined with a legume such as alfalfa (*Medicago sativa* L.) or red clover (*Trifolium pretense*). It is one of the most useful cool-season grasses throughout its range, valuable for hay, pasture, silage and green chop. Its rhizomes and tough root network also make it worthwhile for ground cover and erosion control. Therefore, in this research it was aimed to determine forage quality of the smooth bromegrass during the grazing season in the artificial pastures established in the Mediterranean Region of Turkey.

Materials and Methods

This research was conducted at Suleyman Demirel University Research Farm in Isparta Province (37°45′N, 30°33′E, elevation 1035 m) located in the Mediterranean region of Turkey on three consecutive years from 2010 to 2012. The total precipitation and average temperature data for the experimental area are given in Figures 1 and 2. The major soil characteristics of the research area were as follows: The soil texture was clay loam, the organic matter was 13 g kg^{-1} as determined using the Walkley–Black method, the lime was 71 g kg^{-1} as determined using a Scheibler calcimeter, the total salt was 2.9 g kg^{-1}, the exchangeable K was 122 mg kg^{-1} by 1 N NH_4OAc, the extractable P was 3.3 mg kg^{-1} by 0.4 N $NaHCO_3$ extraction, and the pH of a soil-saturated extract was 7.7. The soil type was *calcareous fluvisol*.

In March 2010, two artificial grazing lands, covering 1.5 ha pasture each were established at the university farm. Pasture was composed of alfalfa (*Medicago sativa* L., 15%) + sainfoin (*Onobrychis sativa* Lam., 15%) + crested wheatgrass (*Agropyron cristatum* L., 35%) + smooth bromegrass (*Bromus inermis* L., 35%). Cutting and maintenance applications were made in the first year. Immediately after sowing pasture, fertiliser was applied at rate of 50 kg ha^{-1} as phosphorus (P2O5) and 50 kg ha^{-1} of nitrogen (N). The same amount of phosphorus as triple superphosphate (46% P2O5) in the October 2010 and 2011 while in 2011 and 2012 March, 50 g ha^{-1} N as ammonium nitrate (33.5% N) was used . Pastures were harvested twice during the end of June and beginning of October in 2010. Animal grazing applications were

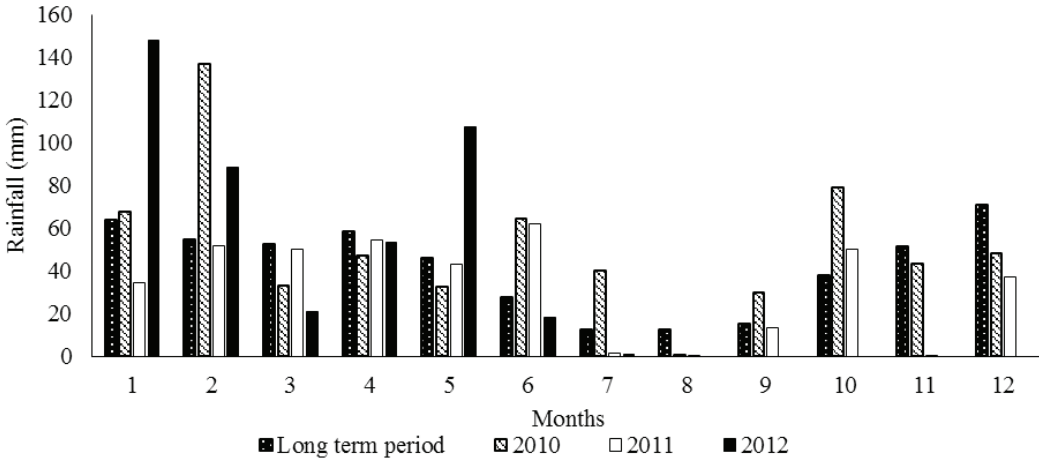

Figure 1. Rainfall values for individual experimental years and over the long term.

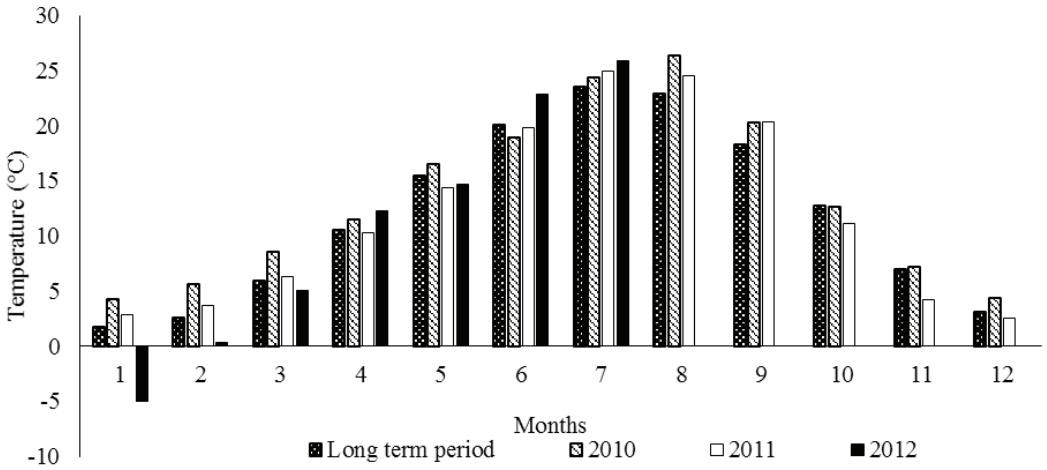

Figure 2. Temperature values for individual experimental years and over the long term.

performed in the second and the third year of the study since the first year covered only the establishment of the artificial pastures. The animals were turned out to pasture for grazing on the 1st of May and the grazing was terminated on the 1st of August each year. Animals were kept on the pasture continuously. 10 Holstein male calves with an average 6 months old were included and allocated evenly to artificially established pasture in the experiment which lasted for 90 days in 2011 and 2012. The animals had a free access to the water during all experimental periods.

Four non-grazed areas within pasture were established in order to determine forage quality changes of smooth bromegrass and fenced with wires by 4×3m size and grass samples were collected by using 0.5m^2 (0.5×1 m) quadrats fortnightly from May to August each year. The crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) contents, total digestible nutrient (TDN) and relative feed value (RFV) were determined as well.

The collected samples (1 May, 15 May, 30 May, 15 June, 30 June, 15 July, 30 July) after the harvest were weighed and dried at 70 °C for 48 h. The dried samples were reassembled and ground to pass through a 1-mm screen. The crude protein (CP) content was calculated by multiplying the Kjeldahl nitrogen concentration by 6.25 (Kacar and Inal, 2008). The acid detergent fiber (ADF) and neutral detergent fiber (NDF) concentrations were measured according to methods from Ankom Technology. Total digestible nutrients (TDN), dry matter intake (DMI), digestible dry matter (DDM) and relative feed value (RFV) were estimated according to the following equations adapted from R.D.Horrocs and J.F.Vallentine (1999):

TDN = (-1.291 x ADF) + 101.35

DMI = 120/NDF

DDM = 88.9-(0.779 x ADF)

RFV = DDM x DMI x 0.775

The data were subjected to the analysis of variance using General Linear Models procedure (MINITAB 2010). The means were compared by pairwise comparison test by Duncan at the 5% level of significance.

Table 1

Results of analysis of variance of the traits determined

Sources of variations	df	CP	NDF	ADF	TDN	RFV
Block (year)	6	ns	*	*	*	*
Year	1	**	*	*	*	*
Grazing (G)	1	**	*	*	*	*
Sampling Times (ST)	6	**	**	**	**	**
G x ST intr	6	ns	ns	ns	ns	ns
G x Year	1	*	ns	*	ns	ns
ST x Year	6	ns	ns	ns	ns	ns
G x ST x Year intr.	6	ns	ns	ns	ns	ns
Error	78					

df: degrees of freedom, ns: not significant, *p<0.05 and **p<0.01

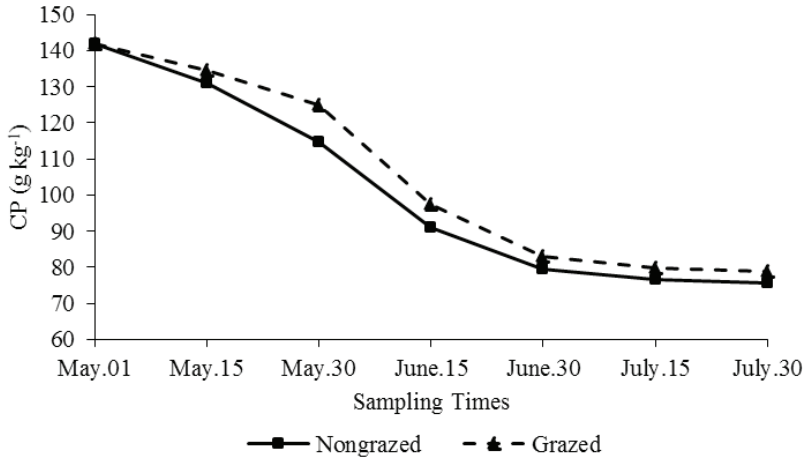

Figure 3. Seasonal variation of CP contents of smooth bromegrass according to averages of 2011 and 2012.

Results and Discussion

The effects of the grazing and sampling times on CP contents were significant (Table 1). The CP contents of smooth bromegrass were decreased throughout the grazing season in the grazed and the non-grazed areas (Figure 3). The highest CP contents were obtained from the beginning of the grazing season while the lowest CP contents were determined at the end of the grazing season. Maturity stage at harvest is the most important factor determining forage quality. Other reports also support that the CP content decreases by advancing stage of maturity (Koc et al., 2000; Rebole et al., 2004), suggesting that animals should be supplemented with protein sources, especially towards the end of the grazing season. As a result of this process, forage quality lessens substantially towards the end of growing season. The CP ratios of the grazed areas were higher than that of non-grazed areas in the present study. This could be associated with the continued re-growth of the plants in the grazed areas because young plant tissues are more nutritious than dead or mature plant (Lyons et al., 1996).

The ADF and NDF contents of smooth bromegrass were significantly affected by both grazing and sampling times (Table 1). Acid detergent fiber and NDF contents were increased during the grazing season in the grazed and non-grazed areas (Figures 4 and 5). This could be explained by the decrease in proportion of leaves and the increase of the stems proportion with advanced maturity. Because, ADF and NDF contents of stems are higher than the leaves. Similar results were reported by Karslı et al. (2003), Kaya et al. (2004), Erkovan et al. (2009), Turk et al. (2014).

The trends in ADF and NDF contents with increasing maturity are normally the reverse of protein (Rebole et al., 2004). Young plant cells has the primary cell wall, but also the secondary cell wall occurs with maturing. This causes being the more fibrous of

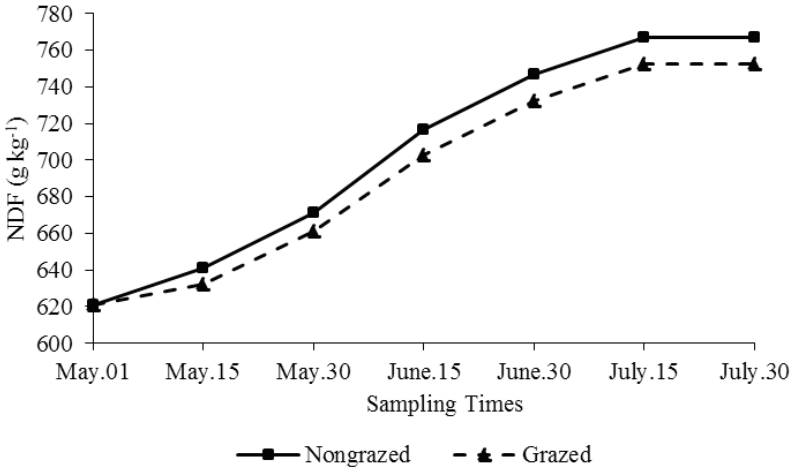

Figure 4. Seasonal variation of NDF contents of smooth bromegrass according to averages of 2011 and 2012.

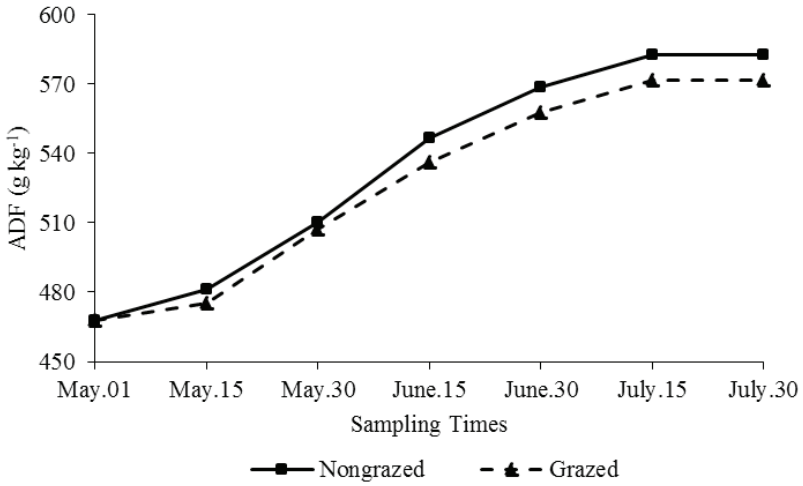

Figure 5. Seasonal variation of ADF contents of smooth bromegrass according to averages of 2011 and 2012.

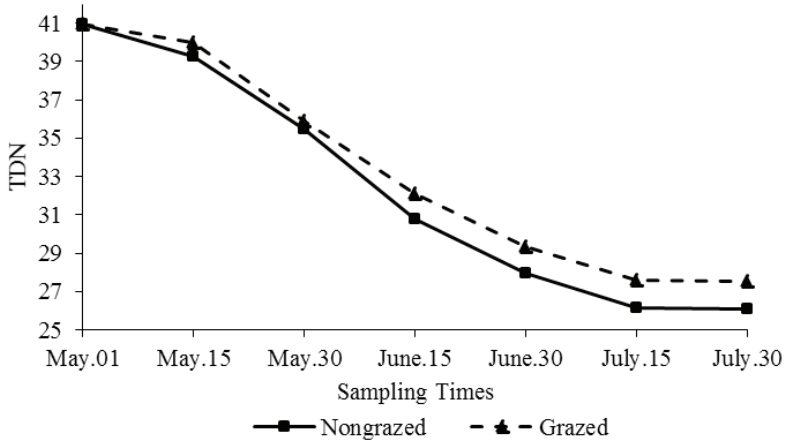

Figure 6. Seasonal variation of TDN values of smooth bromegrass according to averages of 2011 and 2012.

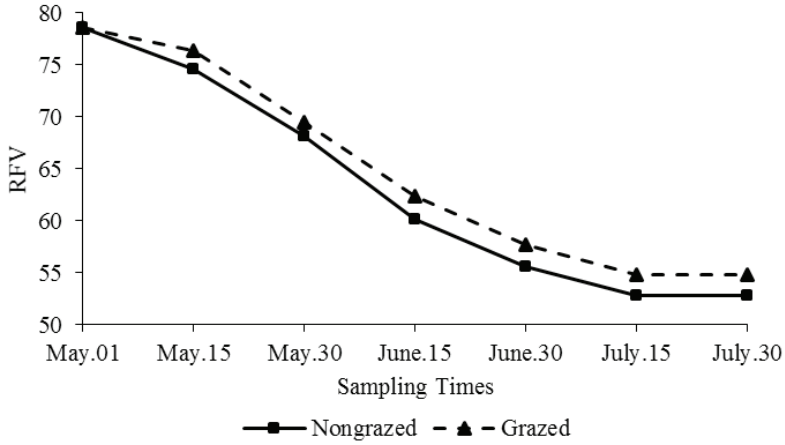

Figure 7. Seasonal variation of RFV values of smooth bromegrass according to averages of 2011 and 2012.

mature plants (Arzani et al., 2004). ADF and NDF contents of non-grazed areas were higher than those of the grazed areas in the present study. This could be explained by the continued re-growth of the plants in the grazed areas.

The highest TDN values were obtained at the beginning of the grazing season, while the lowest TDN values was obtained at the end of the grazing season in the grazed and non-grazed areas (Figure 6). The TDN refers to the nutrients that are available for livestock and are related to the ADF concentration of the forage. As ADF increases there is a decline in TDN which means that animals are not able to utilize the nutrients that are present in the forage (Aydın et al., 2010).

The RFV is an index that is used to predict the intake and energy value of the forages and it is derived from the DDM and dry matter intake (DMI). Forages with an RFV value over 151, between 150-125, 124-103, 102-87 and 86-75, and less than 75 are considered as prime, premium, good, fair, poor and reject, respectively (Lithourgidis et al., 2006). The highest RFV values were obtained on 1st May, while the lowest RFV values were obtained on 30rd July in the grazed and non-grazed areas (Figure 7). The TDN and RFV values of smooth bromegrass in the grazed areas were higher than that of non-grazed areas in this study. It may be associated with the continued re-growth of the plants in the grazed areas because young plant tissues are more nutritious than dead or mature plant (Lyons et al., 1996).

Conclusions

The results from the change in the forage quality of smooth bromegrass in grazing and non-grazing artificially established pastures in Mediterranean conditions of Turkey can be summarised as follows:

1. CP, TDN and RFV decreased throughout the grazing season, while ADF and NDF contents increased in the grazed and non-grazed areas.
2. The ADF and NDF contents of smooth bromegrass in non-grazed areas were higher than the grazed areas.
3. The CP, TDN and RFV values of smooth bromegrass in grazed areas were higher than non-grazed areas.
4. It can be concluded that the harvesting at the late stages caused a reduction in forage quality of smooth bromegrass in the grazed and non-grazing areas.

Acknowledgments

This work was financially supported by TUBITAK (Turkish Scientific and Technological Research Council, project no: 109 O 162).

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THE CHANGES IN NITROGEN CONTENT IN SOIL DEPENDING ON WINTER WHEAT (*TRITICUM AESTIVUM* L.) FERTILIZING SYSTEM

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Abstract
The objective of this study was to evaluate the main plant nutrient: mineral nitrogen (NH_4^+-N and NO_3-N) dynamic in soil under fertilizing for obtaining high grain yields of winter wheat (*Triticum aestivum* L.) and to determine relationships between nutrient uptake and winter wheat productivity. The field study was carried out at the Research and Study farm 'Vecauce' of the Latvia University of Agriculture for two years 2012/2013 and 2013/2014 with winter wheat variety 'Kranich'. Different nitrogen application rates (0, 85, 153, 150, 175, 180 and 187 kg ha-1) and timing were used for winter wheat. The content of nitrates NO_3-N and ammonium nitrogen NH_4-N was determined in the soil layers 0-0.20 m, 0.20-0.40 m, 0.40-0.60 m. Nitrogen management strategy during the plant growth period based on soil N_{min} evaluating can improve N use efficiency and reduce environmental contamination. The maximum of mineral nitrogen content in soil in the vegetation period was observed at the beginning of stem elongation with a tendency to decrease. A significant impact ($p < 0.05$) of nitrogen fertilizer application was noted on the mineral nitrogen content in soil layer 0-0.20 m deep in both trial years. The increasing doses of nitrogen fertilizer raised the amount of mineral N in the soil profile. The significant impact ($p < 0.001$) of nitrogen application and year conditions was observed on grain yield. Close positive correlation significant at 99% probability level was observed between the grain protein content and nitrogen concentration mostly in all soil layers, but it was not found between the grain yield and nitrogen content.
Key words: wheat, nitrates, ammonium nitrogen, fertilizers, soil, yield.

Introduction

The productivity of crop is largely limited by soil mineral nutrients. Nitrogen is the main element affecting the rate of cereal growth and yielding. Nitrogen content in the soil is controlled by climate (especially temperatures), soil management and crops, as reported by different authors (Convertini et al., 2001; Skudra and Ruza, 2002; Timbare and Busmanis, 2002; Ruza and Kreita, 2006). In crop growing it is very important to reduce losses of the ammonium and nitrate nitrogen through the leaching, which is a serious problem because of the possibility of ground and surface water pollution. The European Union Nitrate Directive (91/676/EEC) is intended to reduce soil contamination caused by nitrates from agricultural sources; the content of nitrate nitrogen should not exceed 50 mg kg^{-1} in soil (Council Directive, 1991). If soil nutrient conditions during the growing season could be controlled better, those resources could be more efficiently utilized (Tivy, 2014). Analyses of NO_3-N content can maximize the crop production and minimize environmental impact of nitrogen fertilization. Some authors (Timbare and Busmanis, 2002; Ruza and Kreita, 2006) observed that indices of soil N dynamics in the vegetation period were significantly affected by the temperature and precipitation as well as relationships of their distribution. M.Corbeels et al. (1999) determined that the high level of residual mineral nitrogen in the soil profile resulted from a low N plant uptake relative to the soil N supply and N fertilization, and masked the effect of N fertilization on dry matter accumulation. Nitrogen losses through leaching and denitrification occurred after a heavy rainfall, but were limited. For obtaining high and quality yields, nitrogen provision must correspond to the requirements of the plants and the use of nitrogen from the soil as well.

The objective of the study was to evaluate the main plant nutrient: mineral nitrogen (NH_4^+-N and NO_3-N) dynamics in soil under fertilizing for obtaining high grain yields of winter wheat and to determine the relationships between nutrient uptake and winter wheat productivity.

Materials and Methods

The field study was carried out at the Research and Study farm 'Vecauce' (latitude: N 56°28´, longitude: E 22°53´) of the Latvia University of Agriculture. The 2012/2013 and 2013/2014 experiments were carried out on the fields with rape seed as the previous crop. The soil at the site was *Endostagnic Phaeozem (Loamic)* (WRB, 2014) with humus content 17 – 23 g kg^{-1}, soil pH KCl – 6.6 – 7.2, plant available K – 118 – 150 mg kg^{-1} and P – 50 – 122 mg kg^{-1} and S – 0.67 – 1.34 mg kg^{-1}. The plot size was 20 m^2, with four replicates. Winter wheat variety 'Kranich' was used with sowing rate – 450 germinate able seeds per m^2. While sowing winter wheat, the compound fertilizer was added. In 2012, 300 kg of NPK 5-15-25 were used for fertilizing containing N – 15, P – 20, K – 62 kg ha^{-1}, but in 2013 the same amount of NPK 6-26-30 containing N – 18, P – 34, K – 75 kg ha^{-1}. Different mineral nitrogen rates

and timing were used for winter wheat. In addition, nitrogen fertilizer NH_4NO_3 was applied at the rates of 85 kg ha^{-1} N and 153 kg ha^{-1} N in two splits (85+68). N application 175 kg ha^{-1} was applied in three splits (85+60+30): the first dose as NH_4NO_3, but the second and third as $(NH_4)_2SO_4$. Application 180 kg ha^{-1} N was applied in three splits (85+50+45) in 2013 according to chlorophylmeter (Yara N-tester, Konica Minolta Sensing, inc.) data (Markwell et al., 1995) and 150 kg ha^{-1} N in three splits (85+50+15) in 2014 according to chlorophylmeter data, 187 kg ha^{-1} N in three splits (85+68+34) as NH_4NO_3. The first dose of nitrogen was given in spring at the beginning of wheat regrowth, the second time at the stem elongation and the third time - at the beginning of heading. The BBCH identification key of growth stages was used (Meier, 2001). Herbicide and fungicide applications were made to prevent suboptimal plant growth conditions due to weed infestation or diseases. The crop was harvested at the BBCH 88-92 on August 6, 2013 and August 8, 2014; the yield of plots was dried, weighted and the moisture content was determined. Grain protein content was calculated multiplying the total nitrogen content by coefficient 5.7 determined by Kjeldahl method (ICC 105/2; Kjeltec system 1002, Foss Tecator AB, Sweden).

Soil samples were taken with gauge auger, first in spring during wheat renewing time and, then at different plant development stages: BBCH 32, 51, 69 and 91. The content of nitrates NO_3-N and ammonium nitrogen NH_4-N was determined in the soil layers 0-0.20 m, 0.20-0.40 m, 0.40-0.60 m, according to the method set out by LVS ISO/TS 14256-1. Analyses were done in the Agrochemical Laboratory of the State Plant Protection Service. Each mineral N concentration per unit bulk volume of soil was calculated to the quantity per ha.

Meteorological conditions of the research years differed year by year. The climatic conditions were favorable for the growth of wheat plants in the autumn of 2012 and 2013. The end of vegetation in autumn was observed at the beginning of November (usually they occur in mid-November) in 2012. The permanent snow cover of 5-10 cm lasted from December to February. In March and April the air temperature was lower by 2.6 °C and 1.3 °C than the average for many years (Fig.1.) The vegetation renewed very late – at the end of April. May was favorable for plant development; the air temperature was 3 °C higher than the long-term average and precipitation three times more than the long-term average (Fig. 2.). In June the air temperature exceeded norm by 2.1 °C, but sufficient precipitation was recorded – half the long-term average. In August the weather was favorable for harvesting.

In 2013, autumn weather conditions were comparably good for winter crops. In January of 2014 snow fell on an unfrozen land, but then entered the snow break. From the middle of January till the beginning of February black frost was observed and winter wheat plants partly did not survive. The regrowth of vegetation started early – at the end of March. Temperature in April exceeded norm and weather was warmer. April and May were characterized by lack of rainfall. Moisture in June was optimal for plant growing. At the time of harvesting the weather was dryer than the long-term.

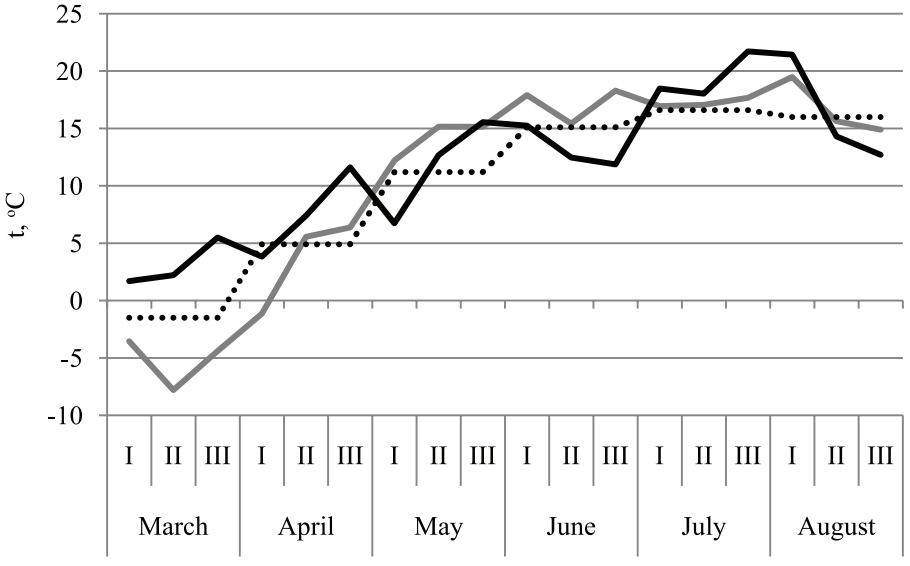

Figure 1. Mean air temperatures, Vecauce, 2013-2014, °C.
(– - 2013, – - 2014, **…**- long term average, I, II, III – ten day period).

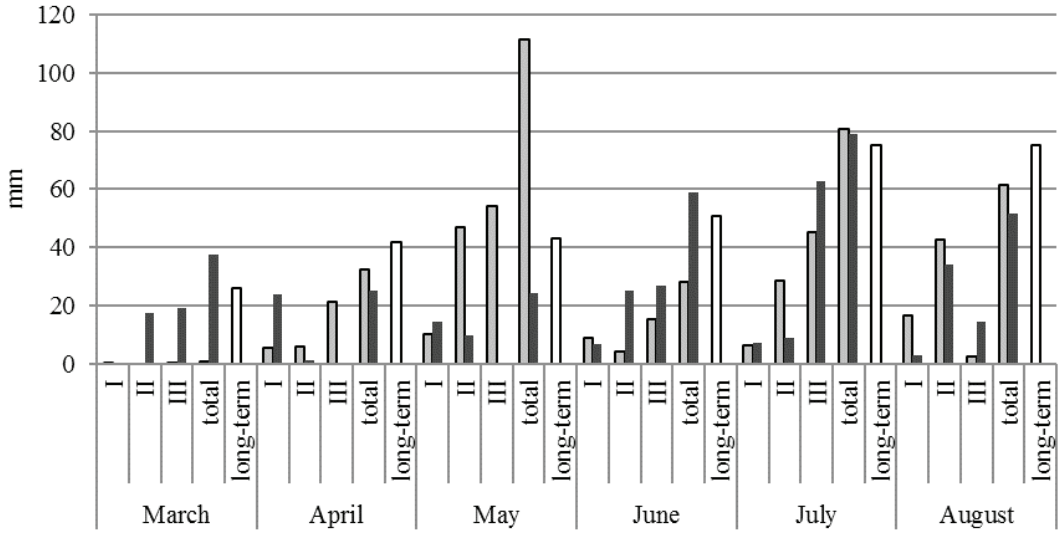

Figure 2. Amount of precipitation, Vecauce, 2013-2014, mm
(■– 2013, ■ – 2014, □ – long term average I, II, III – ten day period, monthly total).

Data were analyzed using ANOVA, correlation and regression analyses. The significance test was performed at probability level of $p<0.05$. Differences among treatment data and sampling dates were separated using Fisher's least significant difference procedure.

Results and Discussion

In 2013 at the regrowth of vegetation (22 April) the mineral nitrogen ($NH_4 + NO_3$) content was determined in the soil layer 0-0.20 m – 26 kg ha^{-1}, at 0.20-0.40 m – 62 kg ha^{-1} and in 0.40-0.60 m – 23 kg ha^{-1}. At this stage in the soil was observed maximum of mineral nitrogen content in vegetation period. Results coincided with A.M. Kibe et al. (2006) and K. Thorup-Kristensen (2009) reports that nitrogen compound is always present in the soil at the beginning of the vegetation period or is produced by mineralization during this period and there also occur losses of nitrogen due to volatilization, denitrification and leaching. The vegetation renewed very late – at the end of April, but in May there were very favorable climatic conditions (warm and wet) for plant development and for plant nitrogen uptake from the soil. As previously mentioned, in June sufficient precipitation was recorded – half a norm, which influenced the high amount of nitrogen in soil, at the BBCH 69 was observed 12-114 kg ha^{-1} (Fig. 3.), depending on fertilizer application. Under the low moisture conditions, the content of mineral nitrogen in topsoil could be a limiting factor with respect to the N nutrition of the plant. In such situation the root system is mainly active and plant N uptake generally occurs in the deeper soil layers (Corbels et al., 1999). Furthermore, in the vegetation period the nitrogen content in all soil layers decreases. Similar data were obtained with spring wheat (Jermuss, 2010).

There was observed a low mineral nitrogen content in the soil layers in the beginning of wheat regrowth in 2014: in the soil layer 0-0.20 m – 10 kg ha^{-1}, 0.20-0.40 m – 2 kg ha^{-1}, 0.40-0.60 m – 6 kg ha^{-1}. The mineral nitrogen content in soil maximum was obtained at the beginning of stem elongation – 39-199 kg ha^{-1} (Fig. 4). The rise of temperature and optimal moisture conditions in plant regrowth period resulted in a rapid increase of mineral N content in soil. During the next growth stages a rapid decline in N content was observed in all soil layers due to the plant uptake. The highest N_{min} content was found in the soil top layer (0-0.20 m) in both investigation years, except N_{min} content at the BBCH 69 in 2013. The nitrogen content in the soil was dependent not only on the duration of the vegetative growth, but also on the level of nitrogen fertilisation, - it was concluded also in the other research (Skudra and Ruza, 2002; Koodziejczyk, 2013). The amount of nitrogen content in the top layer (0-0.20 m) increased with the doses of fertilizers, similar data was obtained in Hungary (Ragasits et al., 1996). A significant impact ($p < 0.05$) of nitrogen fertilizer application was noted on the mineral nitrogen content in soil layer 0-0.20 m in both trial years (by 22% in 2013 and by 30% in 2014). Nitrogen fertilizer application on this parameter was not significant ($p > 0.05$) in soil layers 0.20-0.40 m and 0.40-0.60 m in both trial years. In 2014 a higher average N_{min} content was obtained in the soil layer 0-0.20 m deep (40.48 kg ha^{-1}) and in the 0.20-0.40 m layer (23.80 kg ha^{-1}) in comparison with data in 2013, where the nitrogen content in the top soil layer was lower: 25.29 kg ha^{-1} and 10.87 kg ha^{-1} in 0.20-0.40 m

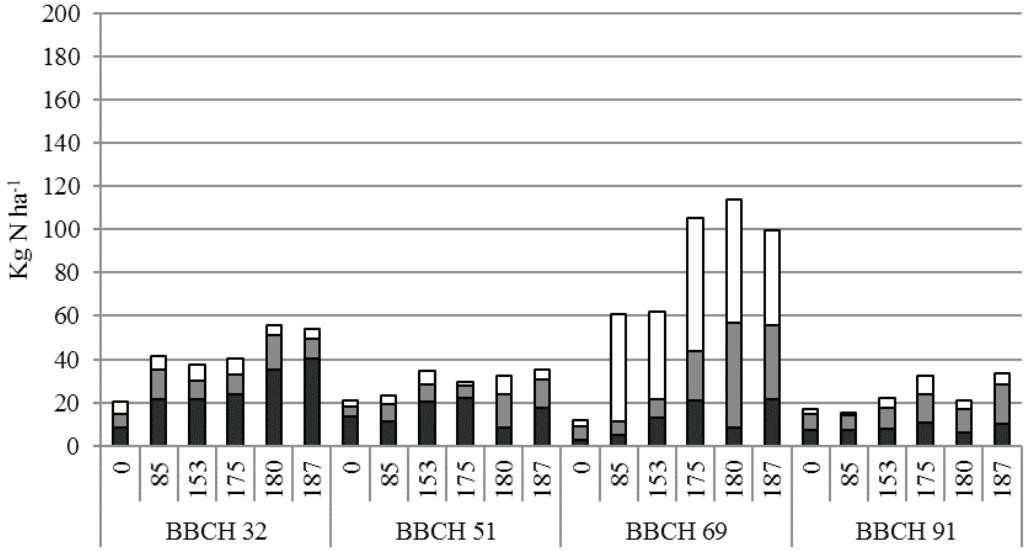

Figure 3. The content of mineral nitrogen in the soil layers in vegetation period 2013 (0, 85, 153, 175, 180, 187 – N norm, growth stage, ■ – 0-0.20 m $LSD_{0.05}$=7.7, ■ – 0.20-0.40 m $LSD_{0.05}$=11.8, □ – 0.40-0.60 m $LSD_{0.05}$=15.5).

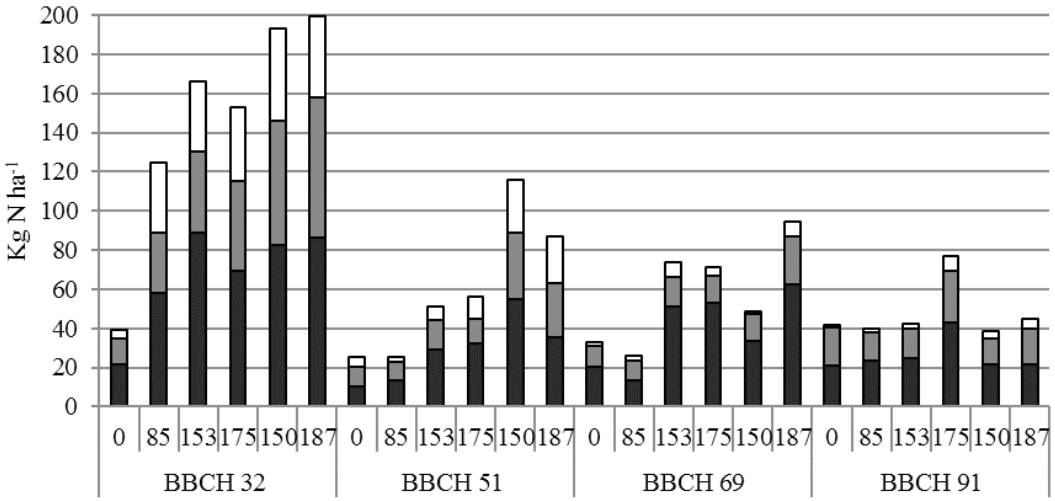

Figure 4. The content of mineral nitrogen in the soil layers in vegetation period 2014 (0, 85, 153, 175, 150, 187 – N norm, growth stage, ■ – 0-0.20 m $LSD_{0.05}$=20.7, ■ – 0.20-0.40 m $LSD_{0.05}$=15.7, □ – 0.40-0.60 m $LSD_{0.05}$=11.9).

layer. Average nitrogen content in the soil layer 0.40-0.60 m was similar in both investigation years (14.36 and 13.54 kg ha^{-1} accordingly). Differences could be explained by meteorological conditions and with differences in plant density – in 2014 after wintering the plant density was lower than in 2013. When the N-doses exceed the requirement of wheat, the soil pollution is significant, but in our investigation in 2014 the maximum NO_3 concentration at the BBCH 91 was 12.6 mg kg^{-1} and did not exceed the norm determined by the Nitrate directive (50 mg kg^{-1}).

In 2013 the winter wheat grain yield ranged between 4.07- 7.64 t ha^{-1} ($LSD_{0.05}$ = 0.47), and between 2.79 – 5.20 t ha^{-1} in 2014 ($LSD_{0.05}$ = 0.12) and had an increasing trend with increasing nitrogen dose. A significant impact ($p < 0.001$) of nitrogen application was observed on grain yield (increase from 2.77 to 3.88 t ha^{-1} in 2013 and increase from 0.52 to 2.41 t ha^{-1} in 2014). These findings are in accordance with other research (Abdin et al., 1996; Sestak et al., 2014) where nitrogen application for increasing grain yield was used. Nitrogen fertilizer norm had

Table 1

Correlation between N_{min} in soil layers and grain yield and protein content

BBCH	Soil layer, m	Correlation coefficients	
		grain yield	protein content
32	0-0.20	-0.28	0.90**
	0.20-0.40	-0.36	0.86**
	0.40-0.60	-0.46	0.81**
51	0-0.20	-0.12	0.78**
	0.20-0.40	-0.13	0.75**
	0.40-0.60	-0.15	0.74**
69	0-0.20	-0.23	0.91**
	0.20-0.40	0.52	0.21
	0.40-0.60	0.92**	-0.41
91	0-0.20	-0.56	0.73**
	0.20-0.40	-0.32	0.73**
	0.40-0.60	0.50	0.39

*- 0.05 probability level = 0.576, **- 0.01 probability level = 0.708

close linear correlation between grain yield (r=0.61, y=0.0587x+2.6521, $p < 0.05$). A significant impact ($p < 0.001$) of the year conditions was observed on grain yield. Another research (Sestak et al., 2014) reported that year properties significantly influenced N availability resulting in different responses of grain yield. A significant correlation was not found between mineral nitrogen content in soil and grain yield, except in soil layer 0.40-0.60 m at the BBCH69, where we got a positive correlation significant at 99% probability level (Table 1). R. Timbare and M. Bušmanis (2002) found a close correlation between grain yield at the variant without nitrogen application and mineral nitrogen in spring, but in our data we did not find such a correlation. It could be explained by diverse meteorological conditions year by year and we should get more data in the next year for a better data interpretation. A close positive correlation was observed between the grain protein content and nitrogen concentration in all soil layers at the all vegetative period significant at 99% probability level, except at BBCH 69 in 0.20-0.60 m layer and BBCH 91 at 0.40-0.60 m layer depth.

Conclusions

The maximum mineral nitrogen content in soil in vegetation period was observed at the beginning of stem elongation with a tendency to decrease. A significant impact ($p < 0.05$) of nitrogen fertilizer application was noted on the mineral nitrogen content in 0-0.20 m deep soil layer in both trial years. The increasing doses of nitrogen fertilizers raised the amount of mineral N in the soil profile. A significant impact ($p < 0.001$) of nitrogen application and year conditions on grain yield was observed. A close linear correlation was found between grain yield and nitrogen application rate. A close positive correlation was observed between the grain protein content and nitrogen concentration mostly in all soil layers, but it was not found between grain yield and nitrogen content. The nitrogen management strategy during plant growth period based on soil N_{min} evaluating can improve N use efficiency and reduce environmental contamination.

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INFLUENCE OF AGROECOLOGICAL FACTORS ON ARTICHOKE YIELD AND QUALITY: REVIEW

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Abstract
Environmental conditions and climate change on a global scale affect the overall agriculture and food supply. Globe artichoke (Cynara cardunculus var. scolymus (L.) Fiori) is widely distributed all over the world. Immature inflorescence, commonly called capitula or head, is used in human consumption. These vegetables are a good source of human health promoting components. Artichokes are widely used in human diet, characterized by low protein and fat, high content of minerals, vitamins, inulin, carbohydrates and polyphenolic compounds. Relationship between plant growth and development is tight and complicated. Many agroecological factors, such as temperature, irrigation and fertilization level, planting and harvesting date, influence processes of growing and development of globe artichoke. Biologically active compounds in plants are dependent on climate conditions, seasonal changes, cultivar properties and maturity. Pre-germination is required for better plant establishment in the field. Better plant growing and development can be ensured by regular irrigation which provides 85 – 100% from evaporation and applied fertilization before planting and during vegetation period according to soil properties. The biochemical quality of artichoke heads differs between cultivars, head fraction, and stage of head development. This indicates possibility to grow artichokes in Latvia.
Key words: *Cynara cardunculus,* germination, quality, irrigation.

Introduction

Cynara cardunculus comprises three taxa: two domesticated forms – globe artichoke (var. *scolymus* L.) and cultivated cardoon (var. *altilis*) - and the wild cardoon (var. sylvestris). Wild cardoon is the ancestor of both cultivated forms (Velez et al., 2012, Durazzo et al., 2013). The globe artichoke (Cynara cardunculus var. scolymus (L.) Fiori), (Fam. Asteraceae) is widely distributed all over the world and especially in the South Europe (Italy, Spain, France, Greece), Middle East (Turkey, Israel), North Africa (Egypt, Tunisia), South America (Argentina, Chile), United States and also in China (Pandinoet et al., 2013). Last year the production of artichokes increased. Artichokes mainly grow in Italy, which had the production of 547 799 tonnes in 2013. The top three leaders in Europe are Italy, Spain (199 900 tonnes) and Greece (28 600 tonnes). In Baltic countries artichokes are commercially grown in Lithuania with the production of 100 tonnes in 2013 (http://faostat3.fao.org/download/Q/QC/E). In Latvia artichokes do not grow in commercial areas, only in home gardens.

Globe artichoke is a perennial herbaceous plant. In Latvia artichoke is cultivated as annual or biennial plant. In the first year the plant develops a rosette of leaves – 1 m and more in height and around 1 m in diameter. The plant's above ground parts in autumn die off, but underground part overwinters. In the second year develop a long stem (1 – 2 m high) and inflorescence. Each plant can develop several heads, aggregated in groups. Iin Latvia artichoke is cultivated mostly as an annual plant because winter periods are characterized with low soil and air temperature. Long periods without snow or thaws are typical in the wintering period. It negatively influences overwintering of perennial and annual plats (Bratch, 2014). Artichokes are usually propagated generatively by seeds and vegetatively by root or shoot cuttings. Generatively propagated artichoke gives yield in the second year. Yield can be developed in the first year if vernalized seeds are used. In the vegetative propagation method, artichoke roots are dug out in late autumn and stored in greenhouses or cellars over the winter period. In spring when the plant starts sprouting, it is divided into several plants up to the number of growing centres (Baumane, 1967; Fernandez and Curt, 2005).

Artichoke has a long history as a herbal medicine, it was used in folk medicine since Roman times (Christaki et al., 2012). Leaf extracts are used as a chloretic, hepatoprotective, anticarcinogenic, antioxidative, antibacterial, antifungal, antimicrobical, bile-expelling, cholesterol-reducing and urinative remedy in medicine. It also reduces occurrence of cardio-vascular disease and forms of cancer (Kolodziej and Winiarska, 2010; Colla et al., 2012; Pandino et al., 2011; Lombordo et al., 2015). Artichoke extracts also have stimulating properties on gastro-intestinal activity, blood-clotting time, capillary resistance and neutralizing effect on toxic substances in the human body (Kolodziej and Winiarska, 2010).

The edible part of the plant is immature inflorescence, commonly called capitula or head. It is consumed in the food throughout the world raw, boiled, steamed, or fried. Artichoke heads are characterized by low protein and fat, high content of minerals, vitamins, carbohydrates, inulin and polyphenolic compounds, which include mono- and

di-caffeoylquinic acid and flavonoids (Kolodziej and Winiarska, 2010; Pandino et al., 2013). S. Lombordo et al. refer to particular flavonoids apigenin and luteolin and their glycosides as widely distributed biologically active substances in globe artichoke (Lombordo et al., 2010). All these compounds have strong antioxidant properties, although their content varies between different artichoke varieties. In France 17 globe artichoke varieties (spring genotype biomass – leaves and floral stem) were analysed for total polyphenol content. The amount of polyphenols ranged between 0.72 and 36.44 g kg^{-1} dry mass (Ciancolini et al., 2013).

Other parts of globe artichoke can also be used for food purposes. From seed can be obtained oil, from roots - inulin. Artichoke can also be used as green forage for ruminant feeding and as natural rennet for traditional cheese making. Artichoke can be used also as energy source for solid biofuel or bioethanol (Fernandez et al., 2006; Ciancolini et al., 2013; Costa et al., 2014).

The aim of this literature survey is to give a review on globe artichoke biology and influence of agroecolocigal factors on yield and quality.

Materials and Methods

Monographic method has been used for this review. Scientific literature from different scientific journals all around the world has been used in it. Literature includes information from investigations performed in Romania, Greece, Italy, France, Brazil, Egypt, Iran – the countries well known as artichoke producers in the world. Data obtained in The Netherlands and Poland are included as well, representing countries close to the Nordic boundary of artichoke growing in the Europe.

Results and Discusion

Germination conditions

The International Seed Testing Association defines germination as the emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether it is able to develop further into a satisfactory plant under favourable conditions (Cone and seed…, 2005). Seed germination starts when seeds are provided with optimal amount of water and adequate temperature. Using pre-germination for more effective establishment of plants is quite popular for vegetable crops. Different substrates can be used for seed pre-germination: filter paper, sand, soil, compost, peat etc. Temperature and substrate are important factors influencing germination and germination capacity. Temperature determines germination capacity and rate, as well as breaking and induction of seed dormancy. The optimal germination temperature can vary between varieties of the same species. In Serbia the artichoke seed germination was tested on three different substrates: filter paper, sand and compost under three temperatures regimes: 16 h in the dark, at the temperature of 20 °C and 8 h under the light of 750 lux; constant temperature 20 °C; hardening for 10 days at 10 °C and continued the germination at 25 °C. Filtre paper was saturated with water and sand, and compost was provided with a sufficient amount of water to ensure optimal seed swelling. The highest seed germination was observed on filter paper (95.7%) and compost (96.2%), under constant temperature regime of 20 °C. First seedling counting was done after 7 days and final counting – after 21 days (Lekič et al., 2011). Another experiment has been carried out in Italy by S.A. Raccuia et al. (2004), where 8 wild cardoon (*Cynara cardunculus* L. var. *sylvestris* Lam.) population seeds were placed under salt stresses. Seeds were germinating in Petri dishes on Whatman paper in the NaCl and in the polyethylene glycol solution and placed in incubator in the dark at 20 °C. Results showed that in polyethylene glycol solution the germination was higher than in NaCl solution (Raccuia et al., 2004). This indicates a low abiotic stress resistance of artichoke germs. To prevent negative influence of different factors, the optimal pre-germination conditions for artichoke have to be ensured: suitable substrate, constant temperature regime (20 – 25 °C) and stable moisture.

Growing and development

Drought is one of the main abiotic stresses limiting plant growth and development in Southern countries. Limited water supply is a major factor influencing physiological and metabolic processes in plant. Water stress can significantly reduce plant height, shoot and root dry weight. In Iran significant differences between vegetative parameters were found when different irrigation intervals were applied on artichoke plants. After longer drought period reduced root and leaf vegetative parameters were observed. The total fresh weight with 3 day irrigation interval was 41.78 g, with 6 day irrigation interval - 33.48 g and with 12 day irrigation interval it was only 18.74 g (Tahna et al., 2014). S.V. Archontoulis with colleagues report that artichokes are tolerant to limited water conditions because they have a very deep root system, it can exceed even 5 metres (Archontoulis et al., 2010). However, in Egypt, S.A. Saleh (2012) with colleagues report that artichoke productivity is strongly influenced by the amount of irrigation water. Best plant growth, development and yield can be obtained if the optimal water supply is ensured – 75 to 100% from evaporation. In experiment with 3 different water irrigation levels (daily supplied 85, 100, and 115% from evaporation amount) the best result showed variant with 115% irrigation. Increased water amount positively influences plants vegetative parameters. The same tendency was observed also regarding the

total yield of heads (3960 g per plant) and marketable yield (3374 g per plant). The lowest total yield (3701 g per plant) and marketable yield (3145 g per plant) was harvested from artichoke plants with 85% irrigation from evaporation (Saleh et al., 2012).

G. Colla et al. (2012) observed that higher salinity in the nutrient solution reduces plant growth parameters such as leaf dry biomass and leaf number. In Poland, researchers also observed that irrigation, fertigation, and harvest date significantly influence vegetative parameters of one year old artichoke plants. Best results showed the variant with fertigation, where fresh weight of single plant was 401 g, in irrigation variant – 362 g and in control variant – 267 g at the first harvest time (in August). At the second harvest time (in October) plants were better developed, with a higher number of leaves, longer and wider leaves. Average fresh weight of single plant in fertigation variant was for 50.5% higher than in the control variant; in irrigation variant it was 32.4% higher than in control (Kolodziej and Winiarska, 2010). S. Hejazi (2013) with colleagues report that intensive use of chemical fertilization can reduce crop yield, but organic manure can improve soil properties. They observed that the planting date and fertilization did not significantly influence artichoke vegetative parameters. However, there is limited research about fertilization influence on artichoke growth (Hejazi et al., 2013). In Romania, an investigation with 3 Cynara cardunculus L. var. altilis DC (cardoon) cultivars was established. If two years of investigation are compared, better results were obtained in the second year, when there were more rainfalls and they positively influenced cardoon growing and development (Bolohan et al., 2013, 2014). In Italy an investigation was carried out with the aim to determine the influence of sowing date and plant density on the artichoke yield. Results showed that the best sowing time in Italy was summer, when the yield was about two times higher than for the plants sown in autumn. Plant density 1.7 – 2.5 plant per m^2 decreased the number of heads per plant if compared with plant density 1.25 plants per m^2, however, yield per unit of area increased (Elia et al., 1991). In Iran, 3 different sowing dates (April 19, May 5 and 20) were compared. The highest leaf area was observed for plants sown on the first date. Also a higher globe artichoke forage yield was observed in variant with the first sowing date – 0.93 kg m^2, in the second and third sowing dates, accordingly, 0.63 and 0.69 kg m^2 where harvested (Hosseinzadeh et al., 2013). Environmental and climatic factors, including photoperiod, light intensity, temperature, soil moisture, and fertility influence the leaf number changes in the growing season. Summarizing the findings of others on the influence of agroecological factors on artichoke growth we can conclude that optimal moisture which provides 85 – 100% from evaporation and applied fertilization before planting and during vegetation period are required.

Yield quality

Abiotic stresses have a significant influence on the nutritional quality of artichoke heads. They cause changes in the content of biochemically active compounds in the plants and following the artichoke heads. The balance between stress factors and factors ensuring plant development and growth leads to a high quality artichoke yield. Long term water stress can significantly reduce photosynthetic activity, antioxidant activity and vitamin C amount in artichoke plants. Proline accumulation in plants is a response to osmotic pressure of the soil during drought stress. After a longer drought period the content of proline in artichoke heads was higher. Chlorophyll and carotenoid play an important role in photosynthesis processes - the longer drought period, the lower content of chlorophyll pigments. K and Na content in plants is the most important indicator of ions in plant tolerance opposed to salinity and drought stress (Tanha et al., 2014). G. Colla (2012) with colleagues observed that higher salinity in the nutrient solution improved biochemical composition of artichoke and cultivated cardoon leaf. They observed higher content of ascorbic acid, total polyphenols, chlorogenic acid, luteolin (Colla et al., 2012). Polyphenols have been implicated in various aspects of plant growth, reproduction, response to abiotic stresses and pathogen challenges. G. Pandino (2013) with colleagues observed influence of air temperature and solar radiation on accumulation of polyphenols in globe artichoke in different harvest times. Low air temperature and solar radiation in February positively influenced polyphenol accumulation (13.04 g kg^{-1} of DM). In Poland, researchers observed that irrigation and fertigation positively influence flavonoids content in the plants. Higher amount of flavonoids was observed in the fertigation variant (Kolodziej and Winiarska, 2010). A. Salata (2012) with colleagues in Poland investigated two artichoke varieties and reported that weather conditions influence the content of chemical compounds in artichoke heads. Higher dry weight, L-ascorbic acid, raw fibre, total protein and sugars content in artichoke heads were in the year when higher temperature (19 °C in August and 13 °C in September) and rainfall (45 and 102 mm in August and September) during heads harvesting time were registered. Differences were observed also between cultivars, head fraction, and the stage of head development.

There have also been reported differences in biochemical composition between plant parts (Pandino et al., 2013). From 3 years data obtained by A. Salata

and colleagues mean content of L-ascorbic acid was higher in receptacle (14.54 – 15.82 g 100 g^{-1} fresh weight) than in head bracts (6.49 – 11.29 100 g^{-1} fresh weight). Total protein and total sugars also showed the same tendency – accumulation was more intense in receptacle than bracts. Crude fibre showed a contrary situation – in bracts it was two times more than in receptacle (Salata et al., 2012). In Italy it was observed that inner bracts and receptacles contain higher amount of polyphenols than floral stem and outer bracts, as well as, varied between genotypes (Lombordo et al., 2010). F. Fratianni (2007) with colleagues also studied polyphenolic content changes in different fractions of head. Inner bracts and receptacles contain a higher amount of polyphenols than intermediate bracts and outer bracts, although these observations significantly varied between genotypes (Fratianni et al., 2007).

In Portugal, it was found, that content of phenols depends on the plant part, physiological plant stage, and botanical variety. Cultivated artichokes are richer in phenols than wild artichokes. The highest phenol content is reported for artichoke leaves, but least – for stalks (Velez et al., 2012).

Conclusions

Artichoke growing is becoming more and more popular in the world, - expanding more and more towards North European countries, the growing area increases. Higher yield and quality of artichoke buds can be obtained by ensuring optimal growing conditions. Average air temperature (15 – 20 °C), irrigation (85 – 100% from evaporation) and fertilization level, optimal planting and harvesting time give notably better results. This indicates possibility to grow artichokes in Latvia. Investigations on proper agrotechnological solutions for artichoke growing in Latvian agroclimatic conditions have to be performed.

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THE EFFECTS OF DIFFERENT IRRIGATION SCHEDULINGS ON THE CUT FLOWER PERFORMANCE OF ORIENTAL LILY 'CASA BLANCA'

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Abstract
This study was carried out in 2012 to determine the effects of different irrigation intervals and irrigation water amounts on the cut flower performance of oriental lilium (*Lilium* L.) 'Casa Blanca' cultivated in greenhouse conditions. Different ratios of the plant water consumption (T_r) calculated using exterior radiation values of the greenhouse were applied to the plants as irrigation water. Irrigation water amounts varied between 199.3-589.0 mm, whereas measured plant water consumption (ETa) varied between 314.9-613.8 mm according to the treatments. Different irrigation water amounts and irrigation intervals had statistically significant effects on flower stem length, flower stem diameter, stem weight and number of flowers. In the study the stem length varied between 26.4-74.7 cm, stem weight varied between 72-175 g, stem diameter varied between 5.0-9.5 mm and the number of flowers buds varied between 3.6 and 8.0. The experimental treatment on which about 1.25 times of the potential plant water consumption calculated in two day intervals and the experimental treatment on which about 1.50 times of the potential plant water consumption calculated in four day intervals were selected as irrigation program.
Key words: Lilium, Plant water consumption, Irrigation interval, Deficit irrigation, Irrigation schedule.

Introduction

The worldwide cultivation area of ornamental plants in 2009 was about 1.519×10^3 ha, which reached 1.573×10^3 ha in 2012 by an increase of 3.57%. While outdoor plants are ranked highest among ornamental plants with 893×10^3 ha, it is followed by cut flowers and pot plants with 652×10^3 ha. The worldwide ornamental plant production value was € 44.530×10^3 according to 2009 and reached € 50.276×10^3 in 2012. The Europe is the continent with the highest ornamental plant production share in the world with 37% which is followed by Asia-Pacific with a ratio of 32%. When the world ornamental production values are evaluated according to the activity areas, cut flowers and pot plants are ranked highest with € 28.192 billion production value (Anonymous, 2010; Anonymous 2013). Ornamental plant production in Turkey has increased by 213% during the last fourteen years (1999-2013) and reached 4,512.57 ha in 2013. While outdoor plants are ranked number one in this production area with a share of about 72% (3242 ha), they are followed by cut flowers with a ratio of 25% (1105 ha). According to the 2013 year data of Turkey, ornamental plants export took place as $ 71.345×10^3. $ 35.000×10^3 of this amount was cut flowers, whereas $ 33.000×10^3 was outdoor plants (Kazaz et al., 2015).

Lilium L. is a monocotyledonous genus of the family Liliaceae and comprises over 80 species (Comber, 1947; De Jong, 1974). Lilium is a native of the Northern Hemisphere and mainly spreads over Asia, North America, and Europe (Lim et al., 2000). Lilies are used as cut flowers and pot plants and cultivated all year round (Treder, 2005). Cultivated lilies are classified into six sections, and the most important hybrid groups cultivated for cut flower production are Longiflorum, Asiatic, and Oriental hybrids (Lim et al., 2000). Lily ranks fourth in the sales ranking of cut flowers at Flora Holland auction, where the largest number of cut flowers is sold worldwide, after cut rose, chrysanthemum, and tulip (Anonymous, 2010). In Turkey, it ranks fifth in terms of production area (51.8 ha) after carnation (*Dianthus caryophyllus* L.), cut rose (*Rosa hybrida* L.), chrysanthemum (*Chrysanthemum morifolium* Ramat), and gerbera (*Gerbera jamesonii* Bolus) (Kazaz et al., 2015).

Photosynthesis, biomass production and dry matter play an important role on the visual quality of ornamental plants. One of the most important factors that affects this in plants is water potential (Jones and Tardieu 1998; Peri et al., 2003). Therefore, optimizing water management is an important step for optimizing water management in order to determine the effects of water on the growth period of ornamental plants as well as their visual quality (Carvalho et al., 2005; Lin et al., 2011). Hence, it is obligatory to consider different factors such as soil, plant and water resource in order to irrigate larger areas with the current water resources and to prevent possible efficiency and quality losses. In addition, plant water consumption values in sufficient and limited water conditions during the growth period should be known and water efficiency functions should be generated accordingly. These data can be acquired via many studies for each plant (Doorenbos and Kassam, 1979).

The objective of this study was to determine the effects of different irrigation intervals and irrigation water levels on the quality parameters of oriental hybrid lily cultivated under greenhouse conditions at the Mediterranean climate zone.

Materials and Methods

The research was conducted in a polyethylene-covered greenhouse of 255 m^2 (6 m x 42.5 m) on the Research and Application Farm of the Faculty of Agriculture at Süleyman Demirel University (lat. 37.83° N, long. 30.53° E, altitude 1020 m) in 2012. The soil texture of the greenhouse is clay loam, bulk density is 1.28-1.34 g cm^{-3}, field capacity is 23.19-27.64%, permanent wilting point is 7.63-8.11% and total available water holding capacity in 0-60 cm is 136.9 mm.

The irrigation applications were carried out via drip irrigation method. The dripper lateral space was 20 cm, whereas the dripper discharge was 2 L s^{-1} (Uçar et al., 2011). The soil water content in the root zone of the plant was measured by means of watermarks (Irrometer, Model; Watermark200SS, USA). Watermarks were placed in two different depths (15 and 45 cm) from the soil surface, with each experimental plot containing 2 watermarks. Potential plant water consumption was calculated using equations 1 and 2 developed by N. Katsoulas et al. (2006). Potential plant water consumption can be calculated via this equation using the radiation values outside the greenhouse.

$$T_r = r\,\zeta\,R_{Go} \quad (1)$$

$$\zeta = k_c\,\tau\,\alpha/\lambda \quad (2)$$

In the equation;

ζ: the coefficient depends on plant coefficient, greenhouse covered material, evaporation coefficient and latent heat coefficient, T_r: the calculated potential plant water consumption (Potential evapotranspiration), (mm), r: shading factor, (r=Ps/85<=1), R_{Go}: solar radiation amount in the irrigation interval considered, (kJ m^{-2}), k_c: plant coefficient, τ: greenhouse cover material solar radiation permeability ratio, (%), α: evaporation coefficient, λ: latent heat, (kJ kg^{-1}). The solar radiation values required for calculations were obtained from the nearby agricultural meteorology station (DAVIS, Model 'Vantage Pro II Plus', USA).

Different irrigation water amounts were determined on the basis of the potential plant water consumption (T_r) calculated with Equations 1 and 2. Different irrigation water amounts were computed considering the different rates (150%, 125%, 100%, 75%, 50%, and 25%) of the potential plant water consumption (T_r) calculated. Thus, in the study, 6 different water levels ($T_{r1}=T_r\times1.50$, $T_{r2}=T_r\times1.25$, $T_{r3}=T_r\times1.00$, $T_{r4}=T_r\times0.75$, $T_{r5}=T_r\times0.50$, $T_{r6}=T_r\times0.25$), 3 different irrigation intervals (I_1=2 days, I_2=4 days and I_3: once every 6 days) were used for a total of 18 irrigation schedules carried out as 3 replications according to the split plots experiment pattern. Plant water consumption was calculated using Equation 3 in accordance with the water budget (Kırnak et al., 2013);

$$ET = I + P - DP \pm RO \pm \Delta S \quad (3)$$

In the equation;

ET: plant water consumption (mm); I: irrigation water (mm), P: rain (mm), R: surface flow (mm), ΔS: change in water content at the root zone in mm. Since the experiment was carried out in greenhouse, P and, since irrigation was carried out by way of drip irrigation method, R was accepted as '0'. In cases when the reading limits of the soil moisture sensors were exceeded (199 kPa), soil samples were taken from the experiment plots and soil moisture content was determined by way of gravimetric methods.

The plant materials used for the experiment were Oriental hybrid lily, 'Casa Blanca'. Lily bulbs (18/20 cm in circumference) were planted on 25th of May with 20 × 15 cm spacing. The plants were harvested on September 15. Stem length, stem diameter, number of flower buds and stem weight parameters were examined.

Variance analysis was performed on the acquired data via MINITAB 16 computer software, LSD Multiple Comparison test was applied for the comparison of the averages using MSTAT-C computer software.

Results and Discussion

Plant water consumption and irrigation water amount: All of the irrigation water (121.3 mm) calculated for about 25 days was applied at the start of the experiment to all experiment plots to avoid any problems in the germination emergence of the bulbs, after the emergence was completed, irrigation water was applied to the experiment plots in the ratios given in the method section. The irrigation water amounts applied according to the experiment plots varied between 199.3-589.0 mm (Table 1). The highest water consumption in the study was measured at I_1T_{r1} (613.8 mm) and I_2T_{r1} (608.6 mm) for which 1.5 times of T_r was applied as irrigation water with 2 and 4 day intervals. This was followed by I_1T_{r2} (567.1 mm) and I_2T_{r2} (551.4 mm) for which 1.25 times the plant water consumption amount calculated was applied with 2 day intervals. However, the lowest water consumption was observed in I_3T_{r6} (314.9 mm). As can be seen in Table 1, application of the same amount of irrigation water with different intervals has resulted in variations of the measured plant water consumption. In general, higher plant water consumption values were obtained for plots to which irrigation water was applied at shorter irrigation intervals and higher irrigation water amounts. The reason for the high plant water consumption in plots with small irrigation intervals might be the fact that the soil

Table 1

Plant water consumption and irrigation water amount values in the treatments

Irrigation treatments	Irrigation water, mm			Plant water consumption, mm		
	IW_a	IW_b	IW_T	I_1	I_2	I_3
T_{r1}	121.3	467.7	589.0	613.8	608.6	592.3
T_{r2}		389.8	511.1	567.1	551.4	549.5
T_{r3}		311.8	433.1	510.9	498.2	496.7
T_{r4}		233.9	355.2	472.3	474.8	450.3
T_{r5}		155.9	277.2	411.2	398.4	390.1
T_{r6}		78.0	199.3	332.0	320.5	314.9

IW_a: The irrigation water amount applied to the experimental treatments before making a transition to scheduled irrigation (mm), IW_b: The irrigation water amount applied after making a transition to scheduled irrigation (mm); IW_T: Total irrigation water (mm), I_1: Irrigation interval 2-d, I_2: Irrigation interval 4-d and I_3: Irrigation interval 6-d.

Table 2

The results of variance analysis of the mean values of the investigated parameters

Variation Source	df	Mean Square			
		Stem length	Number flower bud	Stem diameter	Stem weight
Replication	2	1.53	0.1439	0.4096	150.1
Irrigation interval (I)	2	371.98**	0.2917	2.6924**	950.0**
Irrigation level (T_r)	5	2,178.22**	21.8519**	22.0682**	12,858.0**
T_r×I	10	29.59**	0.2826	0.1733	187.7**
Error	34	5.51	0.2804	0.3565	62.5
Total	54	1,2121.53	122.4883	130.3987	7,0491.5

** F-test significant at $p<0.01$, respectively, df: Degrees of freedom.

surface is always wet because it is irrigated more frequently.

Quality parameters: Different irrigation water amounts (T_r) had statistically significant effects on stem length, stem weight, stem diameter and number of buds per plant ($p<0.01$). Irrigation intervals had statistically significant effects ($p<0.01$) on the parameters other than the number of flowers per plant. Whereas T_r×I interaction was determined to be statistically significant with regard to stem length and stem weight at a ratio of 1%, it was not statistically significant for the number of buds and stem diameter. The significance of T_r×I interaction puts forth that the effect of increasing irrigation water differs according to irrigation intervals (Table 2).

The longest stem was observed in I_1T_{r2} (74.7 cm), I_1T_{r1} (73.7 cm) and I_2T_{r2} (73.4 cm). These three experiment plots were in the same group statistically. The shortest stems were determined in I_3T_{r6} (26.4 cm). When examined according to the irrigation water levels, the longest flowers were observed in T_{r1} (72.4 cm) followed by T_{r2} (68.1 cm), T_{r3} (62.1 cm), T_{r4} (52.5 cm), T_{r5} (45.4 cm) and T_{r6} (30.8 cm). The longest stems in terms of irrigation intervals were observed in I_1 followed by I_2 and I_3 plots (Table 3). Although increasing irrigation water amounts in I_2 and I_3 plots and the corresponding increase in the plant water consumption caused an increase in stem length, it caused a decrease in stem length in plots to which the irrigation water was applied with 2 day intervals. This decreasing might be due to excessive irrigation. When Table 3 is examined, it is observed that shorter stem lengths have been obtained from plots with the same amount of irrigation water applied but at less frequent irrigation intervals. As can be seen in Figure 1, polynomial relationships have been observed between stem length and irrigation water in I_1 and I_2, whereas linear relationships were observed in I_3 ($p<0.01$). Similarly, statistically significant polynomial relationships have been observed between plant water consumption and stem length in I_1 and I_2 ($p<0.01$), whereas the relationship in I_3 was linear ($p<0.01$). Thus, it can be stated that after a certain point, applying irrigation to the root zone of the plant increases plant water consumption but does not increase stem length. Similarly, it is observed that the increasing plant water consumption with increasing irrigation had no increase effect on stem length. It has also been emphasized by Harbaugh et al. (1982) that a general increase in applied irrigation water increases

Table 3

Mean values and significance groups of quality parameters of Oriental hybrid lily 'Casa Blanca'

Treatments	Stem length, cm			Average	Stem diameter, mm			Average
	I_1	I_2	I_3		I_1	I_2	I_3	
T_{r1}	73.7 ab	73.4 ab	70.1 bc	72.4 A	9.5	9.4	9.3	9.4 A
T_{r2}	74.7 a	70.5 bc	59.2 de	68.1 B	9.4	9.0	7.9	8.7 B
T_{r3}	68.6 c	62.6 d	55.2 f	62.1 C	7.4	7.1	6.8	7.1 C
T_{r4}	57.7 ef	54.2 f	45.4 gh	52.5 D	6.6	6.3	6.0	6.3 D
T_{r5}	47.7 g	43.4 h	45.2 gh	45.4 E	6.2	6.1	5.5	5.9 E
T_{r6}	32.5 i	33.6 i	26.4 j	30.8 F	6.0	5.6	5.0	5.6 F
Average	59.2 A	56.3 B	50.2 C		7.5 A	7.3 A	6.8 B	
$LSD_{0.01}$	LSD_I:1.59, LSD_{Tr}:2.249, LSD_{IxTr}:3.895				LSD_I:0.4045, LSD_{Tr}:0.572, LSD_{IxTr}:ns			
Stem weight, g					Number of flower buds, number			
T_{r1}	175 a	174 a	167 a	172 A	7.5	7.8	8.1	7.8 A
T_{r2}	173 a	169 a	141 b	161 B	8.0	7.9	7.8	7.9 A
T_{r3}	111 cd	115 c	119 c	115 C	7.1	7.5	6.9	7.2 B
T_{r4}	102 de	117 c	91 e-h	103 D	6.1	6.6	6.3	6.3 C
T_{r5}	95 e-g	97 ef	83 g-i	92 E	5.0	5.1	4.7	5.0 D
T_{r6}	86 f-h	81 hi	72 i	80 F	4.6	4.1	3.6	4.1 E
Average	124 A	126 A	112 B		6.4	6.4	6.2	
$LSD_{0.01}$	LSD_I:5.355, LSD_{Tr}:5.574, LSD_{IxTr}:				LSD_I:ns, LSD_T:0.5073, LSD_{IxTr}:ns			

*The difference among the averages is significant at 5% level, ns: not significance

stem length. It is stated in another study carried out on chrysanthemum that there are statistically significant polynomial relationships between plant water consumption and yield and flower length (Turan, 2013). Safi et al., (2007) have carried out a study on the effects of different irrigation water qualities on the quality parameters of the Elite, Prato and Pollyana type of Lilium in which it was observed that the stem lengths varied between 99.3-106.5 cm, 105.1-110.1 cm and 91.7-94.5 cm, respectively for wastewater, treated wastewater and the combination of these two types of water. Sirin (2011) and Kahraman (2014) have examined the quality parameters of lily at different growth media. Sirin (2011) states that the stem length varies between 42.16 and 45.12 cm, whereas Kahraman (2014) states that it is between 47.63-64.64 cm. The results obtained in this study are in accordance with Kahraman (2014), whereas they are not in accordance with those of Safi et al. (2007) and Sirin (2011). This is thought to be due to irrigation water quality, cultivation methods and environmental conditions. It is stated according to the Dutch Flower Auction criteria, flowers with stem lengths of 70 cm and above are classified as high quality (Anonymous, 2015). According to this evaluation, the flowers obtained from the I_1T_{r1} (73.7 cm), I_1T_{r2} (74.7), I_2T_{r1} (73.4 cm), I_2T_{r2} (70.5 cm) and I_3T_{r1} (70.1 cm) treatments meet the requirements in terms of quality. It can be stated that high quality flowers will be obtained from these programs if these irrigation schedules are applied.

In this study, stem weights were significantly affected by different irrigation intervals as well as irrigation water amounts ($p<0.01$) (Table 2). The highest stem weights were obtained from I_1T_{r1}, I_1T_{r2}, I_2T_{r1}, I_2T_{r2} and I_3T_{r1}. These combinations were in the same group statistically. However, the lowest stem weight was determined in I_3T_{r6}. In terms of irrigation water amounts, the highest stem weight was observed in T_{r1}, the lowest stem weight was observed in T_{r6}. All of the different irrigation water amounts were in different groups statistically. Although there was no statistically significant difference between I_1 and I_2 with the highest stem weight, there was a statistically significant difference between I_3 and I_1 and I_2 with the lowest stem weights. When the irrigation interval and irrigation water amount combinations were evaluated together, it was observed that in general stem weights were higher for treatments to which greater irrigation water as applied and which had shorter irrigation

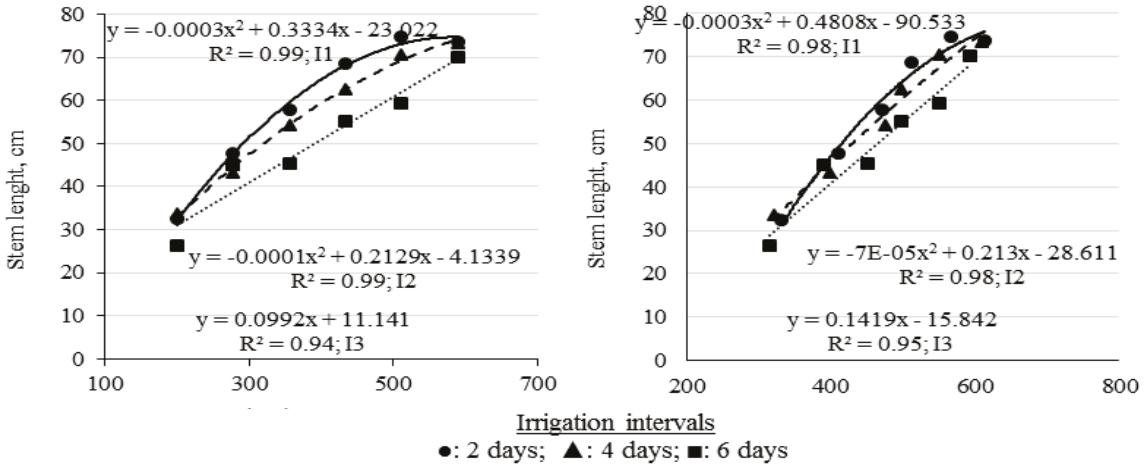

Figure 1. Relationship between stem length and plant water consumption and between stem length and irrigation water.

intervals. This can be interpreted by stating that similar to the stem length, high irrigation water application increases stem weight well.

Stem diameter is an important criteria for determining the strength of the stem. In terms of irrigation water amounts, the largest stem diameter was observed in T_{r1} treatment with 9.4 mm which was followed by T_{r2}, T_{r3}, T_{r4}, T_{r5} and T_{r6}. The stem diameters of these treatments were 8.7 mm, 7.1 mm, 6.3 mm, 5.9 mm and 5.6 mm, respectively. When the irrigation intervals were examined, the highest stem diameter was observed in I_1 with 7.5 mm which was followed by I_2 (7.3 mm) and I_3 (6.8 mm). The largest stem diameter was obtained in the I_1T_{r1} combination, whereas the smallest stem diameter was obtained from the I_3T_{r6} (5.0 mm) combination to which at the least amount of irrigation water was applied with an irrigation interval of 6 days (Table 3). Safi et al. (2007), determined the stem diameter to be between 7.7-10.4 mm. Kahraman (2014) also determined 6.4-7.5 mm under full irrigation conditions. Turan (2013), has stated that increasing water amounts and shorter irrigation intervals in chrysanthemum result in increased stem diameters. The stem diameter results acquired in this study were in accordance with the aforementioned literature data.

The increase of buds per stem in lily increases the attractiveness of the plant by giving it a more aesthetic look. Hence, the number of buds per stem is one of the most important quality criteria for lily marketing. When the number of buds per plant was evaluated in terms of irrigation water amount, it was observed that the highest number of buds has been obtained from T_{r2} plots with 7.9 number. This was followed by T_{r1}, T_{r3}, T_{r4}, T_{r5} and T_{r6}. The difference between T_{r1} and T_{r2} plots was not statistically significant, whereas the difference between other plots was statistically significant. When the number of buds was evaluated with respect to irrigation interval, the average number in I_1 and I_2 plots was 6.4 on average, whereas it was 6.2 for I_3 plots. Kahraman (2014) stated that the number of buds was between 2.25 and 3.55. It was observed that the number of buds was greater than that stated by Kahraman (2014). It is thought that this difference was due to the difference between cultivation conditions and climate differences.

Conclusions

The increase of irrigation water along with a decrease in irrigation interval in this study, which examined the effect of different irrigation water amounts and irrigation intervals on Oriental lily important for cut flowers sector, resulted in an increase in stem length, stem diameter, number of buds and stem weight. Given the stem length and stem weight, the experimental treatments in which the irrigation interval is 2 days and 1.5 times and 1.25 times the Tr are applied as irrigation water (I_1T_{r1} and I_1T_{r2}), and the treatment in which the irrigation interval is 4 days and 1.5 times the T_r is applied as irrigation water (I_2T_{r1}) can be used as irrigation schedules in Oriental lily cultivation. In case savings from irrigation water is desired, the treatment for which the irrigation water of 1.25 times that of T_r calculated with 2 day intervals (I_1T_{r2}) can be used, and if savings from irrigation labor is desired, the treatment for which an irrigation water of 1.50 times that of T_r calculated with 4 day intervals (I_2T_{r1}) can be used. When one of these schedules is used, Oriental lily growth can be carried out without any efficiency loss.

Acknowledgements

The bulbs which were used in this study were provided by Suleyman Demirel University Unit of Scientific Research Project.

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TESTING SAPROPEL (GYTTJA) AS SOIL AMENDMENT: ASSESSMENT OF PLANT GERMINATION AND EARLY SEEDLING DEVELOPMENT

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Abstract
Sapropel or gyttja are the terms that relate to specific water body sediments containing a high level of organic matter formed from the remains of water biota mixed with mineral components. One of the most promising utilisation ways of sapropel is agriculture and forestry where this natural material can be used as soil amendment to enrich soil fertility, neutralise acidity, improve water capacity and reduce phytoavailablity of excess of metallic elements. The aim of this study was to perform plant germination and early seedling tests using various sapropel samples and to reveal response of plant development depending on the type of sapropel to be used as soil amendment. Pure natural sapropel and sapropel/sand substrate of such types as peaty, organic-sandy, cyanobacteria, green algae and carbonatic sapropel, derived from four lakes of eastern Latvia, were tested. Seeds of cucumber *Cucumis sativus* and tomato *Solanum lycopersicum* as dicotyledons and perennial rye-grass *Lolium perenne* as monocotyledon were chosen for the experiment. Seed germination and early seedling tests were performed in PHYTOTESTKIT plates. Seeds were germinated in thermostat at a temperature of 26 °C for 7 days but early seedling development was achieved after 23-30 days (depending on plant species) under daylight conditions at a temperature of 20 °C. Developed radicles and hypocotyls were measured, shoots and roots were weighed. The obtained results showed a distinctive effect of applied sapropel type on the development of plants depending on species and substrate – substrate containing pure natural sapropel is effective for cucumber and perennial rye-grass, but not for tomato.
Key words: freshwater sapropel, gyttja, germination tests, *Cucumis sativus*, *Lolium perenne*, *Solanum lycopersicum.*

Introduction

The terms sapropel, gyttja or dy are used to designate specific organic-rich sediments formed in the bottom of water bodies under the anaerobic conditions from the remains of water biota mixed with mineral components. One of the most promising utilisation ways of this natural material is agriculture and forestry where sapropel can be applied as soil amendment (Kurzo et al., 2004; Stankevica et al., 2013).

Sapropel of different types and origin is found worldwide. For example, the Black Sea deep-water organogene-mineral sediments derived at a depth of more than 1500 m. This sapropel contains relatively low content of organic matter (about 3 g kg^{-1}) but it is rich in calcium minerals (CaO content 14-15 g kg^{-1}). It was detected that applications of such sapropel can reduce phytoaccessibility of metallic elements such as Cd, Pb and Zn in cereals (Angelova, 2008). Experiments with sapropel from the Black Sea showed that it can be applied for neutralization of strong organic acids in peat thus revealing potential for its use as acidity neutraliser in agriculture (Nikolov and Tringovska, 2014). Another example, freshwater sapropel samples derived from the Greater Chabyda Lake and Lake Khomustakh in Yakutia (Russia) were tested to be used as soil fertilizer and the results of the study revealed that in presence of sapropel cereal transpiration is accelerated, photosynthesis was more intensive as well as accumulation of dry matter increased (Myarikyanov et al., 1990). Also in the Baltic region the main interest is paid to the research of freshwater lake sediments. Shallow overgrown and overgrowing inland freshwater lakes usually are rich with sapropel sediments. It is estimated that lakes of the territory of Latvia contain 700-800 million m^3 of sapropel deposits, but counted together with resources settled in peatlands, total estimated sapropel reserves can reach 2 billion m^3 (Stankevica et al., 2014a).

Due to specific peculiarities and diversity of sediments a unified typology of sapropel has not been developed; however, K. Stankeviča and M. Kļaviņš (2013) have worked out the classification of sapropel types applicable for inland lakes. Taking into account the chemical and biological composition of sediments, various types of sapropel can be identified, for example, organic, calcareous, siliceous, cyanobacteria, green algae, peaty, mixed sapropel (e.g., organic-sandy). Moreover, specific types of sapropel can be detected due to various place specific sediment formation conditions (e.g., cyanophyceae-diatomaceous sapropel). Organic sapropel contains a higher content of nitrogen, while rich in phosphorus are organic and organic-calcareous sapropels (Myarikyanov et al., 1990; Liužinas et al., 2005; Stankeviča un Kļaviņš, 2013).

In general, the literature studies reveal that sapropel of different origin applied as soil amendment can improve mechanical structure and texture of soil, increase water balance and moisture capacity in soil, neutralize soil acidity as well as contributes to the balance of microelements and formation processes of humus. It is verified that sapropel applications on soils mutually with mineral fertilizers or alone can reduce toxicity and phytoaccessibility of metallic elements in soil and stimulates growth of plants (Myarikyanov et

Figure 1. Location of sapropel sampling sites in the eastern Latvia, Rezekne District: (A) Lake Pilcine, (B) Lake Pilvelis, (C) Lake Vevers and (D) Lake Padelis.

al., 1990; Яговкин, 2007; Angelova, 2008; Nikolov and Tringovska, 2014; Stankevica et al., 2014b). However, due to various types and origin of sapropel found in water bodies, systematic and profound studies are still needed to investigate specific effects of this material applied as soil amendment.

The aim of current study was to perform plant germination and early seedling tests using various samples of pure natural sapropel and sapropel/sand substratum, and to reveal the response of plant development depending on the type of sapropel to be used as soil amendment. Seeds of cucumber *Cucumis sativus* and tomato *Solanum lycopersicum* as dicotyledons and perennial rye-grass *Lolium perenne* as monocotyledon were chosen for the experiment to assess the germination rate, length of radicles and hypocotyls and weight of biomass (shoots and roots) at early seedling stage.

Materials and Methods

Sampling and analysis of sapropel

Sapropel samples of various types were derived from four small and shallow overgrowing freshwater lakes containing rich organic sediment layers, located in Rezekne district, Latvia (Figure 1).

Sapropel sampling cores in the lakes were carried out in certain points selected according to the lake characteristic description (Table 1) and preliminary data on rich organic sediment layers at the given location. Type of sapropel was defined using classification method of sapropel (Stankeviča un Kļaviņš, 2013). The lakes are inter-hilly inland water bodies with the origin of glacial type. Water surface area of each lake does not exceed 10 ha, and the sediment layers fill the lakes' trench for more than 80% (Pārskats par..., 1998).

Coring of sediments was done using a Russian-type peat sampler equipped with a 1.0 m long (d=10 cm) camera. Every cored sample was put into a non-transparent airtight plastic bucket with a lid and stored at a constant temperature (+4 °C) to achieve *in situ* conditions during the storage.

Five types of sapropel were identified among the collected sediments using sapropel type classification method based on the analysis of microfossils such as remains of vascular plants, algae and aquatic animals, fungi and moss (Кац и др., 1977; Bellinger and Sigee, 2011; Stankeviča un Kļaviņš, 2013).

Characteristic parameters, e.g., the content of organic matter and ash, concentration of carbon (C_{org}) and nitrogen (N_{org}), content of humic substances, pH and red-ox potential of sapropel samples, as well as the content of trace and major elements and diversity of microorganisms were detected in sapropel samples applying developed methodologies (Tan, 2005; Heiri et al., 2001; Stankevica et al., 2015).

Preparation of germination and early seedling tests

Germination and early seedling tests were carried out in PHYTOTESTKIT plates. The PHYTOTESTKIT microbiotest is used to detect changes of germination and early growth development of plants in substrates or contaminated soils, in comparison to the germination and growth rate in a control substrate or reference soil (Baran and Tarnawski, 2013; Phytotestkit for…, s.a.). Tests were performed using seeds of cucumber *Cucumis sativus*, tomato *Solanum lycopersicum* and perennial rye-grass *Lolium perenne* which were

Table 1

Description of derived sapropel samples and sampling sites of sapropel

Code of sample	Description of sapropel sample	Coring coordinates	Description of lake				
	Type	Thickness of layer*, cm	Sampling depth**, cm		Name	Area, ha	Mean water depth, cm
A	Organic-sandy	270	150-180	56°39'45,21" N 27°17'31,40" E	Pilcine	7.0	150
B1	Peaty	60	10-40	56°28'33,23" N 27°7'45,64" E	Pilvelis	9.9	0.90
B2	Cyanobacteria	190	200-230				
C	Green algae	n.	0-30	56°15'53,6" N 27°04'14" E	Vevers	7.5	190
D1	Cyanobacteria	90	110-140	56°14'9,83" N 27°21'1,88" E	Padelis	3.5	150
D2	Carbonatic	280	320-350				

* Fixed thickness of the layer of certain sapropel type at the sampling site; for sample C layer thickness of green algae sapropel was not specified due to the lack of detailed biological composition data of sediment core.
** Measured from the upper layer of sapropel.

obtained from a local seed producer and distributor in Latvia. Germination rate of seeds was determined not lower than 99%.

The current study involved investigation of pure natural sapropel, sapropel/sand mixture at various concentration based on organic matter content, and processed sapropel (after standard heat treatment at 101.325 kPa, 121 °C, 15 min.), but as a control substrate were used pure quartz sand and indifferent cotton material pads saturated with deionised water. In addition, as secondary control, tap water was used. PHYTOTESTKIT plates were filled with sapropel or sapropel/sand mixture (90 cm^3 in each plate) in three replicates. Ten seeds of each plant species were placed in appropriate manner directly onto the substrate and closed with the transparent plate cover according to the PHYTOTESTKIT bench protocol (Phytotestkit for…, s.a.). All the test plates were fastened vertically in a cardboard holder. Incubation process was accomplished in thermostat at a constant temperature of 26 °C for 7 days, but early seedling development was achieved after 23-30 days (depending on plant species) under natural daylight conditions at temperature 20 °C. Development stage of plants was recorded digitally. Developed radicles, hypocotyls and shoots were measured in stored files by using Adobe Photoshop software. The obtained data allowed the estimation of effects of various types of sapropel on seed germination, survival of seeds, length of radicles and hypocotyls. At the end of the tests, plants were carefully extracted from the substrate; roots and shoots were separated and rinsed with deionised water. Fresh biomass of shoots and roots was weighed on analytical scales and dry mass was estimated after drying the plants at temperature 40 °C for 72 h.

Statistical analysis of data was performed using MS Excel by calculation of routine statistical parameters. Calculated mean values from each experimental set were used for further data analysis; standard deviation is indicated in the figures.

Results and Discussion

Characterization of sapropel samples

Analysis of sapropel samples allowed determination of parameters significant for its use as soil amendment. It was detected that peaty sapropel (B1) from Lake Pilvelis was the richest in organic matter (902 g kg^{-1}) and organic carbon (538 g kg^{-1}), while to carbonatic sapropel (D2) from Lake Padelis a higher amount of ash (769 g kg^{-1}) and a higher value of active acidity (pH 6.66) can be attributed, as well as the lowest value of red-ox potential (-24 mV) among all sapropel samples. Organic-sandy sapropel (A) from Lake Pilcine was richer in humic substances (126 g kg^{-1}) counted of organic matter content. Cyanobacteria sapropel sample (B2) from Lake Pilvelis can be characterized with the lowest active acidity (pH 5.35) and the highest red-ox potential (47.55 mV) among all samples. However, the relation between red-ox potential of sapropel samples and

Table 2

Characteristic parameters of sapropel samples

Code	Type of sapropel	Average content of component, g kg^{-1}					Active acidity, pH	Red-ox potential, mV
		Organic matter	Ash	N_{org}	C_{org}	HS in OM*		
A	Organic-sandy	599	401	31	436	126	6.37	-7.75
B1	Peaty	902	98	30	538	90	5.96	14.05
B2	Cyanobacteria	857	143	39	504	39	5.35	47.55
C	Green algae	863	133	43	503	13	5.98	13.10
D1	Cyanobacteria	931	69	37	479	18	6.53	-17.00
D2	Carbonatic	231	769	14	322	8	6.66	-24.00

* Content of humic substances in organic matter

other characteristic properties should be studied further. The determined characteristic parameters of all sapropel samples are summarized in Table 2.

Plant germination intensity and effects on radicles and hypocotyls

The plant germination intensity was assessed by measuring hypocotyls and radicles of tested plant species (cucumber, tomato and perennial rye-grass) on the 5th to 7th day after the beginning of the experiment. Taking into account the measurements on the 7th day, the obtained results revealed that for cucumber and tomato the growth of radicles in natural sapropel was reduced in comparison with the control (Figure 2). But it did not indicate that radicles were of weak development because radicles grew in sapropel, if compared with the control, were shorter but thicker and with strongly developed lateral roots that can be associated with the plant growth specifics in organic-rich substrates where nutrients are available in excess. Results showed that perennial rye-grass was the only plant among the tested species for which the growth in lengthwise of radicles in natural sapropel was more intensive than in the control that can be explained with specifics of *Poaceae* sp. root system development which spreads widely and penetrates deeply in soil (Rich and Watt, 2013).

Measurements of hypocotyls on the 7th day after the beginning of the experiment indicated that the most intensive development of shoots for plants germinated in natural sapropel can be attributed to cucumber for which the length of hypocotyls increased up to 50% in comparison with control (Figure 2). The experiment was supplemented with plant germination in a substrate saturated with tap water. In comparison with the control, the results were similar for cucumber and tomato, but slightly higher for perennial rye-grass.

For tomato plants, the length of hypocotyls did not significantly differ from the control, while for perennial rye-grass the length of hypocotyls slightly increased. It

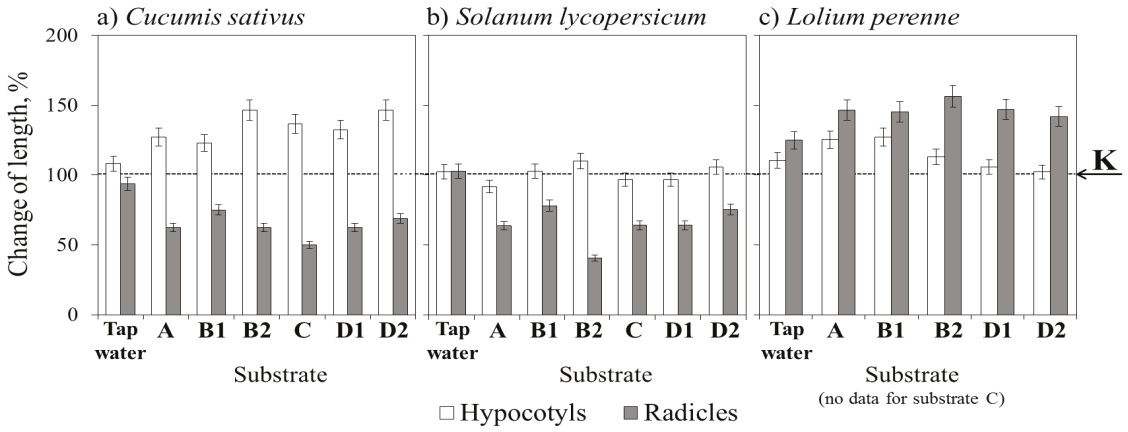

Figure 2. Change of length of hypocotyls and radicles of tested plants germinated in natural sapropel substrate on the 7th day after the beginning of the experiment; type of sapropel: (A) organic-sandy, (B1) peaty, (B2) and (D1) cyanobacteria, (C) green algae, (D2) carbonatic sapropel, according to Tables 1 and 2. Control line (K) is indicated as 100% for length of both, hypocotyls and radicles, based on the results from plant germination tests in control substrate (cotton material pads saturated with deionised water).

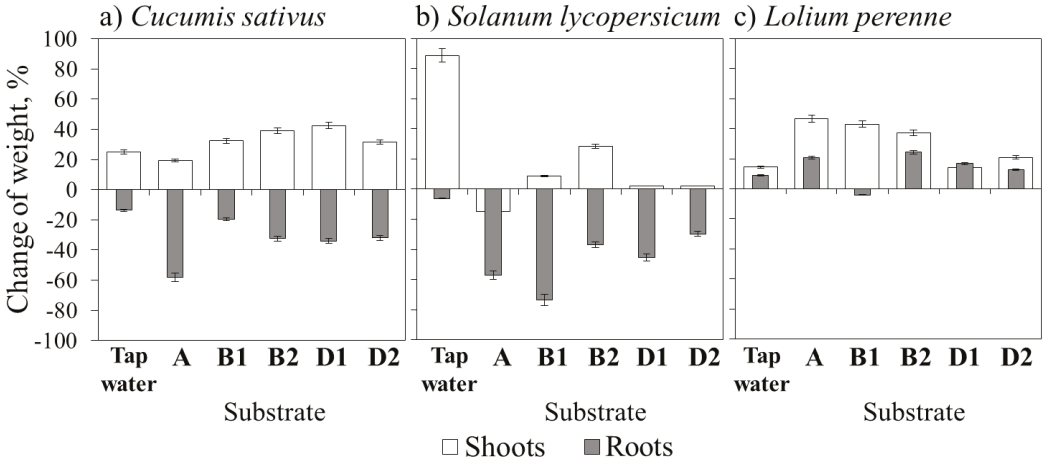

Figure 3. Change of dry weight of shoots and radicles of plants grown in natural sapropel substrate in comparison with control samples; type of sapropel: (A) organic-sandy, (B1) peaty, (B2) and (D1) cyanobacteria, (D2) carbonatic sapropel, according to Tables 1 and 2.

can be associated with preferences for specific growth conditions of tomato plants, e.g., light soil such as peat with perlite is optimal as well as adjustment of pH to 5.6 in substrate is required (Яговкин, 2007), while natural sapropel is of sticky consistence with a high moisture level that should be taken into account if sapropel is used as soil amendment.

It can be noted that among the impact of the tested natural sapropel samples on selected plant species there were no remarkable statistically significant distinctions observed, but differences were obvious among the response of plant species, thus suggesting the need for more specific experiments to be conducted.

Influence of sapropel type on plant biomass

At the end of the experiment, at the early seedling stage of plants, changes in plant biomass in comparison with control were assessed. The results of samples grown in natural sapropel revealed that only for perennial rye-grass the increase in weight of radicles is detectable, while among all tested species the most intensive decrease of root weight was observed for tomato plants (Figure 3). For cucumber and perennial rye-grass, the increase of weight of shoots was up to 40-50%, but for tomato the weight of shoots increased significantly only for samples grown in cyanobacteria sapropel (B2) and slightly for samples grown in peaty sapropel (B1).

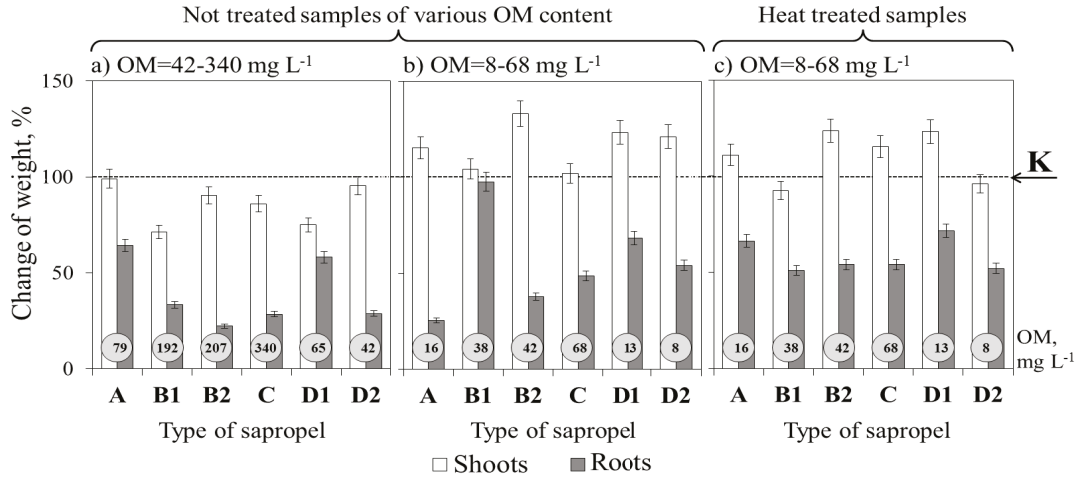

Figure 4. Change of dry weight of shoots and roots of cucumber *Cucumis sativus* germinated in sapropel/sand substrate with various content of organic matter (OM) in comparison with control samples; type of sapropel: (A) organic-sandy, (B1) peaty, (B2) and (D1) cyanobacteria, (C) green algae, (D2) carbonatic sapropel, according to Tables 1 and 2. Control line (K) is indicated as 100% for weight of both, shoots and roots, based on the results from plant early seedling tests in control substrate (pure quartz sand saturated with deionised water).

Certain conditions of growth are of great importance for each plant species as diverse inhibition or stimulation effects of various concentrations of sapropel containing substrates on various plants were detected also in other studies. For example, the use of 10% mineral-enriched sapropel-containing organic fertilizer for substrate enrichment significantly stimulated the growth of radicles, while a higher concentration was inhibiting the development of radicles in some plant species (Grantina-Ievina et al., 2014).

The use of sapropel/sand mixture of various organic matter content revealed that optimum concentration of sapropel can be adjusted to gain the effect of biomass increase. The results revealed that if a higher sapropel concentration based on organic matter was used for germination tests, the growth of cucumber was inhibited in comparison with the samples of lower sapropel organic matter content (Figure 4, a versus b and c). Among the possible reasons for these differences, the size of particles of the organic matter of sapropel can be mentioned, on average the fraction is less than 100 μm of size (Stankevica et al., 2015). Smaller size of particles in substrate can affect the productivity in a way that substrate becomes less aerated and pores are blocked for air and element exchange.

Heat treatment of sapropel was applied to determine the influence of microorganisms on plant germination and development. The results of processed sapropel, which was assessed having negligible microbial activity after the heat treatment at certain conditions, showed that at some level more homogenous development of root system of cucumber can be achieved in comparison with the use of not processed sapropel samples (Figure 4, c). Overall, the absence of microorganisms can inhibit or stimulate development of plants depending on the type of sapropel added. In this case quite similar results to non-processed samples were gained, thus suggesting that presence of microorganisms in substrate is not among the key parameters in the assessment of sapropel used as soil amendment.

Conclusions

Sapropel is a sophisticated mixture of minerals and organic matter with high water saturation, capable to support the fundamental requirements for sustainable use as soil amendment for fertilizing. In order to reach the best application efficiency, careful preliminary analysis of sapropel as well as knowledge on agrochemical properties important for cultivated species in amended soil are of great importance.

The current study revealed that the effect of pure natural sapropel and sapropel/sand mixture used as substrate on plant germination and early stage development was assessed as species-specific, e.g., substrate containing pure natural sapropel is effective for cucumber and perennial rye-grass, but not for tomato. The use of pure natural sapropel for soil enrichment is not the most effective, thus sapropel dilution, pre-treatment or processing should be applied prior the application into soil to adjust pH, moisture content, content of organic matter. The use of sapropel/sand mixture of various organic matter content revealed that the optimum concentration of sapropel can be adjusted to gain the effect of plant biomass increase which is an important matter for agriculture and thus further studies are important. However, the current study will be developed by more detailed statistical analysis among the sapropel characteristic parameters, as well as the macro-nutrient study will be performed.

Acknowledgements

This study was supported by the European Social Fund Project 'Interdisciplinary Team of Young Scientists for Assessment and Restoration of Soil Quality and Usage Potential in Latvia' No.2013/0020/1DP/1.1.1.2.0/13/APIA/VIAA/066.

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MICROBIOLOGICAL CHARACTERISTICS AND EFFECT ON PLANTS OF THE ORGANIC FERTILIZER FROM VERMICOMPOST AND BAT GUANO

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Abstract
There is an increasing demand in the development of new and better types of organic plant fertilizers. The aim of the present study was to evaluate if the beneficial effect of vermicompost on plant growth and development could be further promoted by adding different amounts of bat guano using two model species under controlled conditions, as well as to assess the microbiological characteristics of bat guano and soil after its application. The study was performed at the Faculty of Biology, University of Latvia, during 2013 and 2014. The amount of bacteria was significantly lower in guano in comparison to vermicompost samples. No actinobacteria were present, but yeasts were found in the guano sample. Soil fungal populations after the application of organic fertilizer from vermicompost and bat guano were dominated by potentially plant growth promoting fungi *Trichoderma* and *Mortierella*. However, at increased guano concentration (300 g kg^{-1}) the proportion of potentially plant pathogenic fungi significantly increased. Addition of bat guano to vermicompost fertilizer significantly enhanced the positive effect of the fertilizer on growth and development of winter rye (*Secale cereale* L.) and potato (*Solanum tuberosum* L.) plants.
Key words: bat guano, microbiological diversity, organic fertilizer, plant growth, vermicompost.

Introduction

The contribution of organic farming in agricultural production in Europe and other parts of the world is continuously increasing. Organic fertilizers not only provide the necessary nutrients but also positively affect overall soil fertility, and can be efficiently used in conventional agriculture as a constituent of integrated fertilization systems (Saleque et al., 2004; Mottaghian et al., 2008). Therefore, there is an increasing demand in the development of new and better types of organic plant fertilizers. Vermicompost, produced by a corporate activity of earthworms and their associated microorganisms, represents a promising type of organic fertilizer. Biological aspects of vermicompost application as a rich source of plant-available minerals and efficient plant growth-promoting agent have been recently studied (Ievinsh, 2011; Grantina-Ievina et al., 2013; Karlsons et al., 2015). However, vermicompost preparations usually are not balanced in respect to their mineral nutrient composition (Srivastava et al., 2012; Karlsons et al., 2015). Consequently, other types of organic fertilizers can be used to improve the mineral balance of the resulting fertilizer mixes.

A possible candidate for organic fertilizer mixes can be bat guano, representing highly mineralized faeces of bats, certified for organic farming (Sridhar et al., 2006). In contrast to vermicompost, the studies evaluating the positive effect and possible mechanism of action of bat guano on plants have been extremely scarce until recently (Sridhar et al., 2006; Bhat et al., 2013; Shetty et al., 2013; Tasci et al., 2013; Almohammedi et al., 2014; Sothearen et al., 2014). There is no information available, though, on microbiological characteristics of bat guano-based fertilizers.

The aim of the present study was to evaluate if the beneficial effect of vermicompost on plant growth and development could be further promoted by adding different amounts of bat guano using two model species in controlled conditions, and to assess the microbiological characteristics of bat guano and soil after its application.

Materials and Methods

The study was performed at the Faculty of Biology, University of Latvia, during 2013 and 2014. Quantification of bacteria, *Escherichia coli* and coliforms, enterococci, cultivable filamentous fungi and yeasts, and potentially pathogenic fungi to humans and animals was performed as described previously (Grantina-Ievina et al., 2013). The number of actinobacteria was estimated on Luria-Bertani agar with the following content per 1 l: peptone 10 g (*Pastone*, BIO-RAD, France), yeast extract 5 g (*Pastone*, BIO-RAD, France), D-glucose 1 g (Penta, Czech Republic), NaCl 10 g (*Sodium Chloride pure p.a.*, *Chempur*, Poland), agar 20 g (*Agar Bios Special LL*, Biolife, Italy) (Hamaki et al., 2005). Potentially pathogenic bacteria and fungi were assessed only in guano and vermicompost samples. Genera of cultivable filamentous fungi were determined after 10 days of incubation on Rose Bengal and Mycosel agar, and after subculturing on Malt extract agar (Biolife Italiana S.r.l., Italy) according to the morphological characteristics and light microscopy results. The soil was analyzed only from the winter rye (*Secale cereale* L.) experiment.

Soil from organic potato (*Solanum tuberosum* L.) field was used as a growth substrate in the present study. Analysis of total (extracted in aqua regia) and plant-

available (extracted in 1 M HCl) concentrations of mineral nutrients was performed by standard methods at the Laboratory of Plant Mineral Nutrition, Institute of Biology, University of Latvia (Grantina-Ievina et al., 2014a; Karlsons et al., 2015). Vermicompost was produced from starchless potato pulp and grass by SIA GAHA, bat guano was produced in Madagascar and obtained from Guanomad Europe S.A. Basic characteristics of soil, vermicompost and bat guano used in the present experiments are shown in Table 1. The soil used for organic potato production had acidic pH and relatively high P content together with deficiency in N, K, Ca, Mg, S, Cu, and B. Comparison of plant-available nutrient concentrations in vermicompost and bat guano samples revealed that vermicompost had a higher amount of K and Mg, while bat guano was especially rich in P, Ca, S, Mn, Zn, Cu and Mo. On the downside, guano had a relatively high level of plant-available Na and Cl and extremely high total level of soluble salts as indicated by electrical conductivity of 53 mS cm^{-1}. The degree of mineralization was higher for bat guano (87.3%) in comparison to that of vermicompost (43.5%).

Organic fertilizer was prepared from vermicompost with different amounts (100, 200 and 300 g kg^{-1}) of bat guano, as regular (R) or pelleted (P). Growth substrate for treatments was prepared from soil adding different amounts of fertilizer and thoroughly mixed. For vegetation experiments, winter rye cv. 'Kier' (seed) and potato cv. 'Laimdota' (minitubers from tissue culture-propagated soil-grown plants) were used. Plants were grown in 8 × 8 × 12 cm plastic containers. For winter rye (13 treatments; Table 2), 9 seeds were sown in each container and after 2 weeks thinned to 4 uniform plants. The experiment lasted 5 weeks. Five containers per treatment were used. For potato, 17 treatments were used, consisting of control (soil only) and 1.25, 2.5, 3.75, 5 g of fertilizer per container with 0, 100, 200 and 300 g kg^{-1} of guano supplement for each dose. Tubers were planted individually, with 7 containers per treatment. The experiment lasted for 9 weeks. Substrate was adjusted to 65% water holding capacity and maintained throughout the study with deionized water. Containers were randomly arranged in a growth cabinet at 22/15 °C (day/night) temperature, relative humidity 60%. Illumination was provided by fluorescent lamps at 150 µmol m^{-2} s^{-1} of photon flux density of photosynthetically active radiation, 8/16 h dark/light cycle. Plant height was monitored throughout the experiment. At the end of the cultivation period, both fresh and dry mass of roots and shoots were measured.

Leaf chlorophyll content for winter rye plants was measured using a hand-held chlorophyll meter SPAD-502 (Konica-Minolta Corporation, Japan). For every treatment, the second leaf from five plants was measured with five readings along the leaf.

Table 1

Characteristics of vermicompost, bat guano and soil samples used in the present study in comparison to optimum level of minerals for cultivated plants (Osvalde, 2011)

Characteristic	Vermicompost	Bat guano	Soil plant-available[b]	Optimum for cultivated plants[b]		
	Total[a]	Plant-available[b]	Total[a]	Plant-available[b]		
N	20800	910	10600	882	76	120
P	4900	4578	59300	39240	447	60
K	12400	12400	3000	1250	120	150
Ca	16400	13100	133200	73500	720	800
Mg	5500	3650	3700	2120	70	50
S	3000	250	32500	2400	11	50
Fe	2940	775	6800	800	805	30
Mn	250	236	1840	1250	130	1.5
Zn	74	64	1080	650	4.95	1
Cu	11	4.5	280	10.5	2.0	0.5
Mo	1.9	0.04	–	0.98	0.07	0.02
B	14	7.4	–	6.2	0.2	0.2
Na	320	290	3300	2400	7.5	–
Cl	–	800	–	16750	0	–
Organic matter (%)	–	46.5	–	12.7	–	–
pH (units)	–	7.67	–	6.60	4.86	–
EC (mS cm^{-1})	–	24.8	–	53.1	–	–

[a]Mineral concentration expressed as mg kg^{-1}
[b]Mineral concentration expressed as mg L^{-1}

Table 2

Experimental treatments used in the experiment with winter rye plants

Treatment No.	Type of fertilizer[a]	Vermi-compost[b]	Guano[b]	Dose[c]	Treatment No.	Type of fertilizer[a]	Vermi-compost[b]	Guano[b]	Dose[c]
1	–	–	–	–	8	R	800	200	2.5
2	R	1000	0	2.5	9	R	800	200	5
3	R	1000	0	5	10	P	800	200	5
4	P	1000	0	5	11	R	700	300	2.5
5	R	900	100	2.5	12	R	700	300	5
6	R	900	100	5	13	P	700	300	5
7	P	900	100	5	–	–	–	–	–

[a]R, regular; P, pelleted
[b]Amount of respective fertilizer expressed as g kg^{-1}
[c]Dose of fertilizer expressed as g per container

The significance of differences between means was determined by the Tukey-Kramer test at the $\alpha = 0.05$ level. Correlation analysis was performed with *Excel* (Microsoft, USA). Significance was evaluated at $p < 0.05$ level. Both Pearson correlation coefficients (r) and determination coefficients (R^2) were calculated.

Results and Discussion

The data about the microbiological content of bat guano and vermicompost samples used in the experiments are given in Table 3. The total amount of bacteria was significantly lower in guano in comparison to vermicompost samples, but the number of bacterial coliforms was higher in guano. *E. coli* and enterococci were not present in the analyzed guano and vermicompost samples. No actinobacteria that are characteristic component of organic matter-containing products were present in the guano sample. However, relatively high number of yeasts was present in guano.

In the winter rye experiment moderate and weak correlation, respectively, was estimated that the total number of bacteria increased by increased content of regular vermicompost ($r = 0.56$, $p > 0.05$) and smaller amount of guano ($r = 0.27$, $p > 0.05$) (Fig. 1). The highest number of actinobacteria was detected at 200 and 300 g kg^{-1} of guano [ranging from $(1.06 \pm 0.90) \times 10^5$ to $(1.18 \pm 1.07) \times 10^6$ *vs.* $(5.23 \pm 4.56) \times 10^4$ – $(5.20 \pm 2.71) \times 10^5$] that can be explained by the ability of these bacteria to tolerate high salt concentrations (Keshri et al., 2013), although the guano itself did not contain actinobacteria. The correlation coefficients (r) between the content of guano and actinobacteria were 0.47 in the regular vermicompost treatments and 0.49 in the pelleted vermicompost treatments, indicating moderate correlation. Statistically non-significant differences were observed regarding the total number of cultivable filamentous fungi and yeasts. In the treatments with 300 g kg^{-1} of guano, a higher number of potentially plant pathogenic fungi (members from genera *Verticillium*, *Acremonium*, *Fusarium*, *Thielaviopsis*, *Trichocladium*) was detected (on average 40%) in soil in comparison with the control

Table 3

Microbiological diversity of guano, vermicompost and soil samples, CFU g^{-1} dry mass

Group of microorganisms	Guano	Regular vermicompost	Pelleted vermicompost	Soil
Bacteria	$(1.99 \pm 0.88) \times 10^6$	$(1.20 \pm 0.08) \times 10^7$	$(4.89 \pm 0.18) \times 10^7$	$(4.83 \pm 0.91) \times 10^6$
Coliforms	$(4.24 \pm 0.45) \times 10^4$	$(2.78 \pm 1.18) \times 10^4$	$(2.07 \pm 0.91) \times 10^4$	Not detected
Yeasts	$(6.64 \pm 0.33) \times 10^2$	0	0	0
Actinobacteria	0	$(1.39 \pm 0.79) \times 10^7$	$(1.06 \pm 0.07) \times 10^8$	$(1.77 \pm 0.23) \times 10^6$
Filamentous fungi	$(1.92 \pm 0.28) \times 10^3$	$(1.68 \pm 0.22) \times 10^5$	$(1.03 \pm 0.46) \times 10^5$	$(7.03 \pm 2.19) \times 10^4$
Represented filamentous fungi	*Penicillium* spp., *Mortierella* spp., *Mucor* spp.	*Aspergillus* spp., *Penicillium* spp., *Mucor* spp., *Scopulariopsis brevicaulis*	*Pseudallescheria* spp., *Aspergillus* spp., *Acremonium* spp., *Sporothrix* spp., *Penicillium* spp., *Mortierella* spp., *Mucor* spp.	*Penicillium* spp., *Mortierella* spp., *Mucor* spp., *Paecilomyces* spp., *Trichoderma* spp.
Potentially human pathogenic fungi	0	$(9.04 \pm 1.80) \times 10^3$	$(3.62 \pm 0.86) \times 10^4$	Not detected
Represented potentially human pathogenic fungi	0	*S. brevicaulis*	*Aspergillus fumigatus*, *Geotrichum* spp., *S. brevicaulis*	Not detected

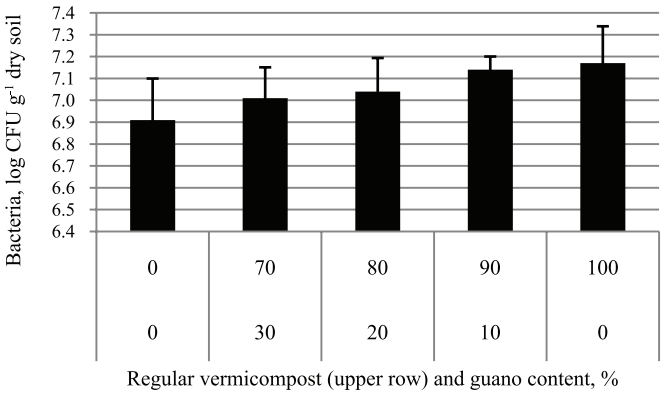

Figure 1. Relationship between the total number of bacteria in growth substrate and content of regular vermicompost and guano in the winter rye experiment.

Table 4

Amount of *Trichoderma* and *Mortierella* (%) from all filamentous fungi in the substrate of the winter rye experiment

Treatment[a]	*Trichoderma*	*Mortierella*	Total	Treatment[a]	*Trichoderma*	*Mortierella*	Total
1	31.34	9.47	40.81	8	40.69	38.63	79.32
2	62.31	0.56	62.88	9	63.76	33.07	96.83
3	45.97	40.59	86.56	10	60.07	6.31	66.39
4	33.00	9.92	42.92	11	10.98	18.67	29.65
5	43.33	56.68	100.00	12	51.77	21.52	73.29
6	85.64	12.31	97.95	13	45.59	3.08	48.67
7	45.18	22.01	67.19	–	–	–	–

[a]Treatments are the same as designated in Table 2

without any fertilizer (8%) and treatments with less guano content (5%) or only vermicompost (20%). However, in the microbiological analysis of guano only plant growth promoting and saprophytic fungi were detected (Table 3). In general, the soil fungal populations were dominated by potentially plant growth-promoting fungi *Trichoderma* and *Mortierella* from 16 to 100% of all fungi (Table 4), and the percentage of them was increased in the treatments with 1000, 900 and 800 g kg^{-1} of regular vermicompost in comparison to the pelleted vermicompost treatments and treatments with 30 g kg^{-1} of guano.

Fungi from genus *Mortierella* are often found in different types of organic fertilizers, e.g., vermicompost from manure and plant residues (Anastasi et al., 2005) and conventional compost (Ryckeboer et al., 2003). Most importantly, *Mortierella* spp. produce arachidonic acid with potentially antagonistic activity against plant pathogenic fungi (Eroshin and Dedyukhina, 2002; Fakas et al., 2009; Grantina-Ievina et al., 2014). Also, *Trichoderma* spp. are well-known plant biocontrol agents (Cordier et al., 2006). On the other hand, increasing the proportion of bat guano up to 30% in the organic fertilizer composition significantly increased the number of potentially pathogenic plant fungi, evoking serious concerns in respect to plant protection. Evidently, as these fungi were not initially present in the guano samples, the use of organic fertilizers with a relatively high content of living microorganisms can lead to changes in soil microbiological diversity through the interaction with native microorganisms.

Growth and development of winter rye plants were significantly promoted by the addition of organic fertilizer to soil. Both fresh and dry mass formation in above-ground parts was enhanced 1.5 to 2.0 times by vermicompost application alone (Fig. 2). Addition of bat guano to the fertilizer further increased plant biomass production and dry matter accumulation up to 500 and 400%, respectively, in comparison to soil-only control plants. There was no significant difference between the application of regular vs. pelleted type of fertilizer. A positive effect of vermicompost and bat guano on physiological status of rye plants was indicated by an increase in leaf chlorophyll content (Fig. 3).

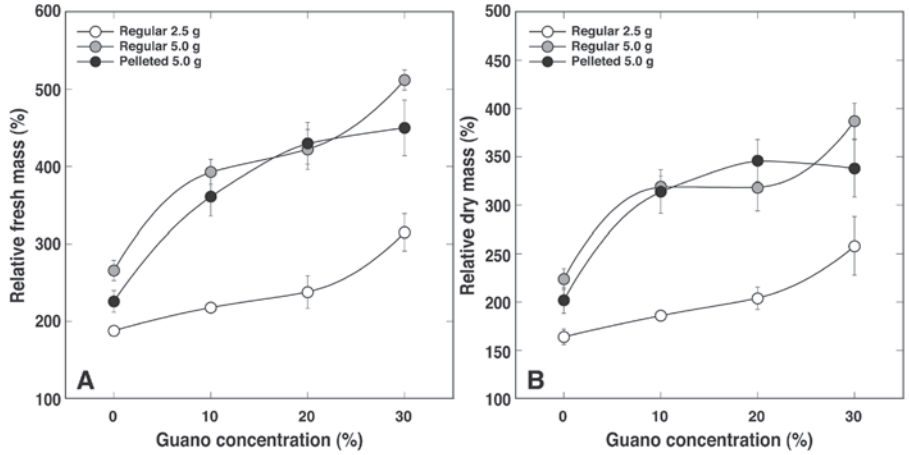

Figure 2. Effect of different concentrations of bat guano in vermicompost-based organic fertilizer on relative fresh (A) and dry (B) mass of shoots of 5-week-old winter rye plants. The data are means ±SE based on 5 replicates with 4 plants each for every concentration.

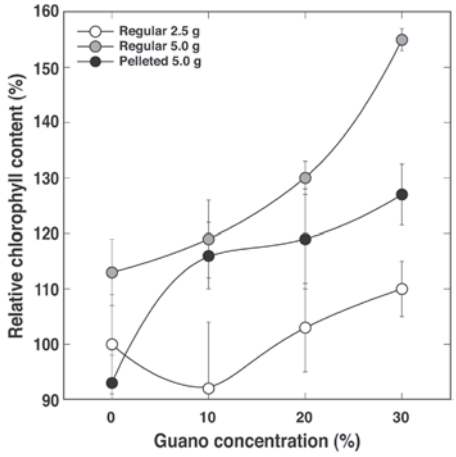

Figure 3. Effect of different concentrations of bat guano in vermicompost-based organic fertilizer on relative leaf chlorophyll content of 3-week-old winter rye plants. The data are means ±SE based on 5 replicates for every concentration.

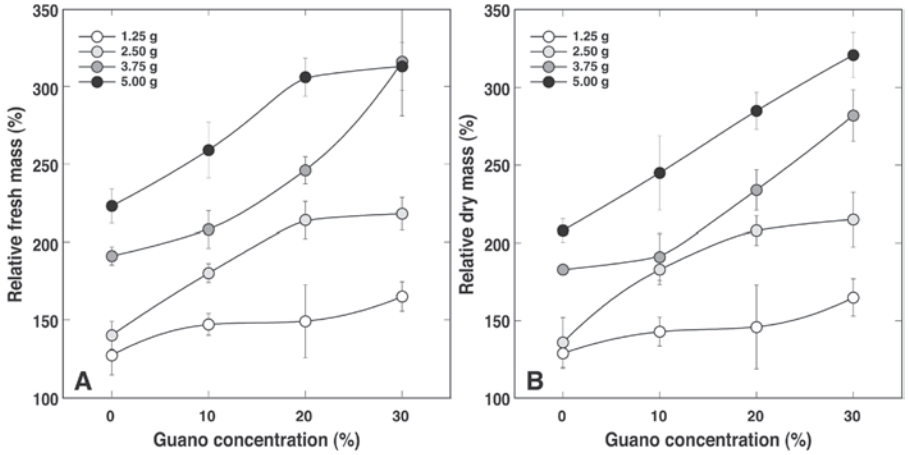

Figure 4. Effect of different concentrations of bat guano in vermicompost-based pelleted organic fertilizer on relative fresh (A) and dry (B) mass of shoots of 9-week-old potato plants. The data are means ±SE based on 7 replicates for every concentration.

A wider range of fertilizer doses (1.25 to 5.00 g per container, roughly corresponding to 2 to 10 t ha^{-1}) was assessed in the experiment with the potato minitubers. It is evident that the addition of guano had a more pronounced positive effect at higher doses of application (Fig. 4). However, an overall increase in accumulation of both fresh and dry mass by fertilizer supplement over unfertilized control was less evident than in the case of winter rye plants.

A clear beneficial effect of guano addition to vermicompost-based fertilizer was seen in the present study both for winter rye and potato plants under controlled conditions. This effect can be partially related to the increased amounts of plant-available mineral nutrients from guano. Similar positive effect in the conditions of limited soil nutrient availability was evident in the study with vermicompost (Karlsons et al., 2015). Plant growth-affecting activity of bat guano samples was not assessed in the present study. However, it is reasonable to suggest that a high microbiological activity in the guano has produced plant growth-promoting substances, leading to an additional positive effect on plant growth and development, similar to that found for other organic fertilizers, vermicompost (Karlsons et al., 2015) and freshwater sapropel (Grantina-Ievina et al., 2014a). Consequently, bat guano is a promising candidate for industrial organic fertilizer mixes.

Conclusions

1. Guano represents organic fertilizer with a high level of mineralization and significant content of living microorganisms, but microbiological diversity differed in comparison to vermicompost samples. The most characteristic was a lower total amount of bacteria, a higher number of coliforms, and the presence of yeasts.
2. Application of guano-containing fertilizer at 300 g kg^{-1} concentration significantly affected the microbiological diversity of the soil, increasing the amount of potentially plant pathogenic fungi. Therefore, microbiologically active organic fertilizers must be used with caution, as overall diversity can be altered due to microbial interaction. Treatment with regular vermicompost positively affected the percentage of potentially plant growth-promoting fungi *Trichoderma* and *Mortierella*.
3. Addition of bat guano to the organic fertilizer from vermicompost significantly increased the stimulating effect of vermicompost on plant growth and development of both model crops - winter rye and potato. This effect increased with an increasing proportion of guano indicating a higher concentration of plant-available minerals and, possibly, growth-promoting substances in the resulting fertilizer mix.

Acknowledgements

SIA GAHA is acknowledged for a kind donation of vermicompost, guano and soil samples used in the present study. Analysis of mineral nutrients was performed at the Laboratory of Plant Mineral Nutrition, Institute of Biology, University of Latvia. We would like to especially thank Dr. biol. Andris Karlsons and Dr. biol. Anita Osvalde for their practical help with experiments and data interpretation.

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THE EFFECT OF CONCENTRATE FEEDING ON COW BEHAVIOUR

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Abstract
In automatic milking systems, where the concentrate and forage components of the ration are offered to the cows separately, lack of control over intakes can result in difficulties balancing the forage and concentrate portions of the diet, leading to problems associated with high concentrate intakes and concomitant low forage intakes. In order to check this as a problem on a dairy unit, the feeding behaviour of a sample of cows was observed by video recording. As a pilot study, four Holstein Friesian cows (two at the highest yield and two at the lowest yield of the milk production range) were selected from sixty lactating cows on the Estonian University of Life Sciences' farm near Tartu, Estonia. The study took place from May 18th till November 4th 2014. The cows were robot-milked and fed a ration comprising, separately, concentrate feed from a robot and a feeder, and a grass/clover silage mix forage at the feed barrier. With the low number of samples the results are indicative and descriptive, but it appears from the raw data that individual variation in visiting times and times spent at the feed barrier are greater than the effect of level of production. Cows spent a significant portion of their time idling at the feed barrier, not actively feeding. It is concluded that care should be taken to presume behaviour from positional data, and there is no evidence that cows with higher and lower milk yields are differentially motivated to feed from a forage source.
Key words: feeding behaviour, concentrate feeding.

Introduction

"Feeding involves a complex series of decisions and depends upon an elaborate array of mental, motor and digestive abilities" (Broom and Fraser, 2007). Cattle spend about five hours a day eating (Broom and Fraser, 2007; Yeiser et al., 2012), and cows modify their feeding behaviour according to the feed supplied, and demonstrate clear preferences for feed (Broom and Fraser, 2007).

To ease feeding management, many producers supplement forage with concentrate based on the average requirement of the whole herd (Lawrence et al., 2015). This means that cows whose production is less than average will receive more concentrate than they require, and cows with milk production higher than the average will receive less concentrate than they need to support their higher milk yield. This means that some cows are overconditioned and others are too thin and are not able to achieve their full milk yield potential. Overconditioned cows at calving are at higher risk for dystocia and in particular retained foetal membranes, which might lead to infertility (Roche, 2006).

Cows feed intake is not only affected by the amount of concentrate given. It depends on lactation (Berry et al., 2006), health and position in the social hierarchy. Feed intake is at its peak in mid-lactation, increases in early lactation and declines at the end of lactation (Berry et al., 2006). Friggens et al., (1998) found that lactation stage affects the intakes of only those cows which are offered a high concentrate total mixed diet. Low concentrate total mixed diets showed no effect on dry matter intake as lactation progressed.

Health disorders show high initial effects on feed intake and milk production (Bareille et al., 2003), already prior to clinical manifestation. The total feed intake decrease is highest during the first occurrence and the first recurrence of ketosis (Barreile et al., 2003), and cows with mastitis show a 1.2 kg day^{-1} decrease in feed intake five days period before diagnosis (Sepúlveda-Varas et al., 2014). Cows with locomotion disorders also show small daily feed intake decreases before diagnosis (González et al., 2008).

The aim of this study was to see if those cows which receive more concentrates in their rations feed less on a partially mixed ration than those receiving a lower rate of concentrates.

Materials and Methods

The trial was carried out on Märja farm of the Estonian University of Life Sciences, Tartu, Estonia. In this pilot study four multiparous Holstein Friesian cows were observed. They were cubicle-housed and milked with an automatic milking system (DeLaval). The mean milk yield for cows which were not offered concentrates was 21.1 kg day^{-1} (±SD 3.3) while for those cows which did, the mean milk yield was 37.7 kg day^{-1} (±SD 5.3). All cows received concentrates at the milking robot, additional concentrate was offered in a partially mixed ration at the feed barrier. Rations contained grass and clover silage and a compound feed of barley, and rapeseed cake. Hay was used if needed to all cows. The percentage of dry matter in partially mixed ration was from 39.8%-48.9%. The concentrate offered at the robot milker contained barley, wheat, rapeseed cake, maize, soya flour, sugar beet, yeast, sunflower meal, rapeseed oil, molasses, salt and Premivit 0.2% Cattle. In the concentrate bin wheat flour, wheat bran and limestone were additionally added; yeast was not included. Metabolizable energy in the concentrate in the bin was 11.9 MJ and metabolizable protein was

112.8 g kg^{-1}, while at the robot there were 11.8 MJ and 103.8 g kg^{-1}, respectively. Four cows were selected for the trial, based only on their concentrate consumption from the concentrate feeding bin. Two cows (one in III and one in IV lactation) received no additional concentrate, while the other two (one in II and one in V lactation) received 2 kg and 4 kg day^{-1}. The automatic feeder brought forage to the feeding barrier twice a day. Cows were observed from a gantry above the feeding area, and feeding behaviour at the feed barrier was video recorded over a 24-hour period from June 2014 till November on 34 separate occasions. Two more cows were filmed, but their videos are currently being analysed.

Behavioural parameters recorded were: time spent feeding, standing and walking, grooming herself, grooming herself with the enrichment spinning brush, grooming another cow (allogrooming), nudging another cow (moving her away), pushing another cow (moving her away), nudged, pushed or scared (moved away) by another cow.

Descriptive statistics for each parameter were calculated with Microsoft Excel: the sum, mean, maximum and minimum.

Results and Discussion

Statistical analyses showed that the mean time spent feeding did not differ much between the treatments (low concentrate cows 4h 35 min and high concentrate cows 6h 15 min). The same thing can be said about the times spent standing (low 1h 28 min and high 2h 01 min), walking (low 18 min and high 26 min) and drinking (low 15 min and high 11 min) behaviours. Soca et al. (2014) and Lawrence et al. (2015) have found that cows receiving more concentrate in their diet feed less on forage. Since cows observed were fed from feeding barrier not bins, it is impossible to calculate individual cow dry matter intake.

Aggression was also observed. Cows who did not receive concentrate from the feeders pushed other cows away more (mean 116 times) than cows who received concentrate from feeders (mean 70 times). When we looked at which group of cows were pushed away more from the feed barrier, it emerged, that cows which received concentrates were pushed away more often (a mean of 70 times) than cows which did not receive concentrates (a mean of 49 times). This was primarily because one of the cows that received concentrates, which was in its second lactation, was pushed away by the other cows more than the other three.

When we looked at individual cows, the oldest cow (V lactation) was the least aggressive. She pushed other cows away 61 times in 24-hour period. Other cows were also less aggressive towards her than they were to each other. She was pushed away from the feed barrier 29 times. The youngest cow (II lactation) received the most aggressive behaviour toward herself. She was pushed away 111 times. This may have been because she was established as an older member of the heard, or because her greater age established herself in a high position in the social hierarchy.

Conclusions

Because of the low number of samples it is not possible to confirm or otherwise comment whether those cows receiving a higher amount of concentrate spend less time feeding on forage. Aggression was observed several times near the feeding barrier. As expected, the oldest cow received very little and the youngest one received the most aggression. We are planning to observe and video record more cows to provide more reliable results.

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THE BODY CONDITION SCORE AND LIVE WEIGHT INFLUENCE ON PREDICTED NITROGEN EXCRETION WITH URINE

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Abstract
The purpose of research was to analyze dairy cows (*Bos taurus*) milk productivity according to the calving body condition score (BCS) and to predict the nitrogen output with urine according to BCS and live weight. The research was carried out at the Research and Study farm 'Vecauce' of Latvia University of Agriculture. Data were collected from 55 dairy cows during October 2013 to October 2014. Dairy cows were from different breeds (Holstein Black and White, red breed cows with Holstein blood more than 40% and crossbreeds) and different lactations. Cows were grouped in two groups for the estimation of BCS effect on the analyzed traits: BCS≤2.5 and BCS≥2.6. BCS was estimated at calving and in monthly recording control days. Nadir value of BCS≥2.6 group was 2.64 ± 0.06 points on the third control day, but 2.46 ± 0.08 points of BCS≤2.5 on the second control day. Calving live weight of BCS≤2.5 and BCS≥2.6 groups were 613.8 ± 13.3 kg and 651.1 ± 11.4 kg. The highest milk yield was observed in BCS≥2.6 group until the third control day, the highest fat content was observed in this group, but the highest protein content was in BCS≤2.5 group. Milk urea content was not significantly affected by calving BCS, but milk urea content of BCS≤2.5 group ranged from 23.2 ± 1.86 to 30.9 ± 1.98 mg dL^{-1}, and from 20.6 ± 1.53 to 30.2 ± 2.27 mg dL^{-1} in BCS≥2.6 group. A significantly higher urinary nitrogen output was observed from BCS≥2.6 group on the second control day – 237.8 ± 8.1 g day^{-1} ($p<0.05$).
Key words: body condition score, milk urea, nitrogen output.

Introduction

Balanced nutrition is a basic condition for good animal health and environmental protection. Dairy cows (Bos taurus) produce a high milk yield if nutrition is unbalanced. Negative energy balance can be observed in early lactation stage and it depends on nutrition. Excess nitrogen losses pollute environment if dairy cows intake too much protein. Total pollution is collected from feces, urine and various other types of nitrogen pollution. The dietary protein degradability efficiency is characterized by nitrogen losses in environment (Jordan et al., 1983). The risk of pollution increases and reproductive performance decreases when nitrogen metabolism is unbalanced. The ova viability is low when concentration of nitrogen in body is high (Kohn et al., 1997). The scientists recommend using special additive for balancing nitrogen metabolism and reduction of nitrogen losses (Chacher et al., 2014). The highest nitrogen output was observed from cows with highest live weight. Nitrogen output can be affected by milk yield, crude protein content in diet and intake nitrogen quantity (Yan et al., 2006).

Different formulas can be used for urinary nitrogen output calculation. Urine is one of the basic end products of nitrogen metabolism and it is very important to predict nitrogen output with urine. For calculation of urinary nitrogen output it is necessary to determinate the milk urea nitrogen (MUN). MUN can vary 9.50 – 19.50 mg dL^{-1} ant it can be affected by live weight, breed and other factors. Formulas were developed for predicted nitrogen output with urine calculation for Holstein, Jersey breeds and all breeds (universal formula) by scientists from The Ohio State University. The breed effect lost its significance when live weight is included as a factor in the model. The universal formula is with high determination – R2=0.98 (Whang et al., 2000; Kauffman and St-Pierre, 2001). Other scientists developed formulas, but the coefficient of determination was not so high (Jonker et al., 1998).

Scientists have observed low positive relationship between body condition score (BCS) and milk urea nitrogen. When BCS milk urea nitrogen increases, these traits also change and become opposite – both traits decrease. It has allowed to conclude that similar relationship is between BCS and predicted nitrogen output with urine (Miglior et al., 2007). According to the previous studies, the predicted nitrogen output with urine ranges from 150 – 225 g day^{-1} from Holstein cow (Castillo et al., 2001).

The purpose of the research was to analyze the dairy cows milk productivity according to the calving BCS and to predict nitrogen output with urine according to BCS and live weight.

Materials and Methods

The research was carried out at the Research and Study farm 'Vecauce' of Latvia University of Agriculture. Data were collected from 55 dairy cows during October 2013 to October 2014. Dairy cows were from different breeds (Holstein Black and White, red breed cows with Holstein blood more than 40% and crossbreeds – F1 progeny of Holstein Black and White, and red breeds) and different lactations. Cows were kept in loose house system and milked three times

per day. Dairy cows were *at libitum* access for total mixed ration. Ingredients of total mixed ration 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.

Milk productivity and quality data for the study were obtained from the state agency 'Lauksaimniecības datu centrs'. Milk samples were analyzed in 'Piensaimnieku laboratorija', Ltd. Milk yield (kg), fat content (g kg^{-1}), protein content (g kg^{-1}), milk urea content (mg dL^{-1}) and somatic cell score were analyzed in the paper. Fat content, protein content, milk urea content was analyzed according to ISO 9622 / IDF 141:2013, but somatic cell count according to LVS EN ISO 13366-2:2007. The first control day was on 17.9 ± 0.72 day of lactation, second control day – 48.4 ± 0.65 day of lactation, third control day – 80.7 ± 0.64 day of lactation, fourth control day – 110.8 ± 0.88 day of lactation. Live weight and BCS were measured at calving and on the monthly recording control days. Live weight was measured with verified type. BCS was evaluated in 5 point system with increment of 0.25 (1 point – extremely thin, 5 point – very obese). Body condition measuring system was described by J.M. Bewley and M.M. Schutz (2008), but developed by B.G. Lowman et al., (1976).

Somatic cell score (SCS) was calculated by formula (Schutz and Powell, 1993):

$$SCS=\log2 (\text{Somatic cell count}/100000)+3 \quad (1)$$

For the calculation of predicted nitrogen output with urine, the milk urea nitrogen (MUN) has to be calculated. MUN was calculated using formula (Spiekers and Obermaier, 2012):

$$MUN= \text{Milk urea content} \times 0.46 \quad (2)$$

The predicted nitrogen output with urine (UN) was calculated using MUN and live weight traits. Two formulas were used for the result comparison. Formula (3) included two factors – MUN and live weight (LW), but formula (4) included only factor MUN. Formula (4) was developed for Holstein breed.

Formula (3) was as follows (Kauffman and St-Pierre, 2001):

$$UN=0.0259 \times LW \times MUN \quad (3)$$

Formula (4) was:

$$UN=17.6 \times MUN \quad (4)$$

Cows were grouped in two groups for the estimation of BCS effect on the analyzed traits: BCS≤2.5 (n=20) and BCS≥2.6 (n=35), without the use of lactation as factor, because calving BCS of primiparous (2.78 points) and multiparous (2.86 points) was not significantly different. T-test for independent samples was used for significance determination ($p<0.05$). Pearson correlation was used for analyzing relationship between live weight, BCS and predicted nitrogen output with urine. SPSS and MS Excel software was used for data mathematical processing.

Results and Discussion

Differences between BCS at calving and on the first, seconding and third control days were significant ($p<0.05$). BCS values range from 2.47 ± 0.08 to 2.79 ± 0.06 points in BCS≤2.5 group, but BCS observed from 2.64 ± 0.06 to 2.99 ± 0.05 points in BCS≥2.6 group. After having analyzed the changes of BCS, we found that BCS had decreased a little in BCS≤2.5 group. Calving BCS was 2.47 ± 0.08 points in this

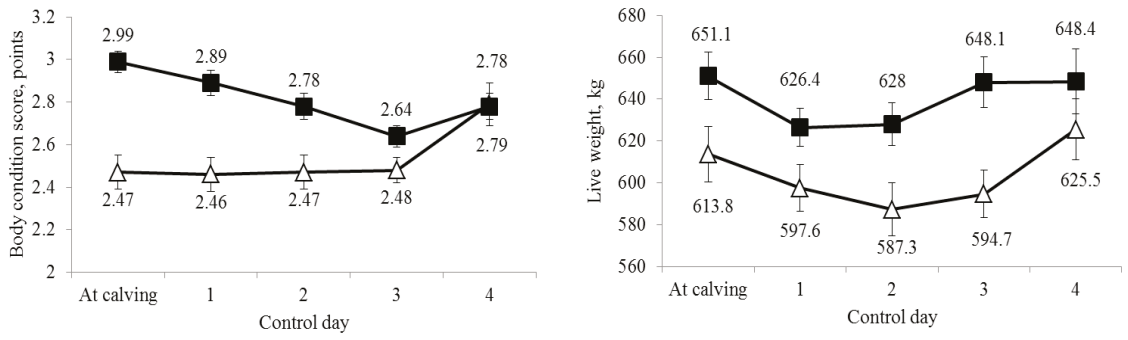

Figure 1. Body condition score and live weight changes: Δ – BCS≤2.5; ■ – BCS≥2.6.

Table 1

Milk productivity and quality traits

Group	Control day			
	1.	2.	3.	4.
	Milk yield, kg			
BCS≤2.5	34.8 ± 1.07	39.3 ± 1.78	40.3 ± 1.45	43.0 ± 2.67
BCS≥2.6	37.2 ± 1.12	42.6 ± 1.34	41.3 ± 1.28	40.5 ± 2.27
×	Fat content, g kg^{-1}			
BCS≤2.5	46.4 ± 1.51	33.9 ± 1.24	38.0 ± 1.72	32.2 ± 1.82
BCS≥2.6	44.1 ± 1.04	36.4 ± 0.99	39.5 ± 1.23	34.8 ± 1.65
×	Crude protein content, g kg^{-1}			
BCS≤2.5	33.3 ± 0.39	34.1 ± 0.43	34.0 ± 0.49	32.3 ± 0.51
BCS≥2.6	32.9 ± 0.36	33.8 ± 0.32	33.6 ± 0.42	33.1 ± 0.39
×	Milk urea content, mg dL^{-1}			
BCS≤2.5	23.2 ± 1.86	26.7 ± 0.71	27.7 ± 1.62	30.9 ± 1.98
BCS≥2.6	20.6 ± 1.53	29.3 ± 0.98	28.8 ± 0.92	30.2 ± 2.27
×	SCS			
BCS≤2.5	2.50 ± 0.33	1.66 ± 0.34	2.33 ± 0.49	1.06 ± 0.55
BCS≥2.6	2.40 ± 0.27	2.04 ± 0.30	1.80 ± 0.30	2.37 ± 0.36

1. control day – 17.9 day of lactation, 2. control day – 48.4 day of lactation, 3. control day – 80.7 day of lactation, 4. control day – 110.8 day of lactation.

group, but BCS increased to 2.79 ± 0.06 points on the forth control day. Calving BCS of other group was 2.99 ± 0.05 points, bud nadir value was observed on the third control day.

Live weight was significantly different at calving and third control day ($p<0.05$). Calving live weight of BCS≤2.5 group was 613.8 ± 13.3 kg, bur nadir value was observed on the second control day. Calving live weight of BCS≥2.6 group was 651.1 ± 11.4 kg and nadir value was found on the first control day when it decreased to 626.4 ± 9.1 kg (Figure 1).

According to the Irish researchers, the average calving BCS and live weight of Holstein dairy cows was 3.14 ± 0.42 points and 564 ± 77.9 kg, respectively (Berry et al., 2011).

M.D.P. Komaragiri et al. (1998) concluded that dairy cows lose major part of BCS by the fifth lactation week and further reduction is small. Other researchers, similar to Irish scientists, found that calving BCS of Holstein cows varied from 3.00 to 3.25 point, but it decreased to 2.60 points by the seventh lactation week (Roche et al., 2006; Yamazaki et al., 2011).

We did not find calving BCS significant influence on milk quantity and quality traits. Variation of average milk yield had a different trend between the control days. The milk yield of BCS≤2.5 group was 34.8 ± 1.07 kg on the first control day, but it increased with each next control day until the fourth recording or 110.8 lactation day when the milk yield was 43.0 ± 2.67 kg. The highest milk yield of BCS≥2.6 group was found on the second control day (42.6 ± 1.34 kg), but milk yield insignificantly decreased over the next control days. According to D. P. Berry et al. (2007), it was concluded that milk yield was highest from dairy cows with the highest BCS. We found similar result by the third control day or 81st lactation day.

Analyzing milk fat content we found a trend that fat content was highest in BCS≥2.6 group, except on the first control day when a peak of milk fat 46.4 ± 1.51 g kg^{-1} was observed. Lower fat content of both groups was observed on the fourth control day.

The highest crude protein content was found from BCS≤2.5 group by the third control day, but highest crude protein content of BCS≥2.6 group was observed on the fourth control day.

Milk urea content of BCS≤2.5 group ranged from 23.2 ± 1.86 to 30.9 ± 1.98 mg dL^{-1}, but from 20.6 ± 1.53 to 30.2 ± 2.27 mg dL^{-1} in BCS≥2.6 group. Milk urea content depends on the season, parity and lactation stage. Researchers from Latvia found that milk urea varied from 12.6 to 52.9 mg dL^{-1}, but average value was 28.3±1.25 mg dL^{-1} and it means that our results are close to the previous papers (Jonkus and Paura, 2011; Ruska and Jonkus, 2014).

The highest somatic cell logarithm was observed on the first control day from groups 2.50 ± 0.33 of BCS≤2.5 group and 2.40 ± 0.27 of BCS≥2.6 group, respectively (Table 1).

Analyzing the predicted nitrogen output with urine using formula (3) we ascertained that the highest value of the first control day was in BCS≤2.5 group – 168.1 ± 12.9 g day^{-1}. Predicted nitrogen output of both groups increased in each next control day. Difference was significant between groups on the second control day when nitrogen values of BCS≤2.5 and BCS≥2.6 groups were 219.3 ± 7.8 and 187.3 ± 7.4 g day^{-1}, respectively. Using formula (4), we observed that values of predicted nitrogen output were higher.

Table 2

Predicted nitrogen output with urine, g day^{-1}

Group	Control day		
	1.	2.	3.
	Formula (3)		
BCS≤2.5	168.1 ± 12.9	187.3 ± 7.4[a]	195.2 ± 11.7
BCS≥2.6	150.8 ± 11.6	219.3 ± 7.8[b]	223.3 ± 8.5
×	Formula (4)		
BCS≤2.5	192.1 ± 14.8	215.6 ± 5.5[a]	222.9 ± 12.5
BCS≥2.6	164.2 ± 13.4	237.8 ± 8.1[b]	233.7 ± 7.6

[a,b] –trait is significantly different between BCS groups using the same formula ($p<0.05$)
1. control day – 17.9 day of lactation, 2. control day – 48.4 day of lactation, 3. control day – 80.7 day of lactation.

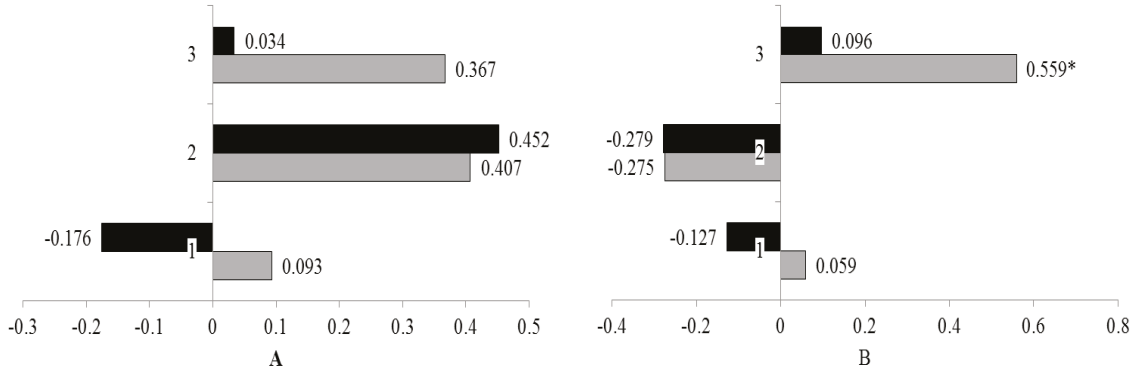

Figure 2. Phenotypic correlation between live weight and predicted nitrogen output with urine in BCS≤2.5 group (A) and BCS≥2.6 group (B) for calculation using: ■ Formula (3); ■ Formula (4) (* $p<0.05$).

Scientists accented that predicted nitrogen output with urine is affected by animal nutrition but not so much by BCS (Noftsger and St-Pierre, 2003). Researchers from China found that nitrogen output with urine depending on animal diet can range from 123 to 196 g day^{-1} (Zhai et al., 2007). Estimation of urinary nitrogen can be predicted by using formula (3), because the target MUN have decreased from 8.5 to 11.5 mg dL^{-1} for most dairy herds compared with the previous target concentrations of 12 to 16 mg dL^{-1} (Kohn et al., 2002). However, models are very sensitive, because nitrogen output can be affected by different factors, for example, nitrogen intake, crude protein content and degradability of protein in diets (Kebreab et al., 2002). Taking into account the nutrition recommendations, the recommended nitrogen output should be 168 g day^{-1}, but, on average, farmers appeared to feed 6.6% more nitrogen and it resulted in increase of nitrogen output with urine, milk and feces (Jonker et al., 2002).

Analyzing phenotypic correlation between live weight and predicted nitrogen output with urine of BCS≤2.5 group using formula (3) we found that correlation was moderate positive in the second and third recording, respectively, rp= 0.407 and 0.367. Using formula (4) moderate positive correlation was found only in the second recording. We observed significant correlation between live weight and predicted nitrogen output in the third recording ant it was moderate (rp = 0.559; $p<0.05$). Quite opposite situation was in the second recording, compared with BCS≤2.5 group, the correlation between live weight and predicted nitrogen output was negative, furthermore, it was low. The first recording results were quite similar as in BCS≤2.5 group; using formula (3) for predicted nitrogen output with urine calculation correlation was positive, but using formula (4) – negative. Relationships are illustrated in Figure 2.

Phenotypic correlation between BCS and predicted nitrogen output of BCS≥2.6 group was different compared with BCS≤2.5 group. Correlation between BCS and predicted nitrogen output of this group was positive in the first recording, but negative in the third recording using both formulas. Using formula (3) correlation was positive, but using formula (4) it was negative in the second recording. Significantly correlated BCS and predicted nitrogen output in the second recording using formula (3) and (4). Both relationships were positive, but using formula (3) it was strong (rp = 0.840; $p<0.05$), while using formula

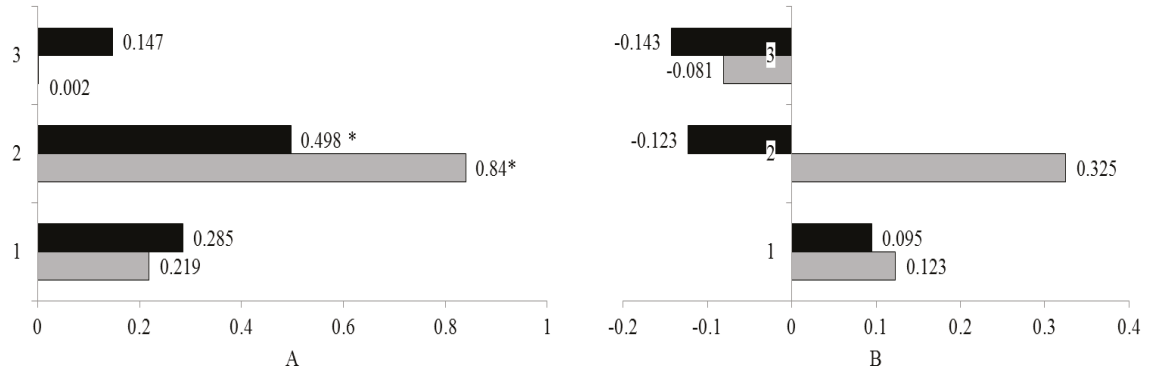

Figure 3. Phenotypic correlation between body condition score and predicted nitrogen output with urine in BCS≤2.5 group (A) and BCS≥2.6 group (B) for calculation using: ■ Formula (3); ■ Formula (4) (*p<0.05)

(4) it was moderate (rp = 0.498, p<0.05). These relationships are showed in Figure 3.

Balanced diets for dairy cows and correct management of manure help improve nitrogen utilization efficiency in farms. Recycling of nitrogen is very important for environment (Gourley et al., 2012). According to the calculations done by the researchers, dairy cows intake 0.33 to 0.67 kg nitrogen, but utilization efficiency varies from 0.21 to 0.42 (Whelan et al., 2013).

Conclusions

Nadir value of BCS≥2.6 group was 2.64 ± 0.06 points in the third recording, but 2.46 ± 0.08 points of BCS≤2.5 in the second recording.

Calving live weight of BCS≤2.5 group was 613.8 ± 13.3 kg, bur nadir value was observed in the second recording. Calving live weight of BCS≥2.6 group was 651.1 ± 11.4 kg and nadir value was found in the first recording when it decreased to 626.4 ± 9.1 kg.

BCS did not affect milk productivity traits significantly, but the highest milk yield was observed in BCS≥2.6 group until the third recording, the highest fat content was observed in this group, but the highest protein content was in BCS≤2.5 group.

Milk urea content was not significantly affected by calving BCS, but milk urea content of BCS≤2.5 group ranged from 23.2 ± 1.86 to 30.9 ± 1.98 mg dL^{-1}, and from 20.6 ± 1.53 to 30.2 ± 2.27 mg dL^{-1} in BCS≥2.6 group.

Using formula (3) and formula (4), the highest predicted nitrogen output with urine was observed in BCS≥2.6 group, furthermore, significant differences were in the second recording between groups.

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LIGHT - EMITTING DIODES (LEDs) FOR HIGHER NUTRITIONAL QUALITY OF *BRASSICACEAE* MICROGREENS

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Abstract
The aim of this study was to investigate the effect of industrially designed light-emitting diode (LED) lamp lighting on the nutritional quality of *Brassicaceae* microgreens. Red pak choi (*Brassica rapa* var. *chinensis* 'Rubi F_1'), tatsoi (*Brassica rapa* var. *rosularis*) and mustard (*Brassica juncea* L. 'Red Lion') were grown in a greenhouse (20±2/18±2 °C) during winter season, and the solar daily integral (DLI) was ~3.46±1.16 mol m^{-2} d^{-1}. The light spectra of lamp consist of 8 violet (420-430), 16 blue (460-470 nm), 8 orange (610-615 nm), 3 red (620-630 nm), 56 red (660-670 nm), 8 white (contain blue (400-500 nm), green (500-600 nm) and red (600-700 nm)) LEDs. The treatments of ~150 and ~250 µmol m^{-2} s^{-1} LED irradiance levels (LED 150 and LED 250) for 16 h d^{-1} in comparison with high pressure sodium (HPS) lamps (~150 µmol m^{-2} s^{-1}) as a control were performed. Photophysiological response to the artificial light varied among *Brassicaceae* species. Microgreens treated with LED 150 and LED 250 were significantly ($P \leq 0.05$) shorter and formed smaller hypocotyls. The photooxidative changes were evoked by both lighting treatments and led to higher phytochemical (phenols, ascorbic acid, flavonols, anthocyanins) and mineral element (Ca, K, Mg, Na, P, Fe, Zn) contents, and the DPPH and ABTS free radicals scavenging activities in all microgreens. Significantly lower content of nitrate was obtained with LED 150 treatment. Finally, LED lamps have the potential to be used as the main light source for growing high nutritional quality microgreens in greenhouses.
Key words: antioxidant, *Brassica,* greenhouse**,** light – emitting diode, microgreen, mineral element.

Introduction

Light is one of essential environmental factors influencing photophysiological responses of all higher plants (Ma et al., 2014). Plants are enabled to sense very broad light spectrum from UV to far red (280-750 nm) by photoreceptors. Photoreception mechanism is involved in response of photoreceptors to red-far red light (phytochromes), blue-UVA light (cryptochromes, phototropins, 'Zeitlupes') and UVB (UVR8) light (Galvão and Fankhauser, 2015; Casal et al., 2014; Pierik, de Wit, 2014). Besides light quality (wavelength), light intensity (irradiation) and photoperiod (duration of day/ night) also play a significant role in plant morphogenetic and photosynthetic responses (Björkman et al., 2011).

Greenhouse is an artificial growing system and requires the application of supplemental light sources to ensure plant growth. Light emitting diodes (LEDs), as the main or supplemental lighting source, despite having intensity, spectral and energy advances, can be used for target manipulation of metabolic responses in order to achieve high plant productivity and quality (Olle and Viršilė, 2013; Darko et al., 2014; Carvalho and Folta, 2014; Duchovskis et al., 2015). LEDs represent a promising light source in greenhouses for lettuce (Ouzounis et al., 2015; Sirtautas et al., 2014; Muneer et al., 2014; Samuolienė et al., 2013a; Samuolienė et al., 2011), red and green leaf microgreens (Samuolienė et al., 2012a; Brazaitytė et al., 2013), cucumber seedlings (Hernández and Kubota, 2014; Novičkovas et al., 2012), tomato (Lu et al., 2012; Brazaitytė et al., 2009), sweet pepper (Samuolienė et al., 2012b; Brown et al., 1995). Light combination with other climatic conditions (temperature, air humidity) and agronomic practices (water availability, soil/ substrate conditions, fertilizers) influence plant reaction to the environment. Plants have the adaptive mechanisms to thrive under environmental conditions which are typically found in greenhouses. Many antioxidants play a key role in plant adaptation to biotic stress. Antioxidants produced by plants in response to stress are secondary metabolites such as phenolic compounds or ascorbic acid (Oh et al., 2009), and are associated with the reduction of human chronic diseases like cancer or cardiovascular disease (Pinto et al., 2015). Biosynthesis of phenols is directly linked to blue light receptors (cryptochromes and phototropins) (Kang et al., 2008). Phytochromes participate in regulating processes of anthocyanin formation and accumulation (Iwai et al., 2010). Besides, biosynthesis of anthocyanins requires enzymes whose expression is regulated by light (Yamazaki et al., 1999). Ascorbic acid is also involved in anthocyanin biosynthesis (Iwai et al., 2010). Ascorbic acid metabolic pathway interacts with photosynthetic and respiratory electron transport chains, and the accumulation of ascorbic acid depends on quantity and quality of light (Bartoli et al., 2006). Minerals are also known to have protective benefits against oxidative stress related diseases, and found in plants as ions involved in secondary metabolite biosynthesis, or inorganic and organic salts and compounds (Mihaljev et al., 2014; Konieczynski et al., 2015). However, vegetables can also accumulate nitrate (NO_3^-) due to growing in closed environment, like greenhouse. Nitrate accumulation in plants mostly depends on nitrate reductase (NR) activity, which can be stimulated by red light (Lillo and Appenroth, 2001). Nitrate reduced to metabolites like toxic nitrite

anion (NO_2^-) or nitric oxide (NO) can lead to human disorders, especially in children (Santamaria, 2006). Availability to reduce nitrate in vegetables before human consumption is very advisable (Pinto et al., 2015).

Horticultural *Brassicaceae* plants are excellent source of fibres, vitamins, minerals and antioxidants (Brazaitytė et al., 2015a; Vale et al., 2014; Björkman et al., 2011). In addition, it can be cultivated under different growth conditions and growth as microgreens from a wide range of seeds all the year round. Microgreens have a central stem with two fully developed cotyledon leaves and mostly one pair of small true leaves. In recent years, microgreens are gaining popularity as a 'functional' food due to high nutritional quality and as a culinary ingredient due to intense flavour, colour and tender texture (Pinto et al., 2015; Brazaitytė et al., 2015b). Unlike sprouts, microgreens are produced under the light.

The aim of this study was to investigate the effect of industrially designed light-emitting diode (LED) lamp lighting on the nutritional quality of *Brassicaceae* microgreens.

Materials and Methods

Experiments were performed at the Institute of Horticulture, Lithuanian Research Centre of Agriculture and Forestry. Microgreen species of a *Brassicaceae* family were selected for their known ability to grow as nutrient rich vegetables under artificial LED light. Red pak choi (*Brassica rapa* var. *Chinensis* 'Rubi F_1'), tatsoi (*Brassica rapa* var. *rosularis*) and mustard (*Brassica juncea* L. 'Red Lion') microgreens were grown from seed to harvest time for 8 days in greenhouse during winter season. Day/ night temperatures of 20±2/ 18±2 °C were maintained and the relative air humidity was 55±5%. 1g of seeds (CN Seeds, Ltd., UK) were sown in 18 x 11 x 6 cm plastic pots filled with pea substrate (N 100-120, P_2O_5 30-80, K_2O 120-200 mg L^{-1}; microelements Fe, Mn, Cu, S, Mo, Zn; pH 5.5-6.5) (Profi 1, Durpeta, Lithuania). Four pots of each microgreen species for each treatment were used. The seeded pots were sprayed daily with tap water as needed. The industrially designed lamps (HL8000, HORTILED, Lithuania) used in the present study were 49,5 x 28 x 8 cm in area and built with 8 violet (420-430 nm), 16 blue (460-470 nm), 8 orange (610-615 nm), 3 red (620-630 nm), 56 red (660-670 nm), 8 white (contain blue (400-500 nm), green (500-600 nm) and red (600 - 700 nm)) LEDs (280 W). Three light treatments were imposed: (1) high pressure sodium lamps (SON – T Agro, Philips, UK) at ~150 µmol m^{-2} s^{-1} (HPS, as a control); (2) LED light intensity at ~150 µmol m^{-2} s^{-1} (LED 150); (3) LED light intensity at ~250 µmol m^{-2} s^{-1} (LED 250). The 16/ 8 light/ dark photoperiod of artificial light was maintained. Photosynthetic photon flux density (PPFD) was measured daily by photometer-radiometer RF 100 (Sonopan, Poland). The natural light was limited due to internal shading from installed lamps. The solar daily light integral (DLI) was measured at ~3.46±1.16 mol m^{-2} d^{-1} inside a greenhouse.

The edible biomass (cotyledons with stems) of microgreens (8 days old) was harvested. From each light treatment ten randomly selected plants were used for biometric measurements. The conjugated biological samples of fresh weight (FW) of randomly selected microgreens (0.5 – 1.0 per sample) were used for phytochemical analysis. Three analytical replications were performed for each phytochemical measurement. The leaf area (cm^2) was measured by an automatic leaf area meter (AT Delta-T Devices, UK). Ascorbic acid was determined spectrophotometrically according to the method published by Janghel et al. (2007). In this assay ascorbic acid reduces methyl viologen to form blue coloured free radical ion. The absorbance of the radical ion was measured by UV/ Vis spectrophotometer at 600 nm (M501, Spectronic Camspec Ltd., UK). The total phenolic content in the extract was determined according to the Folin-Ciocalteu method as outlined by Ragaee et al. (2006). Frozen in liquid nitrogen microgreen FW samples were extracted with 80 % methanol (1:10). Absorbance was measured at 765 nm (M501, Spectronic Camspec Ltd., UK). The results were expressed as gallic acid equivalents. Non-destructive measurements of the flavonol index of the microgreen leaf were performed using Dualex meter (Force-A, France). The 2, 2- diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was evaluated as described by Ragaee et al. (2006). The sample extracts were the same as the ones used in the total phenols assay. The absorbance was measured at 515 nm at 16 min of the reaction (M501, Spectronic Camspec Ltd., UK). The 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity was determined according to Kadri et al. (2013). The methanolic (80%) extracts were diluted with $ABTS^+$ solution (A=0.7±0.2). The decrease of absorbance was measured after 15 min at 734 nm (M501, Spectronic Camspec Ltd., UK). The total anthocyanin content was estimated using pH-differential spectrophotometric method according to Stanciu et al. (2009). The absorption values of sample extracts were measured at 420, 520, 700 nm (Genesys 6, Thermospectronic, USA). Anthocyanins were expressed as cyanidin 3-glucoside equivalents, using a molar extinction coefficient of 25.740 mol^{-1} cm^{-1} and a molecular weight of 485 g mol^{-1}. In order to prepare the plant material for the determination of nitrates, plant tissue samples were dried in a drying oven (Venticell, MBT, Czech Republic) at 70 °C for 48 h.

Table 1

Growth parameters of microgreens cultivated under different light treatments

Light treatment	Hypocotyls length, cm	Plant height, cm	Leaf area, cm^2
Tatsoi			
HPS	4.59±0.46	5.63±0.36	0.73±0.15
LED 150	2.46±0.23[b]	3.52±0.27[b]	0.57±0.09
LED 250	2.81±0.33[b]	3.88±0.31[b]	0.57±0.07
LSD_{05}	0.53	0.49	0.20
Red pak choi 'Rubi F_1'			
HPS	3.66±0.33	4.86±0.51	0.89±0.03
LED 150	2.79±0.25[b]	3.98±0.30[b]	0.90±0.10
LED 250	2.59±0.35[b]	3.96±0.30[b]	1.00±0.10
LSD_{05}	0.30	0.46	0.37
Mustard 'Red Lion'			
HPS	3.39±0.22	4.71±0.24	0.91±0.10
LED 150	2.56±0.11[b]	3.84±0.15[b]	0.81±0.07
LED 250	2.05±0.06[b]	3.47±0.10[b]	0.85±0.13
LSD_{05}	0.22	0.24	0.27

Values are expressed as mean ± SD (n=10). LSD_{05} - Fisher's protected least significant difference (P≤0.05); b - the value is significantly lower than control (HPS). FW - fresh weight.

Nitrate concentration in microgreens was measured by a potentiometric method (Geniatakis et al., 2003) using ion meter (Oakton, USA) and combined nitrate ion selective electrode HI4113 (HANNA instruments, USA). The mineral elements (Ca (II), K (I), Mg (II), Na (I), P (I), Fe (II) and Zn (I)) contents in microgreens were determined by microwave digestion technique combined with inductively coupled plasma optical emission spectrometry (Marin et al., 2011). A complete digestion of dry microgreen material (0.5 g) was achieved with 65% HNO_3 and 30% H_2O_2 (5:3) using microwave digestion system Multiwave GO (Anton Paar GmbH, Austria). The mineral element profile was analysed by ICP – OES spectrometer (Spectro Genesis, SPECTRO Analytical Instruments, Germany).

All data are expressed on a fresh weight basis and presented as mean values ± standard deviation. All measurements were evaluated for significance by an analysis of variance (ANOVA) followed by the least significant difference (LSD) test at the P≤0.05 level.

Results and Discussion

The results of biometric measurements of microgreens influenced by different light treatments are shown in Table 1. Differences of growth responses of three *Brassicaceae* species were clearly observed after providing LED 150 and LED 250 treatments.

The height of all microgreens of the LED 150 and LED 250 treatments was significantly lower (~1.4 – 1.6 times) compared with those of HPS treatment (Table 1). The same tendency was observed measuring

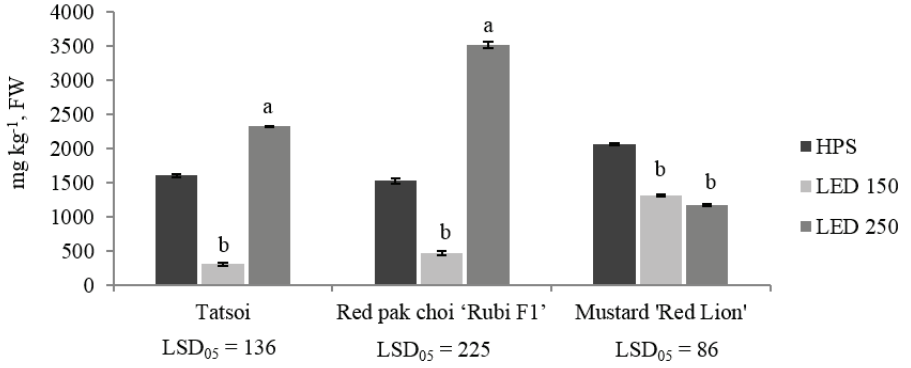

Figure 1. Nitrate contents in *Brassicaceae* microgreens cultivated under different light treatments
Values are expressed as mean ± SD (n=3). LSD_{05} –Fisher's protected least significant difference (P≤0.05); a – the value is significantly higher than control (HPS), b – the value is significantly lower than control (HPS). FW – fresh weight.

hypocotyl length. LED light prevented from an undesirable microgreen elongation. Increasing LED irradiation resulted in decreased hypocotyl length of red pak choi, mustard and tatsoi. It is known that hypocotyl elongation could be prevented by adding at least 15 µmol m^{-2} s^{-1} of blue light (Darko et al., 2014; Hoenecke et al., 1992). In our experiments, blue (460-470 nm) contains ~16% of common spectra of LED lamps used in LED 150 and LED 250 treatments (~24 and ~40 µmol m^{-2} s^{-1}, respectively). Otherwise, tatsoi treated under LED 150 had lower height and formed shorter hypocotyls in comparison with LED 250. No significant differences of leaf area were determined. However, microgreens cultivated under LED light looked smaller in comparison with plants grown under HPS.

Figure 1 shows the effect of LED light on the content of nitrate in microgreens. Nitrate content was greatly affected by LED 150 treatment – the significantly lower content of nitrate was determined in tatsoi and red pak choi (~5.2 and 3.3 times, respectively). Otherwise, higher LED irradiation (LED 250) led to an increased nitrate content in these microgreens. Nitrate content significantly decreased during LED 150 and LED 250 treatments in mustard (~1.5 and ~1.8, respectively).

As shown in Table 2, the artificial light treatments differentially affected the metabolic system of the investigated microgreens. According to Samuolienė et al. (2013b), a higher ascorbic acid content in red pak choi and tatsoi microgreens resulted in 110 µmol m^{-2} s^{-1} blue (455 nm), red (638 nm), red (665 nm) and far red (731) LEDs irradiation, while no significant differences between irradiation levels (110-545 µmol m^{-2} s^{-1}) were determined in mustard. In the present study, the increased biosynthesis of ascorbic acid was determined under LED 150 and LED 250 treatments in red pak choi (both ~1.2 times) and mustard (~1.1 and ~1.5 times, respectively), and under LED 250 in tatsoi (~1.8 times). It can be assumed that the metabolic pathway of ascorbic acid depended more on spectral quality than the irradiance level of LED light.

The greatest quantity of total phenols was found in the order: mustard> red pak choi> tatsoi (Table 2). Significantly higher content of phenols accumulated in these microgreens cultivated under LED 150 (~1.2 times, respectively to all species) and LED 250 treatments (~1.2, ~1.3 and ~1.5 times, respectively - tatsoi, red pak choi, mustard). A similar trend was also observed in flavonol index. The greatest flavonol index was determined in mustard, the lowest in tatsoi. Significantly higher flavonol index was in microgreens grown under both LEDs treatments. It can be noted that flavonol index changed slightly among LED 150 and LED 250 treatments. The significantly higher content of anthocyanins was determined only in microgreens grown under LED 150 treatment (~1.2 times, respectively to all species). An increased phenolic compound content in microgreens along LED lighting exposure influenced free radical scavenging activity. The methanol extracts of microgreens studied were analysed to determine their antioxidant activity against ABTS and DPPH radicals (Table 2). Microgreens grown under LED 150 and LED 250 treatments demonstrated significantly higher antioxidant activity evaluated using ABTS and DPPH methods.

Table 2

Antioxidant properties of *Brassicaceae* microgreens cultivated under different light treatments

Light treatment	Ascorbic acid, mg g^{-1}, FW	Total phenols, mg g^{-1}, FW	Total anthocyanins, mg g^{-1}, FW	Flavonols, index	DPPH•, µmol g^{-1}, FW	$ABTS^{+}$, µmol g^{-1}, FW
Tatsoi						
HPS	1.33±0.05	1.20±0.03	0.51±0.02	0.31±0,00	2.35±0.07	11.94±0.29
LED 150	1.43±0.08	1.42±0.04[a]	0.66±0.03[a]	0.50±0,03[a]	3.46±0.04[a]	16.69±0.05[a]
LED 250	1.88±0.08[a]	1.47±0.05[a]	0.56±0.06	0.49±0,05[a]	3.98±0.06[a]	17.43±0.15[a]
LSD_{05}	0.19	0.10	0.10	0.11	0.16	0.43
Red pak choi 'Rubi F_1'						
HPS	0.92±0.07	1.32±0.01	0.52±0.02	0.33±0,01	3.75±0.26	14.25±0.10
LED 150	1.08±0.05[a]	1.61±0.05[a]	0.63±0.03[a]	0.57±0,03[a]	4.28±0.28	20.86±0.24[a]
LED 250	1.12±0.05[a]	1.69±0.03[a]	0.53±0.01	0.59±0,04[a]	5.10±0.29[a]	21.86±0.15[a]
LSD_{05}	0.11	0.07	0.03	0.13	0.66	0.46
Mustard 'Red Lion'						
HPS	1.47±0.02	1.41±0.06	0.38±0.02	0.36±0,01	3.95±0.13	18.52±0.11
LED 150	1.58±0.03[a]	1.65±0.02[a]	0.44±0.03[a]	0.58±0,02[a]	4.19±0.12	23.28±0.08[a]
LED 250	2.26±0.06[a]	2.10±0.05[a]	0.43±0.02	0.58±0,02[a]	5.39±0.09[a]	28.07±0.21[a]
LSD_{05}	0.10	0.12	0.05	0.06	0.27	0.33

Values are expressed as mean ± SD (n=3). LSD_{05} - Fisher's protected least significant difference ($P \leq 0.05$); a - the value is significantly ($P \leq 0.05$) higher than control (HPS). FW - fresh weight.

Table 3

Mineral elements content in *Brassicaceae* microgreens cultivated under different light treatments

Light treatment	Ca (II)	K (I)	Mg (II)	Na (I)	P (I)	Fe (II)	Zn (I)
	mg g^{-1}, FW					µg g^{-1}, FW	
Tatsoi							
HPS	1.24±0.02	2.66±0.02	0.33±0.00	0.61±0.00	0.55±0.01	13.26±0.14	13.57±0.32
LED 150	1.23±0.02	3.06±0.04[a]	0.36±0.00[a]	0.80±0.00[a]	0.70±0.01[a]	21.55±0.24[a]	17.63±0.17[a]
LED 250	1.49±0.00[a]	3.04±0.01[a]	0.39±0.00[a]	0.76±0.00[a]	0.72±0.00[a]	16.79±0.07[a]	15.20±0.07[a]
LSD_{05}	0.04	0.05	0.01	0.00	0.02	0.25	0.38
Red pak choi 'Rubi F_1'							
HPS	1.32±0.02	2.84±0.01	0.38±0.01	0.62±0.00	0.56±0.00	14.50±0.14	13.20±0.06
LED 150	1.29±0.02	3.76±0.04[a]	0.39±0.01	1.18±0.00[a]	0.75±0.01[a]	17.67±0.27[a]	15.30±0.11[a]
LED 250	3.67±0.02[a]	7.90±0.02[a]	1.09±0.01[a]	1.80±0.00[a]	1.81±0.00[a]	59.92±0.24[a]	36.66±0.08[a]
LSD_{05}	0.04	0.06	0.01	0.01	0.01	0.55	0.22
Mustard 'Red Lion'							
HPS	0.36±0.00	0.91±0.01	0.09±0.00	0.25±0.00	0.18±0.00	3.42±0.06	1.49±0.05
LED 150	1.58±0.01[a]	3.55±0.02[a]	0.49±0.00[a]	0.92±0.01[a]	1.05±0.01[a]	23.98±0.22[a]	13.80±0.07[a]
LED 250	1.35±0.01[a]	2.62±0.02[a]	0.39±0.00[a]	0.74±0.00[a]	0.79±0.00[a]	30.63±0.27[a]	10.12±0.06[a]
LSD_{05}	0.01	0.03	0.00	0.01	0.01	0.27	0.17

Values are expressed as mean ± SD (n=3). LSD_{05} - Fisher's protected least significant difference ($P \leq 0.05$); a - the value is significantly higher than control (HPS). FW - fresh weight.

Nutritional quality depends not only on antioxidant properties, but also on the mineral elements occurring in plant tissues. The data obtained showed that mineral element content in microgreens can be changed by LEDs lighting (Table 3). The significantly higher contents of Ca, K, Mg, Na, P, Fe and Zn were determined after LED 150 and LED 250 treatments in microgreens. Photophysiological response of mineral elements to LED light may be generated through primary (saccharides) and secondary (flavonoids) metabolite biosynthesis pathways.

Conclusions

The industrially designed LED lamps lighting had a significant impact on *Brassicaceae* microgreen growth and nutritional quality. LED light prevented from undesirable microgreens elongation, led to increased contents of phytochemicals (phenols, total anthocyanin, flavonols and ascorbic acid) and mineral elements (Ca, K, Mg, Na, P, Fe and Zn). A ~150 µmol m^{-2} s^{-1} LED light treatment was rated as the optimal condition for microgreen growth and metabolism processes followed by ~250 µmol m^{-2} s^{-1} irradiation. Microgreens grown under ~250 µmol m^{-2} s^{-1} LED light treatment accumulated the highest contents of ascorbic acid, total phenols, flavonols, also DPPH and ABTS radicals scavenging activity that showed oxidative stress, and nitrates due to intense photosynthesis process.

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THE EFFECT OF GROWING SYSTEMS ON THE QUALITY OF CARROTS

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Abstract
The aim of the research was to evaluate how the quality of carrots is affected by organic and conventional production systems. The experiment was carried out at the Estonian Crop Research Institute in 2009. Conventional treatment received the following amount of nutrients via mineral fertilizers: N 115, P 40 and K 152 kg ha^{-1}. For plant protection, the following pesticides were used: Fenix, Fastac 50, Agil and Signum. In organic cultivation system compost and humic acid solution Humistar were used for fertilization and polypropylene non-woven fabric Agryl P-17 for plant protection. Marketable yield of organic carrots was 8% lower compared to the conventional carrots. Conventional carrots contained pesticide residues and had significantly higher nitrate concentration than organic carrots. The contents of total sugars, phosphorus, potassium, calcium and magnesium did not differ significantly between carrots from different cultivation systems. At harvest, dry matter (DM) content of organically grown carrots was significantly higher, whereas vitamin C and ß-carotene content was significantly lower in organically grown carrots. However, after 5-months of storage, the organic carrots had significantly higher total soluble solids (TSS) and β-carotene content compared to the conventional ones indicating that organically grown carrots were less susceptible to storage conditions.
Key words: β-carotene, conventional, *Daucus carota*, nitrates, organic, yield.

Introduction

The interest of consumption of organic food, including that of vegetables, has been steadily increasing during the last decade in Estonia and also in other countries (Winter and Davis, 2006; Pehme et al., 2007; Dangour et al., 2009; Smith et al., 2009; Matallana González et al., 2010). Vegetables and fruits are important sources of vitamins, minerals, trace elements, dietary fibre and a large variety of beneficial phytochemicals, which might decrease the risk of certain age-related and cardiovascular diseases. Therefore, the dietary guidelines recommend eating at least five portions of fruits and vegetables a day. Several factors are affecting the quality of raw products and the cultivation system is one of them. The composition of organically and conventionally produced vegetables has been studied for years, but further research is still recommended due to insufficient data (Hoefkens et al., 2009; Seljåsen et al., 2013). To add knowledge to the previously mentioned topic, the aim of the present research was to evaluate how the quality of carrots (both at harvest and after storage) is affected by organic and conventional production system.

Materials and Methods

The field experiment was carried out at the Estonian Crop Research Institute at Jõgeva (26°24'E, 58°44'N) in 2009 and was a repetition of the experiment done one year earlier (Bender et al., 2009). Carrots (*Daucus carota*) were cultivated under conventional and organic conditions on plots of 100 m^2 altogether, with four replications per treatment.

For two years before the experiment, cereals were cultivated on the area according to the EU regulations on organic production (Council Regulation No. 843/2007). Soil at the sites was sandy loam. The soil samples were analysed before establishment of the experiment using the following methods: pH – ISO 10390, P, K, Ca, Mg, Cu, Mn – Mehlich III, B – by Berger and Truogi and C_{org} – by NIRS. The nutritional status of the soil was satisfactory and soil acidity favourable for carrot cultivation (Table 1). No pesticide residues, as measured by the method prEN 15662: 2007 NIRS, were present in the soil. All soil samples were analysed in the Laboratory of Agrochemistry at the Estonian Agricultural Research Centre in Saku.

For basic fertilization, 800 kg ha^{-1} of Cropcare 8-12-23 (N 65, P 40, K 152 plus micronutrients) was applied to the conventional plot and 20 t ha^{-1} of green waste compost (analysed by the Laboratory of Agrochemistry at the Estonian Agricultural Research Centre to have N 1.0 g 100 g^{-1}, P 0.20 g 100 g^{-1}, K 0.3 g 100 g^{-1}, pH_{HCl} 6.76) was applied to the organic plot. During the growing period the conventional carrot

Table 1

Soil pH and nutrient status before experiment establishment in 2009

pH_{KCl}	P(ppm)	K(ppm)	Ca(ppm)	Mg(ppm)	Cu(ppm)	Mn(ppm)	B(ppm)	C_{org}(ppm)
7.0	79	105	2800	128	1.9	66	1.15	2.0

plants were fertilized by ammonium nitrate (N 50 kg ha^{-1}) and sprayed with Folicare 18-18-18 (4 kg ha^{-1}), resulting in a total supply of 0.7 kg N, 0.3 kg P and 0.6 kg K ha^{-1}. Organic carrot plants were fertigated at a rate of 1 litre per meter of row with humic acid solution (fertilizer Humistar diluted by 1:20), resulting in a total supply of 1.4 kg K ha^{-1}.

Carrot seeds of orange colored cv. Jõgeva Nantes were sown on 27 May. After seed sowing the organic plots were covered with polypropylene non-woven fabric Agryl P-17 for two months to control carrot psyllid (*Trioza viridula*) and carrot fly (*Psila rosae*). In the conventional system, the insecticide Fastac 50 was sprayed at 0.3 l ha^{-1} on 15 June and 28 July. The fungicide Signum 0.75 kg ha^{-1} was used on 12 August to control black rot (*Alternaria radiciana).* The soil of the conventional plots was treated with the herbicide Fenix at 2.5 l ha^{-1} two days before the sprouting of carrots and 18 days after seed sowing against couch grass with a herbicide Agil 100 EC at 1.0 l ha^{-1}. The organic plots were weeded twice by hand and hoeing. The crop was harvested manually on 8 October. Crop yield was calculated per hectare.

For postharvest experiment, 5 kg of carrots from both treatments from each replicate (all together 20 kg carrots per treatment) were stored in plastic boxes for recording postharvest loss. Additionally two more boxes (10 kg) of carrots from both treatments were stored for analyses. Carrots were stored for five months in coolstore of Estonian University of Life Sciences. Storage temperature ranged from 2 to 4 °C and RH was 85 to 90%. Postharvest loss (weight of carrots with decay or growth symptoms) was recorded monthly. The storage period finished in spring 2010. Quality analyses were performed at harvest and after 5-months of storage.

After harvest the carrot composition and amount of pesticide residues were determined from each of the replicate organic and conventional plots. Pesticide residues were analysed by gas chromatography in the Laboratory of Agrochemistry at the Estonian Agricultural Research Centre. Dry matter (DM), total sugars and ascorbic acid (vitamin C) were determined in the same laboratory; DM by 71/393 EEC methods, total sugars and vitamin C by using the standard methods. Total nitrogen was determined by Copper Catalyst Kjeldahl Method, (984.13); phosphorus by Stannous Chloride method, ISO/ FDIS 15681, AN 5242; calcium by o-Cresolphthalein Complexone method, ISO 3696, AN 5260 in Kjeldahl Digest by Fiastar 5000; magnesium by Fiastar 5000, ASTN90/92 by Titan Yellow method; potassium by Flame Photometric Method, (956.01); nitrates by Fiastar 5000, AN 5201 (nitrate - N, Cd-reduction, ISO 13395), Foss Tecator AB, 2001 (Helrich, 1990). Carrot macronutrients were determined at the Laboratory of Plant Biochemistry of the Estonian University of Life Sciences.

Before and after storage, the total soluble solids (TSS) content (°Brix) was measured using the digital refractometer ATAGO CO., Ltd., Japan and β-carotene was determined in the Health Protection Inspectorate Tartu Laboratory, using T44- HPLC method.

Significant differences between cultivation systems were tested by one-way analysis of variance and the effect of cultivation system and storage by two- way analysis of variance at significance level of $P \leq 0.05$. Mean values followed by the same letter are not significantly different at $P \leq 0.05$ in Figures.

Results and Discussion

In 2009 the rainy period started on 3 June with a low temperature and night frosts. This ameliorated the growth conditions. The marketable yield of conventional carrots (13.4 t ha^{-1}) was 8% higher than that of organic carrots in 2009. In 2008, the marketable yield of organic carrots was 11% higher than the conventional one (Bender et al., 2009). It has been reported earlier that the organic yield of vegetable is lower than the conventional (Fjelkner–Modig et al., 2000; Mäder et al., 2002). However, Dresbøll et al. (2008) found no differences in carrot yields between the two systems. Improved thermal regime and maintained humidity under the Agryl cover probably raised the organic marketable yield in our experiment in both years. Earlier studies on Chinese cabbage, beetroot and sweet pepper (Moreno et al., 2001; Gimenez et al., 2002; Michalik, 2010) have demonstrated the same effect of non-woven cover. However, Rekika et al. (2008) did not detect the effect of Agryl cover on radish yield at the harvest time. In our experiment, the applied humic acid fertilizer Humistar might have been another contributor to the yield of organic carrots. It is well established that humic acids improve soil chemical and physical quality and, in general, enhance root growth and development (Arancon et al., 2003). Although Lada et al. (2004) found that humic acid application to the soil promoted early seedling emergence of carrot, there was no significant yield difference compared to yield from the control treatment. Karakurt et al. (2009) demonstrated that soil humic acid treatment can be successfully used for increasing yield and improving fruit quality of organically grown pepper.

From the afore analysed pesticide residues one (α-cypermethrin below 0.01 mg kg^{-1}) was detected in 2009, two in 2008 (Bender et al., 2009) in the conventionally grown carrots and none in organically grown carrots. These results concur with those reported by Rembiałkowska and Hallmann (2007), who also found that organic vegetables were free of pesticide residues. According to the EU regulation

No 149/2008, the residues found in conventional carrots in our experiment did not exceed the permitted levels.

Cultivation system did not affect the content of total sugars, P, K, Ca and Mg of carrots in 2009 (data not shown) and also in 2008 (Bender et al., 2009).

Nitrogen content was significantly higher in the conventional than in organic carrots (1.5 and 1.1 g $100g^{-1}$, respectively) and the same result has been indicated in 2008 (Bender et al., 2009). In 2009, organic carrots contained nitrates at a low level, significantly lower than the conventional ones (26.6 and 149.6 mg kg^{-1}, respectively). In 2008, nitrates were not detected in organically grown carrots (Bender et al., 2009). Several studies have affirmed that organic carrots contain significantly less nitrogen and nitrate than do conventional ones (Leclerc et al., 1991; Warman and Havard, 1997; Rembiałkowska and Hallmann, 2007; Lairon, 2009; Seljåsen et al., 2013).

DM content of organically grown carrots was significantly higher than that of conventionally grown carrots (10.6 and 10.1 mg $100g^{-1}$, respectively), but organically and conventionally grown carrots did not differ significantly in their DM content in 2008 (Bender et al., 2009). Some earlier studies have shown that organic crops (including carrots) contained significantly more DM compared to conventional ones (Rembiałkowska, 2007; Sikora et al., 2009; Bender and Ingver, 2012). However, not all studies have confirmed this phenomenon. Therefore, review articles have identified only a trend for higher DM content, for instance, no significant differences have been found for organic vegetables (Woëse et al., 1997; Bourn and Prescott, 2002).

TSS content of organically grown carrots was significantly lower than that of conventionally grown carrots (Figure 1). Similarly, conventional carrot samples had higher TSS compared to organic ones in Ireland (Gilsenan et al., 2008). TSS of organically and conventionally grown carrots was not affected by the cultivation technology in 2008 (Bender et al., 2009).

No significant differences were found between organic and conventional cultivation in content of vitamin C in carrots in 2009 (5.7 and 5.0 mg $100g^{-1}$), therefore was significantly lower in 2008 (Bender et al., 2009). Vitamin content of a plant depends on a number of factors such as climate, genetic properties, fertilizer and soil (Mozafar, 1994). Also, according to Lee and Kader (2000), the content of vitamin C in vegetables depends on several factors, among them preharvest climatic conditions and cultural practices. As reported by Worthington (2001), organic crops (among them carrot) contained significantly more vitamin C than the conventional crops. Sikora et al. (2009) also have found that organic carrots contained significantly more ascorbic acid compared to the conventional ones. However, several scientists (Warman and Havard, 1997; Fjelkner-Modig et al., 2000; Brandt et al., 2011; Bender and Ingver, 2012) could not verify significant differences in vitamin C content caused by different cultivation systems.

In our experiment, no significant differences in β-carotene content were found between organic and conventional carrots (Figure 2), but conventional cultivation system significantly increased β-carotene content of carrots in 2008 (Bender et al., 2009). Rembiałkowska (2003) has demonstrated that organic carrots had less β-carotene. Contrarily, Sikora et al. (2009) reported that organic carrots contained significantly more carotenoids, such as β-carotene and lutein. The same was confirmed by Hoefkens et al. (2009) on literature-based comparison. The Danish scientists Søltoft et al. (2011) and Seljåsen et al. (2013) demonstrated in their reviews that the content of carotenoids in carrot roots was not significantly affected by the agricultural production system. Mentioned results indicate that the formation

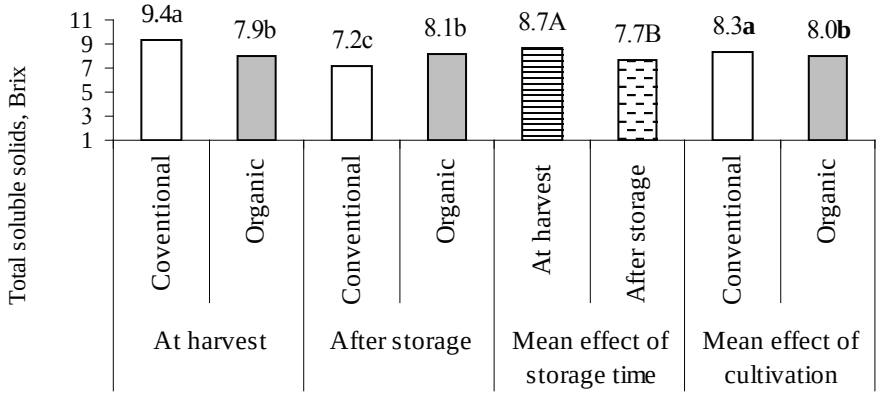

Figure 1. Total soluble solids content of organically and conventionally cultivated carrots cv. Jõgeva Nantes at harvest (October 2009) and after 5-months of storage (March 2010).

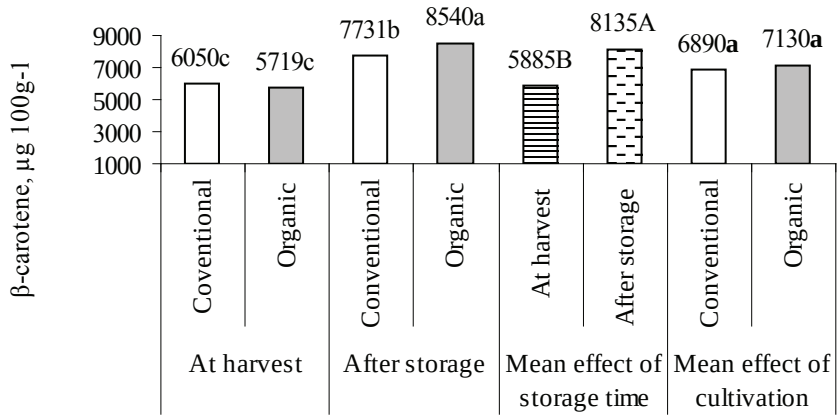

Figure 2. β-carotene content of organically and conventionally cultivated carrots cv. Jõgeva Nantes at harvest (October 2009) and after 5-months of storage (March 2010).

of secondary metabolites is often more dependent on the yearly different weather conditions, and this influence overshadows the possible effect of cultivation system.

After 5 months of storage, the average postharvest loss of organic carrots was 17% and of conventional carrots 19% (data not shown). The cultivation system had no significant effect on postharvest loss. During storage, TSS content of conventional carrots had significantly decreased, whereas TSS content of organic carrots remained unchanged (Figure 1). β-carotene content of both organic and conventional carrots had significantly increased during storage (Figure 2). Previously mentioned result is in agreement with earlier studies reporting that β-carotene content increases at storage temperatures above the freezing point (Howard et al., 1999). It is important to note, that after storage the organic carrots had significantly higher TSS and β-carotene content compared to the conventional ones.

Conclusions

The yield of the conventionally grown carrots was higher compared to organically grown carrots. The cultivation system did not significantly influence the content of analysed mineral elements and total sugars, but the quality of conventional carrots was significantly decreased by their high nitrate content and some pesticide residues. Organic carrots did not contain pesticide residues and contained nitrates at low level. At harvest, ß-carotene content was significantly lower in organically grown carrots. However, after 5-months of storage, organic carrots had significantly higher TSS and β-carotene content compared to the conventional ones indicating that organically grown carrots were less susceptible to storage conditions.

Acnowledgements

Current research was supported by Estonian Science Foundation Grant No 7515 and by target financed project of the Estonian University of Life Sciences No PK 9003.

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MOISTURE CONTENT EFFECT ON EXTRUDED PEA (*PISUM SATIVUM* L.) PRODUCT PHYSICAL PROPERTIES

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Abstract
From legume seeds it is possible to make new products with different physical properties such as size and hardness by using extrusion-cooking. Peas are products that normally need a rather long cooking time, but extrusion-cooking can make them more usable in daily human diet. As protein based food products in markets are less represented than those based on carbohydrates, but for balanced diet protein intake is essential, our aim was to ensure availability of such products, so experiments were carried out in order to establish optimal moisture content for grey pea (*Pisum sativum* L.) flour extrusion.
Three grey pea (*Pisum sativum* L.) based products were obtained with different water amount added before extrusion, and their size, volume mass and colour was analyzed in order to ascertain what amount of water is best for such products.
Results show significant differences for size, volume mass and colour changes, establishing that the best of the products was the one with 9.00±0.01 g·100g^{-1} added water. This product had better characteristics than others, where 11.00±0.01 g·100 g^{-1} and 7.00±0.01 g·100 g^{-1} water was added. The products with 9.00±0.01 g·100 g^{-1} added water were by more than 100 g·L^{-1} lighter than other products, also colour changes compared to non extruded pea flour were fewer than for other samples and their size was the biggest of all obtained products, averagely 11±1mm.
Key words: extrusion-cooking, pea, size, moisture.

Introduction

Extruded products are popular, since they are ready-to-eat, of crispy texture, nicely shaped and coloured. However, they are often regarded as junk food because of their composition mainly based on carbohydrates and fat (Hirth et al., 2014).

The design of extruded snacks and breakfast cereals involves complex molecular transformations such as gelatinization and melting of the mostly starch based raw materials. The raw material has to be sheared, mixed, cooked and expanded to generate the desired product structure. Extrusion cooking is a high pressure, high-temperature, short-time (HTST) process, which can apply the amount of thermal and mechanical energy to raw materials in a relatively short period of time. The short residence time should reduce the undesirable reactions (e.g. degradation or chemical reaction of protein) (Hirth et al., 2014).

The food industry is using HTST technology such as extrusion-cooking for many applications including inactivation of microorganisms and toxins, changes of texture and flavour, improvement of digestibility (Boye et al., 1997) to produce food with high quality attributes and enhanced food safety (Gould, 1995; Jung et al., 2011; Knorr, 1999).

The extent of protein unfolding and exposure of hydrophobic patches would increase with the increase in extent of heating (Boye et al., 1997; Tang et al., 2009). Privalov et al. (1989) estimated that proteins lose almost all secondary and tertiary structures when the temperature exceeds 80 °C, and would adopt a configuration that approaches a fully unfolded, random coil conformation. Thus, these heat-induced changes in protein conformation and polypeptide interactions could dictate the degree of exposure of susceptible peptide bonds and influence the type of peptides generated during enzymatic hydrolysis (Chao et al., 2013), where the amount of added water may play a significant role.

There is an increased interest in utilizing the pea (*Pisum sativum* L.) protein as an alternative for soy protein that has a dominating advantage in the market. The pea is one of the valued crops in the world market (Adebiyi and Aluko, 2011; Tian et al., 1999); like other legume seeds, the pea seed is characteristically rich in proteins (18–30%) with a well-balanced amino acid profile, especially a high content in lysine (Schneider and Lacampagne, 2000). In addition to providing amino acid nutrition level, the ultimate success of using pea protein as a promising food ingredient and an alternative to soy proteins depends largely on its functional properties, including solubility, viscosity, water- and oil-binding properties, gelatinization, foaming and emulsifying properties. To date, pea protein products are very limited in food applications (Liang and Tang, 2013).

Peas contain high levels of protein/amino acids and, accordingly, their potential nutritional value is rather high. However, various anti-nutritional factors in peas interfere with digestive processes, thereby reducing their nutritional value (Gatel and Grosjean, 1990). The major anti-nutritional factors are trypsin inhibitors and, although their levels in peas are 5–20 times lower than in raw soy beans, the amount found in some pea cultivars can be significant and responsible for reduced protein digestibility of pea-

based diets (Al-Marzooqi and Wiseman, 2009). It has been reported (Valdebouze et al., 1980) that the trypsin inhibitor activity (TIA) of winter types was twice that of those sown in spring, and that smooth peas had higher TIA than wrinkled-seeded varieties. Peas also contain appreciable level of starch, although there is considerable variability. For example – the starch content of the smooth seed types is higher than the wrinkled ones (Gatel and Grosjean, 1990). Potentially, peas are a very valuable foodstuff in terms of their energy-yielding potential (French, 1984; Al-Marzooqi and Wiseman, 2009).

The level of trypsin inhibitors may be reduced by heat processing, and the susceptibility of starch to enzymes can be increased by gelatinisation or any other process that destroys the granular structure of starch (Holm et al., 1985); heating also may lead to loss of α-amylase inhibitors (Alonso et al., 2000). Extrusion is a process which involves forcing a material to flow under a variety of controlled conditions to pass through a shaped hole or slot at a predetermined rate. The operating conditions of high temperature, pressure and shear, and low or average water content (all of which can be varied during the process) distinguish extrusion cooking from alternative heating processes and gives the opportunity to ensure that even those low levels of anti-nutritional factors are removed.

It is said (Al-Marzooqi and Wiseman, 2009) that such processing has a limited effect on the nutritional value of peas with low levels of trypsin inhibitors as measured through digestibility of amino acids. However, cultivars with high trypsin inhibitor level may benefit from mild processing (Al-Marzooqi and Wiseman, 2009).

In order to ensure the availability of pea (*Pisum sativum* L.) based products, our aim was to establish the optimal moisture content for grey pea (*Pisum sativum* L.) flour extrusion.

Materials and Methods

Peas (*Pisum sativum* L.) of the variety "Bruno" were used for the experiments. They were milled at "Grauda spēks" Ltd. and 7.00±0.01 g·100 g^{-1}, 9.00±0.01 g·100 g^{-1} and 11.00±0.01 g·100 g^{-1} water was added to flour (moisture – 13.59±0.01 g·100 g^{-1}). Flour and water were carefully mixed in automatic mixer BFJ – I/3 until the mixture was homogenous and extruded with twin screw extrusion-cooker SLG65-III at temperatures 50/150/170 °C and speed 22 Hz. Then the product was dried for 15 minutes in a belt type dryer at 80 °C temperature.

The moisture content (g·100g^{-1}) in the obtained products was determined using ISO 6496:1999. The analyses were done in three repetitions.

The size of the obtained sample pieces (length and height, mm) was measured using the "Electronic digital outside micrometer 08/03" the analyses were done in ten repetitions. The colour was measured in CIE L*a*b* colour system using Tristimulus Colorimeter, measuring Hunter colour parameters by Colour Tec PCM/PSM. Colour values were recorded as L* (brightness) – the vertical co-ordinate runs from L* = 0 (black) through grey to L* = 100 (white); a* (-a, greenness, +a, redness) – the horizontal co-ordinate that runs from -a* (green) through grey to +a* (red) and b* (-b, blueness, +b, yellowness) – another horizontal co-ordinate that runs from -b* (blue) through grey to +b* (yellow). The samples were milled and filled in a Petri dish. The measurements were repeated ten times on different randomly selected locations at the surface of each sample.

The total colour differences of extruded samples were calculated using the formula (1).

$$\Delta E^* = \sqrt{\left[\left(L^* - L_0^*\right)^2 + \left(a^* - a_0^*\right)^2 + \left(b^* - b_o^*\right)^2\right]}; \quad (1)$$

Where ΔE* – total colour difference;
L*, a* and b* are the lightness (L), greenness and (a) and blueness (b) values for extruded samples;
L_0, a_0 and b_0 are the corresponding colour values for non extruded pea flour (Papadakis et al., 2000).

The volume mass (g·L^{-1}) was measured gravimetrically, where each sample was poured up to the mark in a 1L measuring flask and weighed on the balance Precisa 260. The measurements were made at least three times. The hardness (N) was analysed for each sample granule with Texture Analyzer, TA.XT. plus with parameters: pre-test speed 1.5 mm sec^{-1}; test speed 1 mm sec^{-1}; post-test speed 10 mm sec^{-1}, distance 5 mm, selected probe: P/2 DIA stainless steel cylinder. The measurements were made in ten repetitions. The sugar content was determined using ГОСТ 26176-91. The analyses were done in three repetitions.

ANOVA analyses were applied in order to see which moisture content would be the best for further product development.

Results and Discussion

The moisture content in the grey pea flour that was used in experiments was 13.59±0.01 g·100 g^{-1}, so the total moisture content (with added water) in flour before extrusion was 20.59 g·100g^{-1}, 22.59 g·100 g^{-1} and 24.59 g·100 g^{-1}, respectively. All products after extrusion-cooking were dried till 7±1 g·100 g^{-1} moisture. The volume mass was determined in order to examine the aeration of obtained products (Figure 1). The product with added water content 7.00±0.01g·100 g^{-1} – 280±4 g·L^{-1} was the heaviest, but the lightest was the one with added

water content 9.00±0.01 g·100 g^{-1} – 126±2 g·L^{-1} and 241±5 g·L^{-1} for the sample with 11.00±0.01g·100 g^{-1} added water. Sample with 9.00±0.01 g·100 g^{-1} added water had 153 g (33 g·100 g^{-1}) less volume mass than those products obtained using 7.00±0.01 g·100 g^{-1} added water and by 115 g (42 g·100 g^{-1}) for the sample with 11.00±0.01 g·100 g^{-1} added water.

The explanation for these changes could be that the sample with 7.00±0.01g·100 g^{-1} added water was not moisturised enough. As for the high result obtained using sample with 11.00±0.01g·100 g^{-1} added water, literature states that too mush water can reduce product size and make it harder and heavier (Moscicki, 2011), but does not clearly state the reason for such an effect. Mathematical analysis shows significant differences between product volume mass ($\alpha = 0.05$, $p = 1.64 \cdot 10^{-8}$).

The size of granules was measured in order to mathematically describe the samples (Figure 2). The sample with largest granule size was the one with 9.00±0.01 g·100 g^{-1} water added; the mean length was 10.3±0.5 mm and the mean width was 11.7±0.8 mm. The same sample had the lowest volume mass. On the other hand, the samples with 7.00±0.01g·100 g^{-1} and 11.00±0.01g·100 g^{-1} added water were more symmetrical, both having sizes of approximately 7 ± 1 mm; still the distribution of sizes for these products was larger, especially for the sample with 7.00±0.01 g·100 g^{-1} added water – its length was 7±2mm and width 7±1mm, while the sample with 11.00±0.01 g·100 g^{-1} added water measured 7.8±0.8 mm in length and 8±1mm in width. Such fluctuations within driest sample could be caused by too little moisture content, and the flour was not enough hydrated.

Mathematical analysis shows significant differences between the product size for length $\alpha = 0.05$, $p = 9.26 \cdot 10^{-5}$, and for width $\alpha = 0.05$, $p = 2.61 \cdot 10^{-17}$.

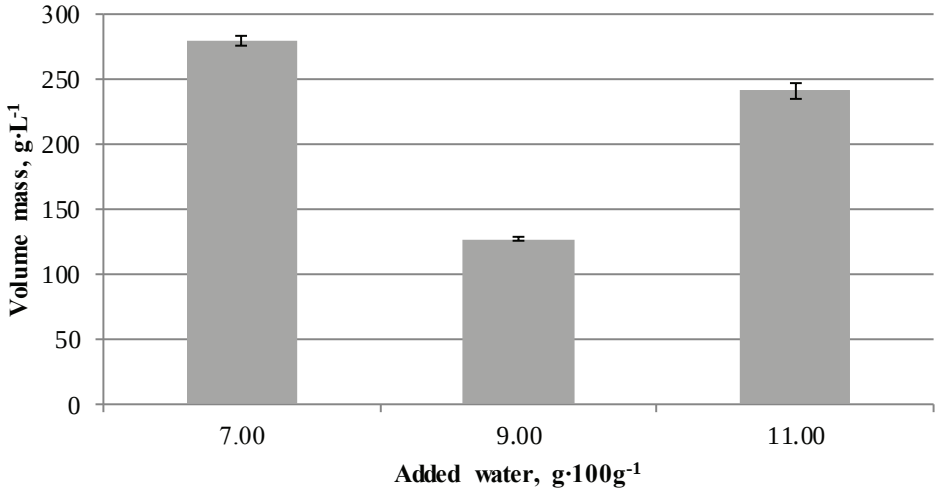

Figure 1. Volume mass of the extruded samples.

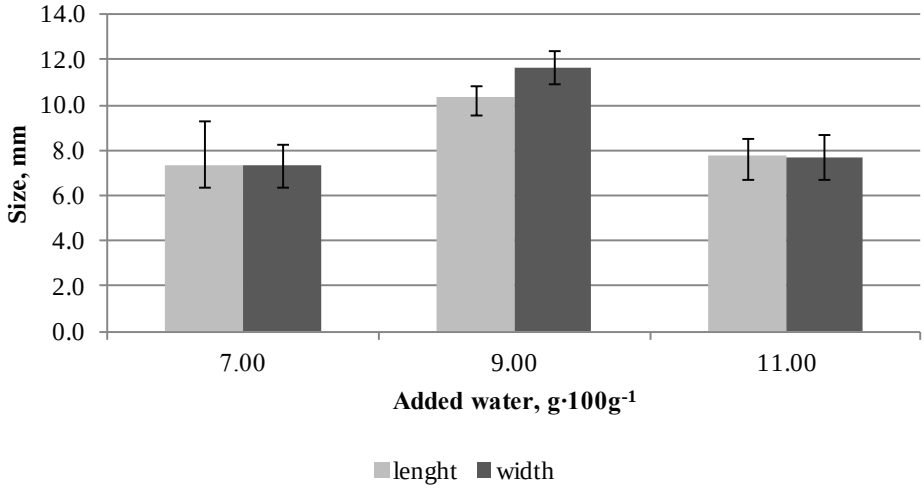

Figure 2. The size of the granules of the extruded samples.

Table 1

The colour values for pea flour and extruded samples, and their comparison to pea flour

Sample, added water, g·100g^{-1}	Colour value			Difference from non extruded flour			ΔE*
	L*	a*	b*	ΔL*	Δa*	Δb*	
0[1]	73.7±0.8	-1.8±0.3	14.3±0.3	-	-	-	-
7.00±0.01	67.6±0.9	0.2±0.5	17.9±0.8	-6.1	2.1	3.5	7.4
9.00±0.01	70.1±0.7	-0.1±0.1	17.7±0.7	-3.6	1.7	3.3	2.5
11.00±0.01	65.9±0.9	0.05±0.6	17.2±0.3	-7.8	1.9	2.9	4.2

[1] flour without added water before extrusion

To describe the possible chemical reactions that could occur in products during high pressure and heat treatment, colour is a good indicator. Colour parameters of non extruded pea flour and extruded products are shown in Table 1. As in Maillard reactions protein is bound with sugar in Amadori compounds (Martins et al., 2000), thus reducing protein availability and nutritional value, so they should be avoided if possible or reduced to minimum. As these reactions change the colour of product, colour was analysed in order to establish if there had been significant changes of colour in products.

The least significant changes in products L parameters were found in the sample with 9.00±0.01 g·100 g^{-1} added water, it got darker only by 3.6 units (8 g 100 g^{-1}), but the biggest changes were found in the sample with 11.00±0.01 g·100 g^{-1} added water 7.8 units (12 g 100 g^{-1}). As the redness of the samples too could be used as indicator of non-enzymatic browning, but the changes were almost the same for all samples, respectively for the sample with 7.00±0.01 g·100 g^{-1} added water – it was redder by 2.1 units, but the least for the sample with 9.00±0.01 g·100 g^{-1} added water – 1.7 units. The same conclusions can be made from the total colour difference ΔE*, where the least changes were observed for the sample with 9.00±0.01 g·100 g^{-1} added water – only 2.5 units, while the largest value of ΔE* was ascertained for the sample with 7.00±0.01 g·100 g^{-1} added water – 7.4 units. Still, these changes probably originated more from other browning processes, as no significant traces of sugar were found in neither the raw material, nor the products.

The hardness of the samples was analysed (Figure 3.) in order to describe crispiness of the granules. The hardness of the sample with 7.00±0.01 g·100 g^{-1} added water was 35±3 N, but the least significant was of the sample with 9.00±0.01 g·100 g^{-1} added water – 8±1 N, meaning that the samples with 7.00±0.01 g·100 g^{-1} added water and 11.00±0.01 g·100 g^{-1} added water were harder than those with 9.00±0.01 g·100 g^{-1} added water, which means they are more airy. Similar conclusions can be made for the volume mass.

The sample with 9 g·100g^{-1} added water is not only the lightest, but also the largest as well as softest, as can also be seen from such parameters as size, volume mass and hardness. It also has the least significant changes in colour values compared to non extruded pea flour.

Conclusions

In order to produce pea (*Pisum sativum* L.) based food products using extrusion-cooking, the optimal

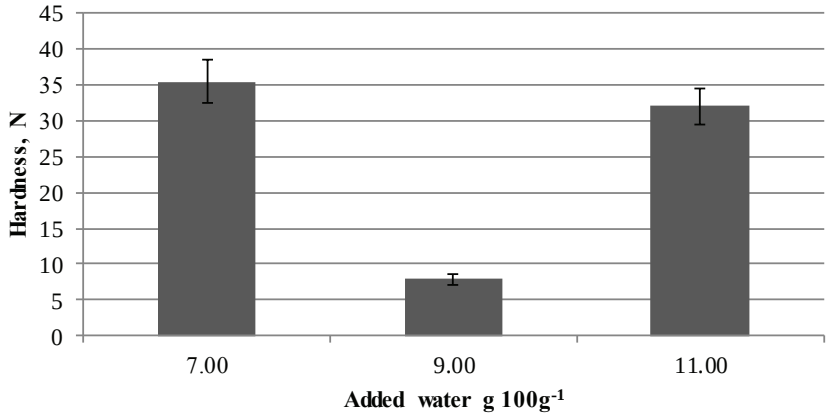

Figure 3. Hardness of the extruded samples granules.

water amount added to flour is 9.00±0.01 g·100 g^{-1}, as such water amount in temperatures 50/150/170 °C gives products better characteristics than using 7.00±0.01 g·100 g^{-1} water and 11.00±0.01 g·100 g^{-1} water. The volume mass for products obtained using 9.00±0.01 g·100 g^{-1} water was 126±2 g·L^{-1}, by 153 g (33 g·100 g^{-1}) less than volume mass for those products obtained using 7.00±0.01 g·100 g^{-1} added water and by 115 g (42 g·100 g^{-1}) for the sample with 11.00±0.01 g·100 g^{-1} added water.

The size of the products obtained adding 9.00±0.01 g·100 g^{-1} water to flour was 10.3±0.5 mm in length and 11.7±0.8 mm in width, larger than those obtained using 7.00±0.01 g·100 g^{-1}.

The hardness of the sample with 9.00±0.01 g·100 g^{-1} added water was 8±1 N. The colour changes from non extruded flour were the slightest for the sample 9.00±0.01 g·100 g^{-1}.

Acknowledgements

This study was supported by the European Union's Seventh Framework project "Eurolegume", project No. 613781.

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INDUSTRIAL POTATO PEEL WASTE APPLICATION IN FOOD PRODUCTION: A REVIEW

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Abstract
Potato (*Solanum tuberosum* L.) is one of the most important agricultural crops for human consumption and high amount is produced worldwide every year. Potato peel waste is a zero value by-product, which occurs in big amounts after industrial potato processing and can range from 15 to 40% of initial product mass, depending on the peeling method. Food waste utilization causes great concern in food industry in Europe and many scientific works were written on this topic in the last years offering solutions and original approaches.
Present article aims to summarize the review of available literature on industrial potato peel waste application possibilities in food production industry. Scientific articles on food waste management, potato peel chemical composition and recycling methods have been studied. The main results show, that there is a big potential for potato peel extract as an antioxidant in food systems due to its high phenol content. In addition, potato peel powder could serve as a partial flour replacement in dough up to 10 g 100 g^{-1} of flour weight without causing significant changes in sensory properties. Potato peel waste can serve as a solid substrate for fermentation. Further investigations in the present field are needed in order to evaluate full potato peel waste application potential.
Key words: potato waste utilization, phenols, dietary fibre.

Introduction

Potatoes (*Solanum tuberosum* L.) are one of the most important agricultural crops for human consumption after wheat (*Triticum* L.), rice (*Oryza* L.) and maize (*Zea mays* subsp. *mays* L.), with 376 million tons produced in 2013 (Compare data…, 2013). In developed countries up to 69.5% (in 2012) of total produced potatoes are processed (U.S. per Capita…, s.a.). Potatoes are usually peeled during processing and production losses in a form of potato peel waste (PPW) can vary from 15 to 40%, depending on the peeling method (Arapoglou et al., 2009). Each year huge quantities of PPW as a by-product remain after industrial potato processing.

Abrasion peeling is typical for chips production, whereas steam peeling is used for dehydrated and frozen potato products (Schieber and Saldaña, 2009). Steam peelers are compact and generate less product losses, but require high investment and operation costs. Because of that, steam peeling is reasonable when high quantities of product (from 8 to 20 t h^{-1}) have to be peeled in limited space and appearance of brown ring (also known as heat ring, or cooking ring) does not cause problems for final product (Steam peeling…, s.a.). Brown ring occurs due to tissue damage and enzyme-catalyzed phenolic oxidation reaction (Bayindirli, 2010). It is reported, that chemical peeling using NaOH could replace steam peeling to avoid heat-ring (Garrote et al., 1993). Otherwise abrasion peeling is to be used.

PPW is not suitable for non-ruminants without further treatment because it is too fibrous to be digested (Birch et al., 1981), but as an inexpensive by-product it contains a large quantity of starch, non-starch polysaccharides, lignin, polyphenols, protein and small amount of lipids. This makes it a cheap and valuable base material for extraction of valuable products (such as natural antioxidants, dietary fibre, biopolymers, etc.) and fermentation processes (Arapoglou et al., 2009; Al-Weshahy and Rao, 2012; Wu et al., 2012).

Many studies were made and articles were written on PPW application possibilities in order to minimize industrial waste amount and find suitable application for PPW as a by-product. The aim of the present study is to summarize the available literature on possible industrial PPW utilization methods and highlight its application possibilities in food production.

Materials and Methods

Monographic method has been used for this study. Available literature on food waste management, PPW chemical composition and recycling methods have been studied with the aim to cover broad spectrum of methods developed for industrial potato waste application possibilities in order to produce new food products.

Results and Discussion

PPW chemical composition

Raw potato peels have high moisture and carbohydrate contents, but overall protein and lipid contents are generally low (Table 1). High content of starch (52 g 100 g^{-1} of dry weight) makes it a good basis for fermentation (Potatoes, raw, skin, s.a.; Arapoglou et al., 2009). In addition, potato peels (PP) contain a variety of valuable compounds, including phenols, dietary fibres, unsaturated fatty acids, amides, etc. (Schieber and Saldaña, 2009; Wu et al., 2012).

Table 1

Chemical composition of raw potato peel, g 100 g^{-1}

Compound	Minimum and maximum values	Average content	Source*
Water	83.3-85.1	84.2	Potatoes, raw, skin, s.a. Arapoglou et al., 2009
Protein	1.2-2.3	1.8	Arapoglou et al., 2009 Potatoes, raw, skin, s.a.
Total lipids	0.1-0.4	0.3	Potatoes, raw, skin, s.a. Arapoglou et al., 2009
Total carbohydrate	8.7-12.4	10.6	Arapoglou et al., 2009 Potatoes, raw, skin, s.a.
Starch	7.8		Arapoglou et al., 2009
Total dietary fibre	2.5		Potatoes, raw, skin, s.a.
Ash	0.9-1.6	1.3	Arapoglou et al., 2009 Potatoes, raw, skin, s.a.

* The first source refers to min value, the second – to max value.

PP contains high quantities of polyphenols which have a role in the defence mechanism against phytopathogens. Therefore almost 50% of phenolics are located in the peel and adjoining tissues and decrease toward the center of the tuber (Friedman, 1997). PP polyphenols can reach almost three times more antioxidant activity than the other plant tissues.

Total phenolic compound content in PPW differs between potato cultivars (Murniece et al., 2014) and it is very heterogeneous class that can be classified in phenolic acids and flavonoids. Phenolic acids are the main phenolic compounds in potatoes (Mäder et al., 2009; Schieber and Saldaña, 2009; Singh and Saldaña, 2011) and a major part of them make neochlorogenic, chlorogenic and caffeic acids (Sánchez Maldonado et al., 2014). Most common flavonoids are flavonols, flavan-3-ols, flavones, flavanones and anthocyanins (Schieber and Saldaña, 2009). Total phenolic content in freeze-dried PP extract can be in range from 1.5 to 3.3 mg of gallic acid equivalents per gram (GAE g^{-1}) (Rodriguez De Sotillo et al., 1994; Al-Weshahy and Rao, 2009). Some studies show up to 4.3 mg of GAE g^{-1} (Kähkönen et al., 1999). The highest phenolic content is obtained from red-colour PP extracts. This may be explained with high amount of anthocyanins which are pigments in red-colour varieties of potatoes (Im et al., 2008; Al-Weshahy and Rao, 2009), and in some coloured potato varieties anthocyanin content in peel can be up to 2.5 times higher than in flesh (Jansen and Flamme, 2006). Major phenolic compounds in PPW freeze-dried extracts are chlorogenic (50 g 100 g^{-1}), gallic (42 g 100 g^{-1}), protocatechic (8 g 100 g^{-1}), and caffeic (0.2 g 100 g^{-1}) acids (Rodriguez De Sotillo et al., 1994). Due to this fact, after potato processing the highest content of phenolic compounds is in peeling by-products (Mäder et al., 2009). Moreover, PP phenols both free and bound-form show higher radical scavenging activity than potato flesh phenols (Nara et al., 2006).

Phenolic compounds contribute to antioxidant activity and protect plants against various biotic and abiotic stresses. As a result, their presence in human diet prevents degenerative diseases (Pourcel et al., 2007; Im et al., 2008). There is a strong correlation between total phenol content in the extract and antioxidant properties, and losses are observed in total polyphenol amounts in PP during storage even at -20 ºC. In the freeze-dried samples moisture level is lower than 2 g 100 g^{-1} and it is still possible that some enzymes can still remain active (Al-Weshahy et al., 2013). Because of that, PP require proper storage conditions to maintain their antioxidant properties (Al-Weshahy and Rao, 2009; Al-Weshahy et al., 2013).

Glycoalkaloids are secondary plant metabolites and they are toxic to humans, animals, microorganisms, insects and viruses (Mäder et al., 2009; Schieber and Saldaña, 2009). The main glycoalkaloids found in PP are α-solanine, α-chaconine and solanidine, and they are suitable for utilization in pharmaceutical industry (Friedman, 2004; Sánchez Maldonado et al., 2014). Studies on rats show that extract derived from PP significantly offsets carbon tetrachloride induced liver injury (Singh et al., 2008). Total levels of solanine and chaconine in PP can range from 84 to 3526 mg kg^{-1} (with ratio of α-solanine to α-chaconine from 2.1 to 2.4) and it depends on variety of factors such as potato cultivars, light, irradiation, conditions of storage and mechanical injury (Friedman, 2004).

PPW is a good source of dietary fibre: primarily insoluble carbohydrates – cellulose, hemicellulose, lignin, pectin, gums, etc. (Al-Weshahy and Rao, 2012) with average content of 40 g 100 g^{-1} (Camire and Flint, 1991) and it depends on the peeling method. Abrasion peeling results in more starch and less dietary fibre (especially lignin) comparing to the steam peeling. In

addition, higher amount of glucose can be recovered from the insoluble fibre fraction of steam peeled wastes, and this fact can be explained by formation of resistant starch (Camire et al., 1997).

Safety of PPW application

PP extracts results in mixtures of phenols and toxic glycoalkaloids and separation is needed prior to phenolic extract application in foods (Hossain et al., 2014; Sánchez Maldonado et al., 2014), where it has huge potential as antioxidant (Mansour and Khalil, 2000; Friedman, 2004; Zia-ur-Rehman et al., 2004; Arapoglou et al., 2009; Al-Weshahy and Rao, 2012). Moreover, at present moment there are no method for the simultaneous recovery and subsequent separation of phenols and glycoalkaloids (Sánchez Maldonado et al., 2014). It is reported, that a glycoalkaloid limit of 20 mg 100 g^{-1} is acceptable for human consumption (Papathanasiou et al., 1999).

Synthetic antioxidants are commonly used in food production, however, due to safety concerns, interest in natural antioxidants is high (Shahidi, 2000). It is reported, that synthetic antioxidants are toxic in high dosages (Ito et al., 1986; van Esch, 1986; Kahl and Kappus, 1993). From the other side, safety limits of natural antioxidants are mostly unknown and there is no guaranty that they are safer than synthetic analogue (Pokorný, 2007). Synthetic antioxidants butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) and natural vitamin E in high dosages induce impairment of blood clotting in animals (are antagonists to vitamin K), but in contrast to BHA and BHT, comparing carcinogenic effect of high dosages, vitamin E is not carcinogenic (Kahl and Kappus, 1993). Further studies on antioxidant toxicity are needed.

It has been reported that in terms of safety as natural antioxidant in foods, PPW phenolic extract is not mutagenic (Sotillo et al., 1998).

PPW application in food industry

Methanol, ethanol and water are the most commonly used solvents for phenolic compound extraction from PP (Singh and Rajini, 2004; Mohdaly et al., 2010; Amado et al., 2014; Sánchez Maldonado et al., 2014). Phenols extracted from PPW show potential in food industry as a natural antioxidant to prevent lipid oxidation (Singh and Rajini, 2004; Mohdaly et al., 2010). It is reported, that PPW extract is able to protect against oxidation of soybean oil (Onyeneho and Hettiarachchy, 1993; Zia-ur-Rehman et al., 2004; Amado et al., 2014) and fish-rapeseed oil mixture (Koduvayur Habeebullah et al., 2010). Soy bean oil samples, containing 1600 and 2400 mg kg^{-1} of PP petroleum ether extract after 60 day storage at 45 °C, showed lower peroxide values (10 and 9 meq kg^{-1}) and free fatty acids content (1.2 g kg^{-1} and 1.1 g kg^{-1}), than the control samples (59 meq kg^{-1} and 3.2 g kg^{-1}, respectively) (Zia-ur-Rehman et al., 2004). Positive results were gained on freeze-dried PPW extracts application in raw beef patties during cold storage at ~5 °C for 12 days. In this study, freeze-dried extract showed maximum antioxidant capacity at pH range 5-6 (Mansour and Khalil, 2000). Moreover, it is capable to retard lipid peroxidation of irradiated meat and antioxidant activity of PP extract was found to be comparable to BHT (Kanatt et al., 2005). It is very important aspect because during irradiation process meat can undergo oxidative changes that affect its quality (Formanek et al., 2003). Ethanol extracts were found to be very effective in retarding lipid and protein oxidation in mackerel mince (*Trachurus trachurus*). Samples of mackerel mince (with PPW ethanol extract concentrations 2.4 and 4.8 g kg^{-1}) were stored at 5 °C for 96 h. Antioxidant effect of the extract resulted in low levels of peroxide value and carbonyl compounds (Sabeena Farvin et al., 2012).

Studies show that potato dietary fibre is able to bind bile acids in-vitro and can be part of the mechanism that lowers plasma cholesterol (Camire et al., 1993). In addition, high intake of dietary fibre has a positive effect on blood glucose profile (Onyechi et al., 1998; Chandalia et al., 2000; Singh et al., 2005). Studies were made by adding dry PPW powder to baked products. The best results were achieved by replacing 10–15% of flour in dough. Prepared samples were darker and harder than control without differences between types of peels. Meanwhile, replacing up to 10% of flour did not make significant sensory changes comparing to control (Toma et al., 1979; Magied, 1991; Arora and Camire, 1994; Camire et al., 1995; Pasqualone et al., 2013).

PPW extract at high concentrations found to have species independent antibacterial and antifungal activities, especially significant compared to streptocycline (an effective antibiotic against bacterial plant pathogens) against *Pseudomonas aeruginosa* (Deviprasad and Pushpa, 2007). Conversely, other studies show that PPW extract is species dependent and is effective against gram negative and one gram positive bacteria and have only bacteriostatic effect – inhibits multiplication of bacteria (Sotillo et al., 1998).

Edible films, films produced from edible biopolymers and food grade additives (Han, 2014), can be produced from PPW (Kang and Min, 2010; Tammineni et al., 2013). H.J. Kang and S.C. Min (2010) developed edible film production method. In their studies, PPW powder was produced, mixed with water and pre-homogenized. To destruct biopolymers, homogenate was treated in three ways: by high-pressure homogenization (138 MPa), gamma irradiation (10-20 kGy) or ultrasound (24 kHz, 120 μm). Glycerol as

plasticizer and soy lecithin as emulsifier were used. Film, prepared by high-pressure homogenisation, showed better moisture barrier, tensile and colour properties. Present results make good basis for further studies, because produced PPW-based film should be further improved to be applied to food products.

There are several potential compounds that can be manufactured from PPW by using fermentation reactions (Salvador et al., 2002), such as ethanol (Arapoglou et al., 2009, 2010; Kheyrandish et al., 2015), lactic acid (Zhang et al., 2006, 2007; Zhang, 2008; Liang et al., 2014), enzymes (Mahmood et al., 1998; Sachslehner et al., 2000; Asgher et al., 2007; Mabrouk and El-Ahwany, 2008).

PPW features a high potential for ethanol production that has a large potential market (Arapoglou et al., 2010; Kheyrandish et al., 2015). PPW contains sufficient quantities of starch, cellulose, and hemicellulose. However, fermentable reducing sugar content is very low, 0.6 g 100 g^{-1} of dry weight and because of this fact fermentation of raw material is not practical and an initial hydrolysis of carbohydrates is necessary. Sugars can be hydrolysed with various enzymes and/or acid, and fermented by *Saccharomyces cerevisiae* var. *bayanis* (Arapoglou et al., 2009, 2010). Studies show that sulphuric acid is an adequate acid to hydrolyse dried potato samples for further fermentation and it is more efficient than enzymatic hydrolysis (Guerra-Rodríguez et al., 2012).

Among different compounds, lactic acid can be produced during hydrolysed PPW fermentation process using *Rhizopus* fungus cultures (Zhang et al., 2007, 2008) and undefined mixed culture inoculated from wastewater treatment plant sluge (Liang et al., 2014). Concentration of 85.7 g L^{-1} was obtained by using *Rhizopus arrhizus* in association with the formation of coalesced loose small pellets (Zhang et al., 2008).

PPW starch can be used for enzyme production such as thermostable α-amylase, a starch hydrolysing enzyme that is widely used in different food industries. Positive results were achieved using *Bacillus subtilis* strains (Mahmood et al., 1998; Asgher et al., 2007) with maximum enzyme production after 48 h of cultivation at pH 7.0 and 50 °C. Enzymes had optimum activity at pH 8.0 and 70 °C, were stable for 1 h at 60 and 70 °C and had suitable characteristics for application in starch processing and food industries (Asgher et al., 2007). In addition, enzyme β-mannanase can be produced using *Bacillus amylolequifaciens* and it is reported that PPW found to be the most effective carbon source, comparing to peel samples of orange, apple, mango, as long as palm seed powder, corn cob and rice husk (Mabrouk and El-Ahwany, 2008). Currently β-mannanase is used in instant coffee processing for coffee mannan hydrolysis to significantly reduce the viscosity of coffee extracts (Sachslehner et al., 2000).

Conclusions

1. Potato peel waste (PPW) can serve as a basis for phenol extraction, ethanol, lactic acid and enzyme (α-amylase and β-mannanase) production through fermentation, and edible film production.
2. PPW extract has a high application potential as antioxidant in food systems. It can prevent lipid oxidation in oils and meat.
3. PPW has potential as a base for fermentation reactions because of high starch content, but due to its low fermentable reducing sugar content, requires initial hydrolysis of carbohydrates.
4. PPW can be used in healthy and functional food production as dietary fibre source. It can be used in bakery production and replace up to 10% of flour amount without changes in sensory quality.

Acknowledgements

Present study was supported by Latvian State Research programme 'Agricultural Resources for Sustainable Production of Qualitative and Healthy Foods in Latvia (AgroBioRes)' (2014–2017). Project No. 4 'Sustainable use of local agricultural resources for qualitative and healthy food product development (FOOD)'.

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TECHNOLOGICAL PROPERTIES OF PEA AND BUCKWHEAT FLOURS AND THEIR BLENDS

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Abstract
Pea and buckwheat flours are gluten free and have high nutritional value; therefore they are advisable for frequent consumption. The addition of pea and buckwheat flours to products changes their nutritional value and technological properties significantly. The aim of the research was to investigate the starch content, colour and rheological properties of pea and buckwheat flours and their blends.
Results showed that pea flour had a higher content of starch than wheat and buckwheat flours, pea-buckwheat flour blends and formed the largest part of dry matter. The peak, holding, final, breakdown and setback viscosities of buckwheat flour, in turn, were significantly higher than those of wheat (control) and pea flours. Buckwheat flour provided higher peak, holding, final, breakdown and setback viscosities and lower starch gelatinization temperature in flour blends. The highest lightness was demonstrated by the control sample, whereas the lowest by the buckwheat flour which had the highest redness value a^* comparing with other flours and blends. Pea flour showed significantly higher yellowness b^* in comparison with other samples, with the exception of flour blend with 60%PF + 40%BF. Colour values could be changed significantly by blending buckwheat and pea flours. It is possible to increase L^* and b^* values of buckwheat flour with pea flour and a^* value of pea flour with buckwheat flour in flour blends. Results of farinograph showed that buckwheat flour was characterized by a long development time, high stability and high farinograph quality number (FQN), whereas pea flour and pea-buckwheat flour blends had short development time, low stability and low FQN.
Key words: pea, buckwheat, viscosity, rheological properties.

Introduction

Most health organizations encourage frequent consumption of legumes (Leterme, 2002) due to their nutritional value. Legumes are rich in starch, protein and dietary fibre with significant amounts of vitamins and minerals (Piecyk et al., 2012; Tharanathan and Mahadevamma, 2003). It is important to understand how the benefits of legumes could be used to produce new products. Many factors, such as the nutritional value, physical, functional and organoleptic properties of legumes, are important by producing new products.

The starch content of pea flour range between 30-50% of the dry matter (Ratnayake et al., 2002; Sadowska et al., 2003). The slow and reduced digestibility of legume starch has been attributed to its amylose activity (Tharanathan and Mahadevamma, 2003). Generally, legume starches contain about 24-65% of amylose (Chung et al., 2008; Hoover and Sosulski, 1991) and processing of legumes may lead to an increase in the net resistant starch content which may have important effects on human physiology (Edwards, 1993). Apart from the energy contribution of starch (it provides the major source of physiological energy of human diet), another important role of starch in most of the processed food systems is to contribute to the texture and, as a result, to the organoleptic properties of food (Tharanathan and Mahadevamma, 2003). Legume starches exhibit a wide variation in swelling power and solubility (Ratnayake et al., 2002). The swelling factor of smooth pea starches ranges from 4 to 27 in the temperature range 50-95 °C (Ratnayake et al., 2001). The swelling properties and gelatinization are controlled in part by the molecular structure of amylopectin, starch composition and granule architecture (Tester, 1997).

Buckwheat is recognised as an important functional food in China, Japan and Taiwan (Lin et al., 2008). Buckwheat has gained an excellent reputation for its nutritious qualities in the human diet (Wijngaard and Arendt, 2006): a well balanced amino acid composition, gluten free, resistant starch and antioxidant activity. Starch content of buckwheat ranges from 69-87% in dry matter (Hatcher et al., 2008). Buckwheat, which is added to food, can provide beneficial health effects and prevent food from oxidation during processing (Lin et al., 2009). However, the addition of buckwheat into products changes their physical, functional and organoleptic properties. Torbica et al. (2010) reported that an increase in the amount of buckwheat flour resulted in longer dough development time due to the increase in fiber content which requires longer period of time to absorb water. Buckwheat starch granules are small (Hatcher et al., 2008); therefore they exhibit high water absorption due to large surface area (Dexter and Matsuo, 1979). Conversely, Hatcher et al. (2008) reported about the differences in the physically-chemical properties of buckwheat and cereal starches and indicated a greater swelling and gelling tendency for buckwheat starch than for wheat starch. Buckwheat flour possesses poor protein quality from the technological point of view (Mariotti et al., 2008); therefore it is required to mix buckwheat with other cereals.

The aim of the research was to investigate the starch content, colour and rheological properties (viscosity, consistency, water absorption, dough development time, dough stability and degree of softening) of pea and buckwheat flours and their blends.

Materials and Methods

Materials

Pea (*Pisum sativum* L.) flour (moisture content 9.2%, protein 20.9 g, lipid 1.8 g, total carbohydrates 59.6 g and energetic value 338.0 kcal / 1435.0 kJ in 100 g), buckwheat (*Fagopyrum esculentum*) flour (moisture content 13.3%, protein 12.0 g, lipid 3.0 g, total carbohydrates 62.0 g and energetic value 345.0 kcal / 1446.0 kJ in 100 g) were obtained from Fasma, Lithuania. Fine wheat flour (moisture content 12.1%, protein 11.0 g, lipid 1.1 g, total carbohydrates 72.6 g and energetic value 352.0 kcal / 1494.0 kJ in 100 g) as control was purchased from Dobeles Dzirnavnieks, Latvia.

Five flours and their blends were analysed (Table 1). Flour blends were chosen based on the results of previous research (Beitane et al., 2014).

Table 1

Description of flours and their blends

Code	Sample
Control - WF	100% Wheat flour
PF	100% Pea flour
BF	100% Buckwheat flour
40%PF + 60%BF	40% Pea flour and 60% Buckwheat flour
60%PF + 40%BF	60% Pea flour and 40% Buckwheat flour

Establishing the starch content

The content of starch in flour was established using a modified MEBAK Diastatic Power (EBC) 3.1.4.6 method with SCHOTT manual volumetric titrator, 20-mL capacity (Jacob, 2011). The measurements were performed in triplicate. The starch content of flour blends was calculated using findings of pea and buckwheat flour according the proportion of flour blends.

Evaluation of dough viscosity

Viscosity of the pea and buckwheat flours and their blends were evaluated using Viscograph –E (Brabender® GmbH&Co KG, Germany). Parameters recorded were starch gelatinization temperature (SGT), peak viscosity (PV), trough viscosity (TV), final viscosity (FV), holding viscosity (HV), breakdown viscosity (BDV, which is calculated PV-TV) and setback viscosity (SBV, which is calculated FV-TV). The measurements were performed in triplicate.

Colour measurement

Colour measurements of flours and their blends were carried out in quintuple using CIE Lab system. The obtained results were expressed in terms of CIE L^*, a^* and b^* values, where L^* measures brightness, a^* represents the red – green coordinates and b^* measures the blue – yellow coordinates.

Evaluation of rheological properties

The rheological properties of the pea and buckwheat flours and their blends were examined using Farinograph®-AT (Brabender® GmbH&Co KG, Germany) according to the ICC method 115/1, AACC method 54-21 and ISO 5530-1. Parameters recorded were consistency, water absorption, dough development time, dough stability, degree of softening and farinograph quality number. The measurements were performed in triplicate.

Statistical analysis

The results were analysed using the analysis of variance (ANOVA). T-test was applied to compare the mean values, and p-value at 0.05 was used to determine the significant differences. Mean ± standard deviation of three replicates was used.

Results and Discussion

The addition of legumes into cereal based products could be a good alternative for increasing their intake (Gómez et al., 2012; De la Hera et al., 2012) and their nutritional value. It is known that the nutrients content of raw materials has an effect on the functional

Table 2

Carbohydrates and starch content of flours and their blends

Flours, their blends	Carbohydrates, g 100 g^{-1} (Data of producer)	Starch, g 100 g^{-1}	Starch content in dry matter, %
Control - WF	72.60	51.25±0.06	58.3
PF	59.60	55.55±0.02	61.2
BF	62.00	45.57±0.03	52.6
40%PF + 60%BF	61.04	49.56	56.1
60%PF + 40%BF	60.56	51.56	57.8

properties of the final product. Several research papers (Debet and Gidley, 2006; Nelles et al., 2000) report that a lower lipid and protein content of cereals is associated with a higher peak viscosity, indicative of higher starch swelling. Starch content of flour influences the technological properties of products. Total carbohydrates and starch content of wheat as control sample, pea and buckwheat flours and their blends as well the starch content in dry matter are shown in Table 2.

Evaluating the data displayed in Table 2, it was obvious that pea flour had the highest content of starch (55.55 ± 0.02 g 100 g^{-1}) in comparison with other flours and their blends and formed the largest part (61%) of dry matter. The obtained results were comparable to those reported in other research papers. Similar results were reported in Kaushal et al. (2012) stating that the starch content of pigeonpea flour was 52.41 ± 0.01 g 100 g^{-1}, and in Chung and Liu (2012) concluding that the total starch content of pea flour was 48.8-50.2 g 100 g^{-1}. Literature usually reports a higher starch content of buckwheat (e.g. 65.52-78.09% in dry matter in Qin et al. (2010) or 69-87% in dry matter in Hatcher et al. (2008)) comparing with the results of this research. The differences in starch content of buckwheat flour could be explained by different cultivars, determination methods and growth conditions. The starch content could be increased by blending buckwheat flour and pea flour, achieving a value similar to the control sample.

The pasting properties of starch are significantly influenced by genetic effects: a smaller amylose chain length and a lower proportion of DP (degree of polymerization) 6-12 branch chains of amylopectin would lead to a low setback and a low viscosity at the end of cooling cycle (Ratnayake et al., 2001). The significant differences of viscosity of flours and their blends are presented in Table 3.

Significant differences in the viscosity of flours and their blends were identified. The peak, holding, final, breakdown and setback viscosities of buckwheat flour were significantly higher than those of wheat (control) and pea flours. Izydorczyk et al. (2014) reported similar results stating that buckwheat starches exhibit distinctive pasting profiles with shorter peak time, and much higher peak, setback and final viscosity values compared with cereal starches. Pea flour showed similar results with control as regards peak and setback viscosity. By blending pea flour with buckwheat flour it has shown coherence. As the percentage of buckwheat flour in the blend increased, the values of peak, holding, final and breakdown viscosities increased, too. The peak, holding, final and breakdown viscosities of both flour blends were significantly higher ($p<0.05$) than those of pea flour. The highest setback viscosity was demonstrated by buckwheat flour and flour blend with 40%PF + 60%BF. The highest final and setback viscosity of buckwheat flour could be explained by a large proportion of DP 6-12 branch chains of amylopectin (Ratnayake et al., 2001). The low setback viscosity of control and pea flour indicated their lower tendency to retrograde (Kaushal et al., 2012). The lowest breakdown and setback viscosities of pea flour suggest that it has a high resistance to

Table 3

Viscosity of pea and buckwheat flours and their blends

Flours, their blends	PV (BU)	HV (BU)	FV (BU)	BDV (BU)	SBV (BU)	SGT (°C)
Control - WF	1227.0^{a}	1188.5^{a}	1062.5^{a}	721.5^{a}	557.0^{a}	58.3±0.1
PF	1257.0^{a}	2083.5^{b}	1739.5^{b}	75.0^{b}	557.5^{a}	67.2±0.3
BF	4430.5^{b}	4452.0^{c}	4413.0^{c}	1506.5^{c}	1347.0^{b}	64.6±0.1
40%PF + 60%BF	3379.5^{c}	4101.0^{c}	3670.5^{d}	1070.0^{a}	1361.0^{b}	64.2±0.2
60%PF + 40%BF	2946.0^{c}	3776.5^{c}	3305.0^{d}	827.5^{a}	1186.5^{c}	66.9±0.2

PV: Peak viscosity, HV: Holding viscosity, FV: Final viscosity, BDV: Breakdown viscosity, SBV: Setback viscosity, SGT: Starch gelatinization temperature.
Viscosity with different superscripts in a column differ significantly ($p<0.05$)

Table 4

The *L, *a** and *b** value intensity of flours and their blends**

Flours, their blends	*L**	*a**	*b**
Control - WF	94.30±0.05^{a}	-1.99±0.01^{a}	10.52±0.08^{a}
PF	92.37±0.18^{a}	-2.39±0.02^{b}	19.37±0.09^{bc}
BF	88.18±0.21^{b}	-0.19±0.04^{c}	10.70±0.12^{a}
40%PF + 60%BF	90.17±0.13^{ab}	-1.99±0.03^{a}	15.05±0.10^{c}
60%PF + 40%BF	91.40±0.17^{ab}	-2.37±0.02^{a}	17.09±0.11^{c}

Values with different superscripts in a column differ significantly ($p<0.05$)

Table 5

Rheological properties of pea and buckwheat flours and their blends

Flours, their blends	Consistency, %	Water absorption, %	Development time (dough), min	Stability (dough), min	Degree of softening, FU	FQN
Control - WF	491±17	61.80±0.87	2.40±0.07	8.54±1.11	41±1	59±3
PF	205±6	52.50±0.75	1.13±0.11	1.12±0.07	59±1	37±2
BF	435±11	60.45±0.11	14.58±0.43	9.25±0.20	67±4	152±6
40%PF + 60%BF	461±6	50.34±0.03	1.44±0.03	1.26±0.09	79±2	26±1
60%PF + 40%BF	497±8	49.84±0.20	1.31±0.09	0.56±0.07	138±4	20±2

FQN – Farinograph quality number

retrogradation and therefore would form stable paste (Kaushal et al., 2012). Pea flour and flour blend with 60%PF + 40%BF had the highest starch gelatinization temperature. It could be associated with the fact that a greater amount of protein in pea flour could induce increased protein starch interaction, which could cause retardation toward swelling, thereby increasing the starch gelatinization temperature (Chung and Liu, 2012). By blending buckwheat and pea flours it was possible to change the viscosity significantly ($p<0.05$) depending on final product. Buckwheat flour provided higher peak, holding, final, breakdown and setback viscosities in flour blends.

Colour characteristics of different flours depend on the botanical origin of plants and on the composition of flour. Colour values of different flours and blends are summarized in Table 4.

The highest lightness (indicated by L^*) was exhibited by the control sample (i.e. wheat flour), whereas the lowest was shown by buckwheat flour which had the highest redness value (indicated by a^*) comparing with other flours and blends. Pea flour, in turn, showed significantly higher yellowness (indicated by b^*) in comparison with other samples, with the exception of the flour blend with 60%PF + 40%BF. Similar results were reported in Izydorczyk et al. (2014) for buckwheat flour where L^* values ranged from 87.9 to 91.3, a^* values from -0.7 to -1.5 and b^* values from 6.0 to 12.2 depending on cultivars. Conversely, Qin et al. (2010) indicated that the common buckwheat flour L^* value was 71.87 ± 0.16, a^* value 1.95±0.10 and b^* value 8.36 ± 0.11. Comparing colour values of pea flour with literature the results of research were close to the findings by Kaushal et al. (2012) where L^* value of pigeonpea was 89.50 ± 0.225, a^* value -0.15 ± 0.145 and b^* value 22.32 ± 0.381.

Colour values could be changed significantly by blending buckwheat and pea flours. It was possible to increase L^* and b^* values of buckwheat flour with pea flour and a^* value of pea flour with buckwheat flour in flour blends.

Rheological characteristics of flours and their blends are presented in Table 5.

Farinograph measurements showed that the consistency of wheat flour (control), buckwheat flour and pea-buckwheat flour blends was similar, whereas pea flour indicated significantly lower consistency which conflicted with findings in other paper. Mohammed et al. (2012) reports that chickpea flour consistency is 608 ± 5.56% and a similar value for wheat flour consistency (491 ± 4.58%). The differences could be explained by determination problems, because the farinograph curve of pea flour did not reach 500 FU. The same problem was with buckwheat flour, whereas the farinograph curves of pea-buckwheat flour blends reached 500 FU. The addition of buckwheat to pea flour resulted in an increase of the consistency of the flour blends.

Evaluating the water absorption of flour and their blends, in case of buckwheat flour the value was similar to that of the control sample (wheat flour) and both values were significantly higher comparing with other samples. Research results showed that the blending of pea and buckwheat flours did not result in an increase of water absorption in blends. In literature, Mohammed et al. (2012) and Sadowska et al. (2003) indicated that water absorption increased with increasing amount of chickpea/pea flour added in wheat flour. For the purposes of the present research, pea and buckwheat flours were blended which could be the reason for result differences in comparison with literature.

Dough development time was significantly longer for buckwheat flour than the control, pea flour and flour blends. Similar tendencies were reported by Torbica et al. (2010) about buckwheat flour, where dough development time was significantly longer comparing with wheat flour, which could be related to a higher fiber content of buckwheat flour. It was interesting to note that the development time of flour blends was not influenced by buckwheat flour. It could be explained by changes of starch and protein proportion in flour blends. Torbica et al. (2010) came to similar conclusions: the blending of rice and unhusked buckwheat flours, which development time were 8.76 and 5.93 min respectively, provided

shorter development time (2.6-3.75 min) in flour blends.

As concerns the dough stability, it appears that the wheat (control) and buckwheat flours exhibited significantly higher stability and resistance to mechanical mixing values than pea flour and pea-buckwheat flour blends. This coincides with the findings of literature saying that wheat, husked and unhusked buckwheat flour have high stability – 11.78 min, 10.96 and 5.66 min, respectively. Evaluating dough stability of blends it could be concluded that the presence of buckwheat flour in blends did not result in a high dough stability and increasing the content pea flour from 40% to 60% lead to a decrease of dough stability in flour blends. It could be related to changes of fiber composition in flour blends. Similar conclusions were reported by Sadowska et al. (2003), Kohajdová et al. (2013) and Mohammed et al. (2012) stating that dough stability decreased as the substitute level of chickpea/ pea increased.

In the case of buckwheat and pea flours the degree of softening was higher comparing with the control sample and it increased significantly with increasing the amount of pea flour in blends. Similar changes in dough characteristics were observed by Mohammed et al (2012) and Sadowska et al. (2003) when blending wheat flour with chickpea/pea flour.

Research results showed that buckwheat flour could be marked as strong flour, because Mohammed et al. (2012) indicated that strong flours were characterized by a long development time, high stability with a small degree of softening, and high FQN, while poor flour weakened quickly, resulting in low quality number FQN.

Conclusions

1. Pea flour had a higher content of starch (55.55 ± 0.02 g 100 g^{-1}) than other flours and their blends and formed the biggest part (61%) of dry matter. The starch content could be increased by blending buckwheat flour and pea flour, achieving a value similar to the control sample.
2. Buckwheat flour provided higher peak, holding, final, breakdown and setback viscosities in flour blends.
3. Colour values could be changed significantly by blending buckwheat and pea flours. It is possible to increase L^* and b^* values of buckwheat flour with pea flour and a^* value of pea flour with buckwheat flour in flour blends.
4. Buckwheat flour was characterized by a long development time, high stability, and high farinograph quality number, whereas pea flour and pea-buckwheat flour blends had short development time, low stability and low farinograph quality number.

Acknowledgements

Research has been supported by the National research programme "Agricultural Resources for Sustainable Production of Qualitative and Healthy Foods in Latvia" (AgroBioRes) (2014–2017), project No. 4 Sustainable use of local agricultural resources for qualitative and healthy food product development (FOOD).

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EVALUATION OF AROMA VOLATILES IN NATURALLY FERMENTED KVASS AND KVASS EXTRACT

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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% by volume. Kvass extracts have longer shelf-life and they are essentially free of ethanol. The aim of this research was to evaluate and compare aroma compounds in naturally fermented kvass and kvass extracts. Experiments were carried out at the Latvia University of Agriculture, Department of Food Technology from November 2014 to February 2015. Three commercially available kvass samples (Bruveris, Bauskas and Liepkalni) were used to produce kvass extracts applying vacuum evaporation. The investigation of volatile compounds in kvass and kvass extracts was performed using solid phase microextraction and gas chromatography mass spectrometry. Dry matter content in kvass extracts was 32.4 ± 0.3% (ISO 2173:2003). In all kvass and extract samples in total 25 volatile compounds were detected. Ten of them were esters, five alcohols, five acids, four aldehydes and three ketones. Such aroma compounds as ethyl acetate (fruity flavour), hexyl acetate (fruit, herb) and ethyl decanoate (grape) were found only in Bruveris kvass, 2,3-butanedione (buttery) and phenethyl butyrate (floral) were found only in Bauskas alus kvass and three volatile compounds were identified only in Liepkalni kvass – acetic acid (sour), furfuryl alcohol (burnt) and carvone (caraway). Less than a half of the main aroma volatiles in kvass were also identified in kvass extracts and total values of peak areas were significantly lower in kvass extracts compared to kvass ($p = 0.01$).
Key words: kvass, kvass extracts, aroma volatiles, dry matter, vacuum evaporation.

Introduction

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, different flavours (Klosse, 2013) and artificial sweeteners. Kvass drinks and malt extract drinks are sometimes produced without the use of yeast, therefore carbon dioxide is added artificially for no fermentation has taken place. Many consumers choose naturally fermented kvass over kvass drinks. Kvass production is similar to the production of beer but alcoholic fermentation is stopped before ethanol level reaches 1.2 % alcohol by volume. Naturally fermented kvass is made from diluted malt extract which is fermented by adding bread yeast *Saccharomyces cerevisiae.* Naturally fermented bread kvass is made from dried rye bread by soaking it in hot water for a few hours. After separating water-bread extract from the soaked bread, it is fermented by adding bread yeast.

The most important sensory characteristics that describe the products are taste and aroma. Food aroma has been investigated in many countries all over the world as the food smell is undoubtedly the most important parameter influencing consumer acceptance. Estonian researchers (Kaseleht and Leitner, 2008) have started analysing volatile compounds in traditional Estonian food (kama and kvass) but the research for aroma compounds in kvass is still ongoing.

Baker's yeast, used for bread fermentation throughout the world, is very important for the bread quality and different commercial baker's yeasts are each highly selected strains of the species *Saccharomyces cerevisiae*. The fermentative activity of baker's yeast is essential not only for the rising action of the dough by production of CO_2, but also in production of wide range of aroma compounds identified in bread (Birch et al., 2013). *Saccharomyces cerevisiae* is also used for kvass fermentation; therefore kvass aroma is highly dependable on the baker's yeast.

Most of the aroma compounds in bread crumb are derived from the metabolism of yeast and the dominating compounds are alcohols, aldehydes as well as 2,3-butanedione (diacetyl), 3-hydroxy-2-butanone (acetoin) and esters. These aldehydes or their corresponding alcohols are formed inside the yeast cell from degradation of the flour amino acids via the Ehrlich pathway (Hazelwood et al., 2008). The esters are produced in the yeast cell by an enzymatic reaction between acetyltransferases, acetyl coenzyme A and various alcohols (Lilly et al., 2000). The di-ketones 2,3-butanedione and 3-hydroxy-2-butanone are formed from acetohydroxy acids leaked from the yeast cell through non-enzymatic chemical reactions outside the yeast cell (Wainwright, 1973). Furthermore, products from oxidation of flour lipids, such as alcohols, aldehydes and ketones, contribute highly to the aroma profile of bread crumb (Birch et al., 2013), whereas the aroma compounds in the crust originates from Maillard reaction occurring at high temperatures and low water activity between reducing sugars and amino acids (Purlis, 2010). Kvass made from bread rusks is fermented twice – once when making bread and the second time when fermenting kvass mash.

Malt is the main ingredient in other naturally fermented kvass and the roasting process clearly influences its aroma and colour. According to Hoff at al. (2012), compounds from the Maillard reaction are responsible for the colour change during roasting and the volatile composition. Pyrazine compounds, including 2,6-dimethylpyrazine, 2-methylpyrazine and 2-ethyl-3-methylpyrazine, and furans such as furfural, 5-methylfurfural, and 2-furanmethanol are Maillard reaction products formed during the roasting process (Riu-Aumatell et al., 2014), these compounds are mainly found in alcohol-free beers. Methionol was also found and is formed during the Maillard reaction or alcoholic fermentation (Pinho et al., 2006). According to Perpète and Collin (2000), ethanol could induce the retention of some volatile compounds, indicating that in low-alcohol beers the perception of some volatile compounds could be more pronounced.

Kvass extracts have longer shelf-life, they are substantially free of ethanol and can be used by consumers that abstain from alcohol for various reasons; kvass extracts can be diluted with still or carbonated water to produce drinks at desired water to kvass extract proportion (the taste could be mild or stronger).

The aim of this research was to evaluate and compare aroma compounds in naturally fermented kvass and kvass extracts.

Materials and Methods

Experimental design

Experiments were carried out at the Department of Food Technology, Latvia University of Agriculture from November 2014 to February 2015. The object of the research was vacuum evaporated kvass extracts. To produce kvass extracts three commercially available kvass samples were used: 'Liepzeme' Ltd. Liepkalni naturally fermented non-pasteurised, non-filtered bread kvass, 'Bauskas alus' Ltd. naturally fermented kvass and 'Bruveris' Ltd. naturally fermented kvass (Table 1). Liepzeme Ltd. kvass was initially experimentally developed at the Department of Food Technology and then adapted to the industrial production process.

Different ingredients and heat treatment or the lack of it can influence aroma composition in kvass and kvass extracts. All investigated kvass samples are made by naturally fermented kvass mash. The main difference among the samples is that only Liepzeme Ltd. produces kvass from rye bread rusks baked at the factory, therefore using the traditional method for kvass production. The aroma compounds in Liepkalni kvass and kvass extract could be more similar to aroma compounds in bread crust and crumb as natural rye bread is used in kvass production. Bruveris and Bauskas alus use water and malt and/or malt extract to obtain kvass mash. These two producers also filter and pasteurize their products contrary to Liepzeme Ltd. When it is pasteurized the microflora in kvass is eliminated and it can be stored for longer periods of time compared to non-pasteurized kvass.

Kvass extract production

Carbon dioxide content was reduced in all kvass samples using Magnetic stirrer MSH 300 for 15 min to reduce foaming during vacuum evaporation. The initial dry matter content was determined in kvass samples with digital refractometer DR301-95 (ISO 2173:2003), as well as in kvass extracts. Kvass extracts were produced using rotary vacuum evaporator Heidolph Laborata 4000 Efficient in two stages; kvass samples were 200 ml. The parameters of extraction method were 50 °C temperature of all extraction process, 30 rpm for the first 30 min, from 50 to 60 rpm for over an hour until fixed dry matter content. Dry matter content was continuously measured during the second evaporating process every 10 minutes.

Detection of volatile (aroma) compounds in kvass and kvass extracts

Volatile compounds were determined in kvass and kvass extract samples using solid phase

Table 1

Comparison of kvass used in the research

Kvass	Producer	Ingredients	Shelf-life
Classic naturally fermented kvass	Bruveris Ltd.	Water, malt, sugar, rye malt extract, acidifier: lactic acid, yeast	6 months
Naturally fermented kvass	Bauskas alus Ltd.	Water, sugar syrup, barley and rye malt extract, carbon dioxide, acidifiers: lactic acid and citric acid, yeast	4 months
Liepkalni bread kvass (non-pasteurised and non-filtered)	Liepzeme Ltd.	Water, rye bread rusks 10% (rye flour, wheat flour, sugar, rye malt, salt, yeast, barley malt extract caraway), sugar, barley malt, wheat malt, acidifier: citric acid, yeast	2 weeks

micro extraction (SPME) in combination with gas chromatography/mass spectrometry (GC/MS) according to the methods of Sabovics et al., 2010; 2013. The SPME fibre was coated with a thin bipolar polymer film – Carboxen/Polydimethylsiloxane (CAR/PDMS). The film thickness was 85 μm with polarity (Supelco, Inc., USA).

A sample of 5.00±0.05 g was weighed into 20 ml glass vial which was covered with a rubber gasket and sealed with a cork. The vial with the sample was heated for 30 min at 40 °C to excrete volatile compounds above the liquid phase. After 30 min CAR/PDMS fibre was inserted into the vial through the rubber gasket and held above the sample for 30 min at 40 °C temperature. During this time volatile compounds were absorbed onto the fibre.

Volatile compounds from the fibre were thermally desorbed in GC/MS injector. Separation of volatiles was carried out in the Elite-Wax (PerkinElmer, Inc., USA) capillary column (60 m × 0.25 mm i.d., polyethylene glycol coating thickness 0.25 μm).

GC–MS analysis was performed with the following parameters: the initial temperature was 40 ºC, held for 7 min, then ramped from 40 °C to 160 °C at a rate of 6 °C min^{-1} and from 160 ºC to 210 °C at a rate of 10 °C min^{-1} then held for 15 min at 210 °C. The total run time was 47 min for a sample. Mass spectrometer in Electron impact Ionization mode was set to 70 eV as the electron energies, while the ion source temperature was set to 250 °C and the inlet line temperature was set to 250 °C. Injections were performed in split mode (2:1) and helium (He) was used as the carrier gas at a constant flow of 1 ml min^{-1}; acquisition parameters in full scan mode – m/z 40-300. Compounds were identified by comparison of their mass spectra with mass spectral library Nist98 and the amount of compounds was measured as peak area units (PAU).

Data analysis

The obtained data processing was performed with the Microsoft Excel 13 for Windows; mean values and standard deviations were calculated. ANOVA and Tukey's test were used for data cross-comparison. For the interpretation of the results it is assumed that α=0.05 with 95% confidence. The Principal component analysis (PCA) was done using Multibase2015 statistics program.

Results and Discussion

Dry matter in kvass and kvass extracts

The initial dry matter content was different in all kvass samples: 8.6% (Liepkalni), 7.9% (Bauskas alus) and 12.1% (Bruveris), it could be explained with

Table 2

Volatile compounds (PAUx10^7) in Bruveris kvass and kvass extract

Volatile compounds	Odour (Gas chromatography ..., 2004; Odor Descriptors, 2015)	Kvass	Kvass extract
Ethylacetate	fruity	0.40±0.00	-
3-methylbutanal	fruity, almond-like, toasted, malty, green, herbaceous	-	0.08±0.00
4-penten-2-ol	fruity	**6.81±0.38**	**3.89±0.07**
isoamyl acetate	banana	**1.37±0.06**	-
ethyl hexanoate	apple peel, fruit	0.54±0.08	-
3-methyl-1-butanol	whiskey, malt, burnt	0.63±0.04	-
hexyl acetate	fruit, herb	0.20±0.00	-
3-hydroxy-2-butanone	butter, cream	-	0.07±0.00
5-methyl-1-hexanol	-	0.09±0.01	-
ethyl octanoate	fruit, fat	0.79±0.04	-
acetic acid	sour	-	0.28±0.04
furfural	bread, almond, sweet	0.27±0.01	-
ethyl decanoate	grape	0.07±0.01	-
furfuryl alcohol	burnt	-	0.12±0.01
2-phenylethyl acetate	rose, honey, tobacco	0.69±0.07	0.02±0.01
hexanoic acid	fatty type	0.12±0.01	0.10±0.00
phenylethylalcohol	rose	0.25±0.01	0.29±0.00
octanoic acid	sweat, cheese	**1.55±0.10**	0.23±0.02
decanoic acid	rancid, fat	0.36±0.08	0.06±0.01
	The sum of peak area	**14.15±0.89**	**5.14±0.17**

Table 3

Volatile compounds (PAUx10^7) in Bauskas kvass and kvass extract

Volatile compounds	Odour (Gas chromatography ..., 2004; Odor Descriptors, 2015)	Kvass	Kvass extract
3-methylbutanal	fruity, almond-like, toasted, malty, green, herbaceous	-	0.07±0.00
4-penten-2-ol	fruity	**2.03±0.00**	**1.32±0.13**
2,3-butanedione	buttery type	0.64±0.05	-
isoamyl acetate	banana	0.33±0.05	-
3-methyl-1-butanol	whiskey, malt, burnt	**1.27±0.02**	0.14±0.03
furfural	bread, almond, sweet	0.12±0.01	-
benzaldehyde	almond, burnt sugar	-	0.25±0.01
furfuryl alcohol	burnt	-	0.07±0.00
2-phenylethyl acetate	rose, honey, tobacco	0.09±0.00	-
phenylethylalcohol	floral type	0.37±0.03	**0.93±0.06**
octanoic acid	sweat, cheese	0.24±0.03	0.14±0.00
phenethyl butyrate	floral type	0.15±0.01	-
decanoic acid	rancid, fat	0.31±0.01	0.26±0.00
palmitic acid	waxy type	-	0.09±0.01
	The sum of peak area	**5.54±0.21**	**3.28±0.24**

a higher water to malt ratio in Bruveris Ltd. kvass. After vacuum evaporation, the dry matter content in all kvass extract samples was 32.4 ± 0.3%.

Aroma volatiles in kvass and kvass extracts

Headspace-solid phase micro extraction was used to characterize the volatile compounds present in three types of kvass and kvass extracts. Twenty five volatile compounds were isolated and characterized by GC–MS analysis. The identified volatile compounds belong to esters, alcohols, acids, aldehydes and ketones.

There were 19 volatile compounds identified in Bruveris kvass and the total sum of peak areas of Bruveris kvass was 14.15×10^7 PAU. The amount of compounds in kvass extract was about 3 times lower (5.14×10^7), a total of 10 volatiles were found in the kvass extract. The highest value of peak area (6.81×10^7) among all detected volatile compounds was detected for 4-penten-2-ol (alcohol) in Bruveris kvass, which gives fruity aroma, but in Bruveris kvass extract it was about 50% lower (Table 2). Fatty acid – octanoic acid (1.55×10^7) and ester – isoamyl acetate (1.37×10^7) had the second and third highest peak area values, thus giving odours of sweat and cheese, and banana, respectively.

A more various volatile compounds profile and higher total sum of peak area Bruveris kvass compared to other two kvass samples could possibly be explained by significantly higher dry matter content ($p = 0.011$) in Bruveris kvass, however, the dry matter can also consist of compounds that do not affect the aroma or compounds from which aroma volatiles are not synthesized, and non-volatile flavour-active compounds (lipids, sugars, etc.) which can affect the final flavour perception (Lozano, 2011).

The total sum of peak area in Bauskas kvass extract (3.28×10^7) is about 1.6 times lower than in kvass (5.54×10^7). In total there were 10 volatiles identified in Bauskas kvass and 9 volatiles in Bauskas kvass extract. Analysing the volatile compounds in kvass of Bauskas alus Ltd. alcohol 4-penten-2-ol has the highest peak area value (2.03×10^7) as well, but it was lower comparing to Bruveris kvass (Table 3). Significant amount of 3-methyl-1-butanol (1.27×10^7) can be found in Bauskas alus kvass and smaller amounts in kvass extract, giving to both samples whiskey and malt burnt odour. But in kvass extract phenylethylalcohol has the second highest peak area value (0.93×10^7) after 4-penten-2-ol, forming floral type odour in Bauskas kvass extract.

17 volatile compounds were identified in Liepkalni kvass and 13 in Liepkalni kvass extract. Alcohol 4-penten-2-ol had the highest peak area value (3.64×10^7) in Liepkalni kvass (Table 4) and it was lower comparing to Bruveris kvass (Table 2) and higher comparing to Bauskas kvass (Table 3). Peak area of carvone is the second highest (2.03×10^7) in Liepkalni kvass, but it was not detected in Liepkalni kvass extract. Rye bread which is used in Liepkalni kvass production contains caraway; this explains carvone in Liepkalni kvass aroma profile as carvone and limonene form the main portion of essential oils in caraway fruits (Sedláková et al., 2003).

Volatile compounds found in all three kvass sample forms base aroma profile which includes fruity (4-penten-2-ol), banana (isoamyl acetate), whiskey,

Table 4

Volatile compounds (PAUx10^7) in Liepkalni kvass and kvass extract

Volatile compounds	Odour (Gas chromatography ..., 2004; Odor Descriptors, 2015)	Kvass	Kvass extract
4-penten-2-ol	fruity	**3.64±0.11**	**1.40±0.02**
isoamyl acetate	banana	0.22±0.04	-
ethyl hexanoate	apple peel, fruit	0.13±0.01	-
3-methyl-1-butanol	whiskey, malt, burnt	0.63±0.02	0.39±0.00
3-hydroxy-2-butanone	butter, cream	-	0.01±0.00
nonanal	aldehydic type	-	0.03±0.01
ethyl octanoate	fruit, fat	0.39±0.05	0.02±0.00
acetic acid	sour	0.37±0.06	0.48±0.03
furfural	bread, almond, sweet	-	0.10±0.01
furfuryl alcohol	burnt	0.20±0.03	0.10±0.01
carvone	caraway	**2.03±0.06**	-
2-phenylethyl acetate	rose, honey, tobacco	0.10±0.02	-
hexanoic acid	fatty type	0.09±0.01	0.07±0.00
phenylethylalcohol	floral type	0.16±0.01	0.69±0.02
octanoic acid	sweat, cheese	0.72±0.04	0.46±0.08
decanoic acid	rancid, fat	0.25±0.00	0.19±0.00
palmitic acid	waxy type	-	0.11±0.03
	The sum of peak area	**8.94±0.46**	**4.05±0.20**

malt, burnt (3-methyl-1-butanol), rose, honey, tobacco (2-phenylethyl acetate), floral (phenylethylalcohol), sweat, cheese (octanoic acid) rancid and fat (decanoic acid) aroma.

Alcohols can be formed in the metabolism of yeast when long-chain and complex alcohols are produced, but aldehydes and ketones can be formed from alcohols (de Smidt et al., 2008). Acetic acid is formed in yeast fermentation process from yeast and gives acidic and vinegar flavour. Secondary metabolism of yeast can form 3-methyl-1-butanol, which gives malty flavour to product. According to Schieberle (1996), the amount of flavour compounds can be affected by yeast amount and activity, fermentation time and fermentation temperature.

The identified isoalcohols, 3-methylbutanal and phenylacetaldehyde are typically fermentation compounds likely formed via the Erhlich pathway in

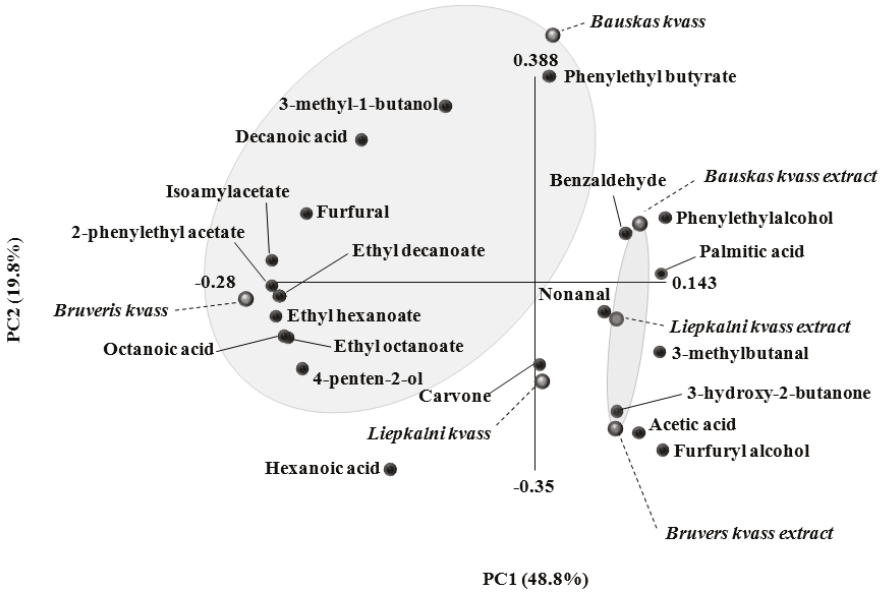

Figure 1. Distribution of volatile compounds in the kvass and kvass extract samples.

the yeast cell (Hazelwood et al., 2008). The identified 2,3-butanedione and 3-hydroxy-2-butanone were most likely formed by oxidative decarboxylation and decarboxylation, respectively of acetohydroxy acids outside the yeast cell. The yeast cell has been found to be responsible for the synthesis and excretion of these acetohydroxy acids (Wainwright, 1973).

The principal component analysis of volatile compounds in kvass and kvass extract samples is shown in Figure 1.

The samples of kvass and kvass extract in the score plot show how the samples relate to each other. Samples close to each other have similar volatile compounds profile, whereas samples far from each other have dissimilar volatile compounds profile. The volatile compounds loading plot shows which volatile compounds are influential from the model and how the volatile compounds are correlated to each other. Forty nine percent of contribution (component 1) means that 51% of original information is lost and component 1 represents about half of the original data. The contribution of the second component is 21% and accumulated contribution of component 1 and component 2 goes up 69%, which means that the scatter plot between PC1 and PC2 covers 69% of original data (Figure 1).

In principal component analysis it can be seen that the volatile compounds profile and value of peak areas are dissimilar in each sample of kvass, where the highest amount of volatile compounds is in Bruveris kvass. In kvass extract samples aroma volatiles profile changes and the value of peak areas decreases comparing to kvass which can be explained by evaporation of water and volatiles during extraction process; evaporation can concentrate new volatile compounds which cannot be detected in kvass samples because of their small amount, and it can also promote the synthesis of new aroma volatiles. Evaporation process has a significant influence ($p=0.01$) on the volatile compounds profile and value of peak areas.

Such aroma compounds as ethylacetate (fruity flavour), hexyl acetate (fruit, herb) and ethyl decanoate (grape) were found only in Bruveris kvass, 2,3-butanedione (buttery) and phenethyl butyrate (floral) were found only in Bauskas alus kvass and three volatile compounds were identified only in Liepkalni kvass – acetic acid (sour), furfuryl alcohol (burnt) and carvone (caraway). 2-phenylethyl acetate can be found in all kvass samples, but from extracts it was found only in Bruveris kvass extract. Benzaldehyde which gives almond and burnt sugar flavour was identified only in Bauskas kvass extract. Liepkalni kvass extract had 3 volatile compounds which were not identified in other extracts – nonanal (aldehydic odour), ethyl octanoate (fruit, fat) and furfural (bread, almond and sweet). Furfural and its derivatives are Maillard reaction products formed during the roasting process of geminated grains and bread baking.

Conclusions

1. Twenty five volatile compounds were identified in all analysed kvass samples and extracts: eight esters, five alcohols, five acids, four aldehydes and three ketones.
2. The highest peak area value of alcohol 4-penten-2-ol, which gives fruity odour was detected in Bruveris kvass, but the lowest in Bauskas kvass; it means that the 4-penten-2-ol is the main volatile compound of aroma.
3. Evaporation process has a significant influence ($p=0.01$) on the volatile compounds profile and value of peak areas: less than a half of the main aroma volatiles in kvass were also identified in kvass extracts and the total values of peak areas were significantly lower in kvass extracts compared to kvass.
4. Liepkalni kvass extract had three volatile compounds which were not identified in other extracts – nonanal, ethyl octanoate and furfural which add aldehydic, fruit, fat, bread, almond and sweet odour to the kvass extract.

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RHEOLOGICAL PROPERTIES OF WHOLE GRAIN WHEAT, RYE AND HULL-LESS BARLEY FLOUR BLENDS FOR PASTA PRODUCTION

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Abstract
Whole grain flour can be considered as a good candidate for pasta fortification due to the health benefits. Literature reports pasta dough fortification with non-traditional ingredients and cereals. Therefore, the purpose of the current research was to investigate rheological properties of whole grain flour blends for pasta production. Flour blends were made from wheat flour (type 405) in a combination with other flours (whole grain wheat, rye or hull-less barley flour) in various proportions (from 10% to 50%). Wheat flour type 405 was used as a control. Rheological properties of dough were analysed using Farinograph AT (Brabender, GmbH&Co.KG., Germany) and starch gelatinization properties of flour starch using Amylograph-E (Brabender GmbH&Co.KG., Germany); moisture content of flour samples (AACC 44-15A from 2000). The results of present research demonstrate that rheological properties of dough decrease if the amount of whole grain flour in blend increases. It was concluded that water absorption and dough development time of dough with whole grain flour blends addition is less than the parameters of control wheat flour (type 405). However, a higher starch gelatinization was obtained for flour blends with whole wheat grain flour, comparing to blends with whole rye and hull-less barley grain flour, which mainly could be explained with a higher gluten content of whole wheat grain flour.
Key words: whole grain, flour blends, pasta, farinograph, amylograph.

Introduction

Cereal-based foods have been staples for humans for millennia. Cereal grains contain the macronutrients (carbohydrate, protein and fat) required by humans for growth and maintenance. They also supply important minerals, vitamins and other micronutrients essential for optimal human health conditions (Topping, 2007).

Traditionally, pasta products are made from wheat semolina, although more recently other cereals have been used to partially replace it (Chillo et al., 2008, Manthey et al., 2004 and Petitot et al., 2010). Common wheat flour also can be useful for precooked pasta products, but because of the low protein content, addition of high protein components such as whole grain flour may enrich the products and result in improved functional properties and quality when the right processing conditions are used (Chillo et al., 2008). Wheat (*Triticum spp.*) is the main cereal crop used for human consumption in many areas of the world. Traditionally, pasta is manufactured from durum wheat (*Triticum durum* D.) with protein <15%, 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 (Sissons et al., 2005; Troccoli et al., 2000). Rye (*Secale cereale*) could be exploited more efficiently in new types of pasta products due to its positive health effects. Nowadays, its use is limited mainly as a result of the problems arising from its pentosan and water-soluble proteins. 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 (Andersson et al., 2004).

Characterization of rheological properties of dough is effective in predicting the processing behaviour and in controlling the quality of food products (Song and Zheng, 2007). When wheat flour is mixed with water, with the required amount of energy, dough is formed. The behaviour of the resulting dough when submitted to mechanical energy input is determined by dough rheological properties (Bloksma, 1990). The Farinograph results reveal dough-mixing properties which are ascribed to wheat gluten, starch, lipid and water contents, as well as the amount and activity of α-amylase.

Gliadin and glutenin are the two primary types of grain protein which are responsible for the elastic and viscous properties, respectively, which help to form a continuous spatial network in the dough (Koehler et al., 2010). These properties derive largely from the gluten proteins, which form a continuous viscoelastic network within the dough. 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 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 (Weegels et al., 1994). Gluten is the main base of the wheat dough and is the protein

that only exists in wheat and rye. Wheat flour dough simultaneously exhibit characteristics of a viscous liquid and of an elastic solid and hence are classed as viscoelastic materials (Bagley et al., 1998). 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). Dough mechanical properties depend on a large variety of factors including flour mixing time, etc. (Bagley et al., 1998). Bran and germ particles also disrupt the continuity of the protein network, resulting in weaker, less firm pasta (Manthey and Schorno, 2002).

The Amylograph results reveal starch properties which are ascribed to grain. Starch generally consists of two D-glucose homopolymers, the linear polymer amylose and a highly branched glucan amylopectin that connects linear chains. Starch granules contain an amylose and amylopectin. Amylopectin structurally contributes to the crystalline organization of the starch granule in cereals. Amylopectin chain length distribution also affects gelatinization, retrogradation, and pasting properties of starch (Jeon et al., 2010). Starch gelatinization is a process that breaks down the intermolecular bonds of starch molecules in the presence of water and heat, allowing the hydrogen bonding sites (the hydroxyl hydrogen and oxygen) to engage more water (Sobkowska, 2001). Starch is essential to determine the pasta cooking quality (Delcour et al., 2000). Starch affects the water absorbency, gel consistency and integrity of the gluten matrix during cooking (Edwards et al., 1999). Bran from whole grain flour can interfere with water migration during this step, increasing water retention within the pasta (Villeneuve and Gélinas, 2007).

In the scientific literature it is reported, that when pasta dough is fortified with non-traditional ingredients, it behaves differently. Therefore, the purpose of the current research was to investigate rheological properties of whole wheat, rye and hull-less barley flour blends for pasta production.

Materials and Methods

The study was carried out at the scientific laboratories of Faculty of Food Technology at Latvia University of Agriculture (LLU) and at the laboratory of the JSC "Jelgavas dzirnavas" (Latvia).

Conventional rye (′Kaupo′) and hull-less barley (line ′PR 5099′) grains of 2014 cultivated at State Priekuli Plant Breeding Institute (Latvia), wheat (′Zentos′) grain cultivated at LLU research center „Peterlauki" (Latvia) were used in the experiments. For the flour blend obtaining wheat flour type 405 from JSC "Dobeles dzirnavnieks" (Latvia) was used. Rye, wheat and hull-less grain were ground in the laboratory mill PLM3100/B (Perten, Sweden) obtaining fine whole grain flour. Wheat flour type 405 was used as a control. Blends were made from whole rye, hull-less barley or wheat flour in combination with wheat flour (type 405) in various proportions. As a result, fifteen several flour blends were developed: flour blend with whole rye or hull-less barley, or wheat flour. Experimentally, a part of wheat flour type 405 was replaced with whole grain flour from 10% to 50% (Table 1).

Table 1

Sample composition per 100% of flour blend

Sample code	405 type wheat flour, %	Whole grain flour type		
		Rye, %	Wheat, %	Hull-less barley, %
100% W	100	–	–	–
10% WR	90	10	–	–
10% WW	90	–	10	–
10% WH	90	–	–	10
20% WR	80	20	–	–
20% WW	80	–	20	–
20% WH	80	–	–	20
30% WR	70	30	–	–
30% WW	70	–	30	–
30% WH	70	–	–	30
40% WR	60	40	–	–
40% WW	60	–	40	–
40% WH	60	–	–	40
50% WR	50	50	–	–
50% WW	50	–	50	–
50% WH	50	–	–	50

Moisture content of flour samples was determined using air-oven method (AACC 44-15A from 2000).

For analysis of rheological properties Farinograph-AT (Brabender GmbH&Co.KG., Germany) was used according to the international standard method (AACC No. 54-21, ICC No. 115/1). For all samples the following parameters were determined: water absorption (WA) of flour and flour blends, stability of dough (S), development time of dough (DDT), and the degree of softening (DS).

For analysis of starch gelatinization properties Amylograph-E (Brabender GmbH&Co.KG., Germany) was used according to the international standard method (AACC standard 22-10; ICC No. 126/1). The acquired diagram was evaluated for the gelatinization maximum and the gelatinization temperature (ICC Standard 126/1, 1992).

Microsoft Excel software was used for the research purpose to calculate mean values and standard deviations of the obtained data. 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 ($p<0.05$). Analysis was realised in triplicate.

Results and Discussion

Moisture content of flour used in the research was from 10.23% (whole wheat) to 14.16% (wheat flour), but in flour blend samples – from 12.20% to 13.79%. In the present experiments a significantly higher ($p<0.05$) moisture content was obtained for wheat flour type 405, comparing with whole grain flour and flour blend samples.

Water absorption (WA) is a parameter indicated as the amount of water needed to develop the standard dough of 500 farinograph unit (FU) at the peak of the curve. Stronger wheat flours have the ability to absorb and retain more water as compared to weak flours (Mis, 2005). In the present experiment it was observed that water absorption of analysed flour sample increases by adding whole grain flour (Figure 1).

At the same time, the smaller water absorption was obtained for the control wheat flour sample (59.1 ± 0.2%), while significantly higher ($p<0.05$) – for whole rye grain flour (71.5 ± 0.2%). Present results demonstrate that the value of water absorption for whole wheat or hull-less barley increases by ~ 2%, but for whole rye by ~ 4%. Obtained results could be explained with lower moisture content and higher bran content of analysed whole grain flour. However, the inclusion of a higher amount of bran in the dough formulation usually resulted in increased dough water absorption due to the higher levels of pentosans present in bran (Sanz-Penella et al. 2008). Besides, bran from whole grain flour can interfere with water migration during this step, increasing water retention within the pasta (Villeneuve and Gélinas, 2007).

Gluten has viscoelastic behaviour in which gliadin and glutenin fractions represent viscous and elastic behaviour, respectively. Both quality and quantity of gluten proteins affect the flour processing quality. The gluten in the manufacture of pasta has two main functions: providing dough plasticity linking all the components which are fed through the binder matrix

Table 2

Moisture content in flours and flour blend samples

No.	Samples	Moisture, %
1.	Flour blend with 10% whole rye	13.79
2.	Flour blend with 10% whole wheat	13.78
3.	Flour blend with 10% whole hull-less barley	13.77
4.	Flour blend with 20% whole rye	13.42
5.	Flour blend with 20% whole wheat	13.40
6.	Flour blend with 20% whole hull-less barley	13.37
7.	Flour blend with 30% whole rye	13.05
8.	Flour blend with 30% whole wheat	13.03
9.	Flour blend with 30% whole hull-less barley	12.98
10.	Flour blend with 40% whole rye	12.68
11.	Flour blend with 40% whole wheat	12.65
12.	Flour blend with 40% whole hull-less barley	12.59
13.	Flour blend with 50% whole rye	12.31
14.	Flour blend with 50% whole wheat	12.27
15.	Flour blend with 50% whole hull-less barley	12.20

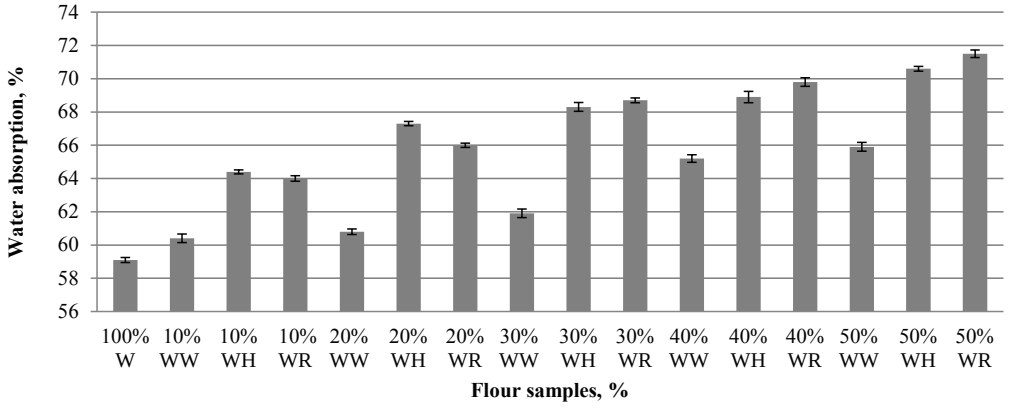

Figure 1. Water absorption for flour blend samples.

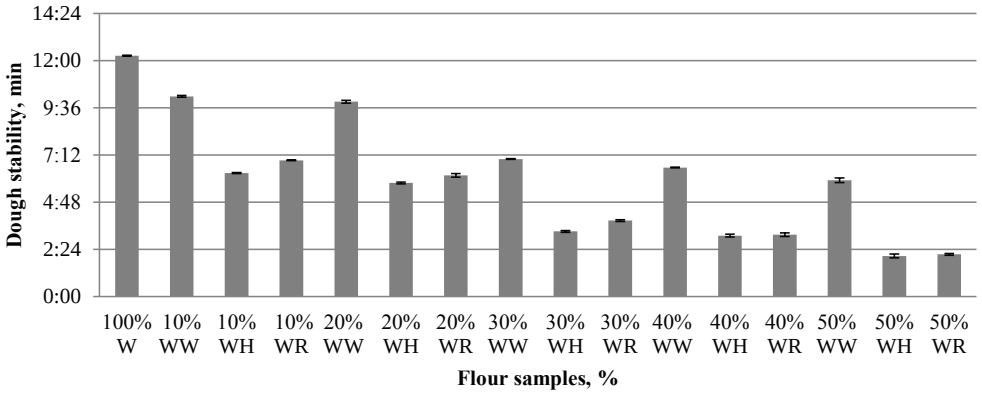

Figure 2. Dough stability for flour blend samples.

and starch grains in one mass to keep a form extruder matrix. Gluten is unique for pasta production, because it keeps the gluten matrix in starch granules (Осипова, 2009).

Pasta structure is as a compact matrix of protein network formation and starch granules (Cunin et al., 1995). As a result, the gluten structure is responsible for the nutritional characteristics of pasta and the formation of various technological stages (Scanlo n et al., 2005). Variation in protein content alone is not responsible for the differences in dough properties and suitability for end-products amongst the cultivars (Zhu and Khan, 2002). However, dough stability (DS) is as the time difference between the point where the top of the curve first intercepts the 500 FU line and the point where the top of the curve leaves the 500 FU line. Dough stability indicates the time when the dough maintains maximum consistency and is a good indication of dough strength. Good wheat quality dough has stability of 4–12 min (Kulhomäki and Salovaara, 1985). Stability time of control wheat dough sample was 12:15min. However, dough stability time decreases by increasing of whole grain wheat, rye and hull-less barley flour (Figure 2) additive.

Bran and germ particles also disrupt the continuity of the protein network, resulting in weaker, less firm pasta (Manthey and Schorno, 2002). Such changes could mainly be explained with possible decreases of gluten content in the analysed whole grain flour samples. Short dough stability time mainly could indicate non-acceptable dough properties during kneading and formation of pasta. There dough stability time less than 4 min could be non-acceptable. As a result, the obtained products as, for example, pasta could be with non-acceptable quality properties. Therefore, for obtaining of dough with good properties, the additive of whole rye and whole hull-less barley grain flour could be 20%, but of whole wheat grain flour – 50%.

Wheat flour (control) showed the lowest dough development time (DDT) (2:22 min), but the higher development time (3:36 min) was obtained for whole wheat grain flour, for whole rye grain flour (6:36 min) and for whole hull-less barley grain flour (6:52 min) (Figure 3). Increased dough development time could mainly be explained with differences in chemical composition of whole grain flour, as elevated dietary fiber content, especially, and, possibly, more proteins.

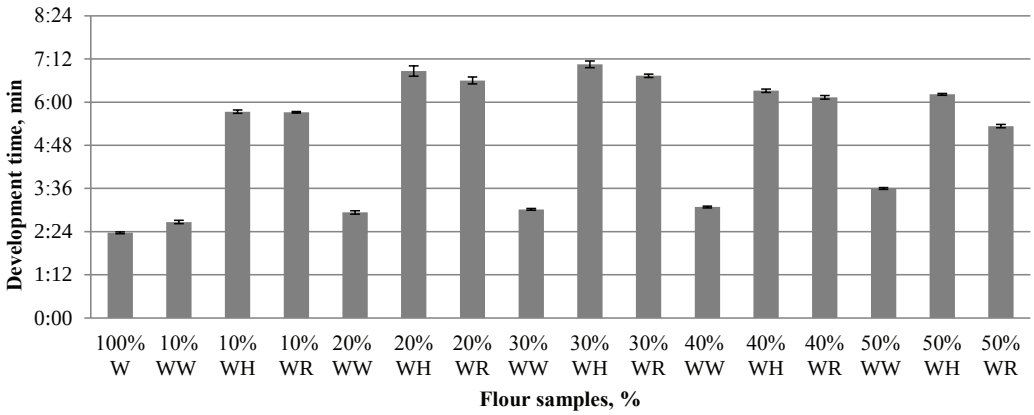

Figure 3. Development time for flour blend samples.

Table 3

Starch gelatization parameters of flour blends

Flour blend samples	Beginning of gelatinization, °C	Gelatinization, °C	Gelatinization maximum, AU
Wheat flour	60.3*	85.4*	1100*
Flour blend with 50% whole wheat	61.8**	90.9**	1095*
Flour blend with 20% whole rye	60,3*	85.3*	1097*
Flour blend with 20% whole hull-less barley	59.9*	85.7*	1100*

* – there is no significant difference between samples ($p>0.05$);
** – there are significant differences between samples ($p<0.05$).

Similar results were found in the *Zhang 2014*, as the dough development time for waxy wheat flour (1.5 min) was shorter than that of wheat flour (2.1 min) - an advantage for improving output during the actual production. The results of the pasta dough rheological properties demonstrate that of wholemeal flour amount to be added whole grain wheat flour – 50%, whole grain rye and hull-less barley – 20%.

To ensure high-quality pasta production already in the raw materials it is necessary to ensure a high protein content and quality, good starch properties (Cubadda et al., 2007; Delcour et al., 2000). Gelatinization process occurs at 62.5 °C, when the amount of water absorbed by the starch granules is 4–5 times for obtaining of a viscous fluid (Осипова, 2009). Starch is essential to determine the pasta cooking quality (Delcour et al., 2000). During pasta cooking, protein existing network restricts the diffusion of water and limits the swelling of starch granules in the central zone of pasta. The extrusion process causes damage to the protein matrix; the resulting pasta is compact and a continuous protein network (Stefano and Marco, 2009). Starch gelatization temperature is very important for the development of pasta technological production parameters, especially extrusion temperature. Therefore (Table 3), the determination of starch gelatization temperature of obtained flour blends with chosen rheological parameters (80% wheat type 405 + 20% whole hull-less barley; 80% wheat type 405 + 20% whole rye; 50% wheat type 405 + 50% whole wheat and 100% wheat type 405 flour) was recommendable.

In the present experiments a significantly higher ($p<0.05$) was obtained for wheat flour type 405, comparing with whole wheat, whole rye and whole hull-less barley grain flour. Obtained results demonstrate that a higher gelatinization temperature (90.9 °C) was for whole wheat grain flour, lower – for whole rye grain flour (85.3 °C). The higher gelatinization temperature demonstrates a higher thermal stability in the future processing (Marti et al., 2010). Wheat starch gelatinization temperature at around 52–85 °C and processing by heating the dough to 80–90 °C in a presence of water is sufficient to initiate starch swelling and gelatinization (Thomas and Atwell, 1999).

Conclusions

1. In the present experiment it was demonstrated that water absorption of analysed flour samples increases by replacing part of wheat flour type 405 with whole grain wheat, hull-less barley or rye flour.
2. A higher dough stability was obtained for flour blends with whole wheat grain flour, comparing to blends with whole rye and hull-less barley grain flour, which could be explained with a higher gluten content of whole wheat grain flour.
3. Wheat flour (type 405) showed the lowest dough development time 2:22 min, but the higher dough development time (6:30 min) was obtained for whole wheat grain flour, whole rye grain flour (6:36 min) and for whole hull-less barley grain flour (6:52 min).
4. To obtain dough with good properties for pasta production, the 20% of wheat flour type 405 could be replaced with whole rye and hull-less barley grain flour and with 50% of whole wheat grain flour.
5. A higher gelatinization temperature was obtained for flour blends with whole wheat grain flour (90.9 °C), comparing to blends with whole rye (85.3 °C) and hull-less barley grain flour (85.7 °C).

Acknowledgement

This research and publication has been prepared within the Local resources research and sustainable use Programme “Agricultural Resources for Sustainable Production of Qualitative and Healthy Foods in Latvia (AgroBioRes) (2014–2017)”. Project No. 4 Sustainable use of local agricultural resources for qualitative and healthy food product development (FOOD).

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DIVERSITY OF LACTIC ACID BACTERIA IN RAW MILK

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Abstract
In this study we described the diversity of lactic acid bacteria and their representatives in raw and thermally treated milk, focusing on their potential in cheese production influencing cheese quality. The aim of the present study was to analyse the concentrations and representatives of lactic acid bacteria in raw milk and to detect the changes of lactic acid bacteria microflora during thermal treatment of cheese milk at a dairy processing plant. The analysis carried out in the study showed a seasonal variation in the microbial composition and quantity of raw milk. The most frequently isolated lactic acid bacteria: lactococci, lactobacilli, leuconostoc were found at low level in raw milk (mean 9.27×10^3 CFU mL^{-1}) and the most frequently identified species were *Lactococcus lactis*, *Lactobacillus brevis* and *Lactobacillus fermentum.* The microflora of raw and pasteurised milk is similar to the analysed lactic acid bacteria representatives in the samples. Interestingly, we found the same species in raw milk and pasteurised milk, for example, *Lactobacillus brevis* and *Lactobacillus fermentum* were detected in the same samples in raw milk and pasteurised milk. Our study showed that lactic acid bacteria concentration was quite low in pasteurised milk (0-76 CFU mL^{-1}), but they grow rapidly in cheese during ripening; therefore the definition of limits of the non–starter lactic acid bacteria colony forming units in milk should be reasonable for selection of appropriate raw milk quality for cheesemaking.
Key words: lactic acid bacteria, total plate count, lactobacilli, raw milk.

Introduction

Raw milk is a natural growth medium for microorganisms. The composition and quality of raw milk microflora are determined not only by hygienic observation in the places of milk production and processing, rapidity of milk cooling and temperature, but also by microflora in the air of dairy environment, and on the surfaces of equipment and premises. An integral part of raw milk microflora is lactic acid bacteria – *Lactobacillus casei* subsp. *paracasei, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus curvatus, Lactobacillus brevis, Lactobacillus fermentum; Leuconostoc lactis, Leuconostor cremoris; Enterococcus faecium, Enterococcus faecalis, Enterococcus durans and Pediococcus* spp.*: P. pentosaceus, P. acidilactici.*

Hygienically produced raw milk may contain 10^2 lactobacilli mL^{-1}. Pasteurization regime selected in dairy industry is able to destroy essential microflora, enzymes and pathogens in milk. It should be noted that inactivation level of microorganisms depends on the count of microorganisms, growth phase and other factors. Although lactobacilli are inactivated by pasteurisation, some strains may survive the heat treatment and proliferate in dairy products production and storage (McSweeney et al., 1999; Jordan and Cogan, 1999).

Non-starter lactic acid bacteria (NSLAB) are found in cheeses made from raw and pasteurized milk. In cheese made from pasteurized milk, they are normally present in relatively low numbers, probably $< 10^3$ CFU g^{-1} at the beginning of ripening, but they grow rapidly during ripening to levels of $\sim10^8$ CFU g^{-1} within 2 to 4 months, depending on the species, cheese, and the ripening temperature (Coppola et al., 1997).

Bactofugation, microfiltration, and application of food additives, cannot significantly decrease the proportion of *Lactobacillus* spp. and *Leuconostoc* spp. in milk. Defects caused by non-starter lactic acid bacteria are found in all dairy products, but the most problematic they are in cheeses.

Taking into account the impact of non–starter lactic acid bacteria on the formation of cheese flavour, various solutions are recommended for manufacturers to assure the quality of cheeses and one of the solutions is the definition of limits of the non–starter lactic acid bacteria colony forming units in milk. The critical limits of mesophilic non–starter lactic acid bacteria are stated 10^2 CFU in 1 mL of milk (Fox et al., 2000). In Latvia mesophilic non–starter lactic acid bacteria are not detected in raw milk, we do not have critical limits for NSLAB.

According to the study of A. Mikelsone (2011) and her research conclusions, the representatives of *Lactobacillus* genus and its colony forming units differ between same cheese varieties manufactured at different plants. This proved that manufacturing and ripening conditions at cheese plant have a significant impact on the diversity of microflora however, the main source of cheese microflora still remains raw milk. From this point of view, the aim of the present study was to analyse the concentrations and representatives of lactic acid bacteria in raw milk and detect the changes of microflora of lactic acid bacteria during thermal treatment of cheese milk at a dairy processing plant.

Materials and Methods

Research was performed from January 2014 to February 2015 at:

- ✓ the Laboratory of Microbiology of the Department of Food Technology of Latvia University of Agriculture;
- ✓ the laboratory of the dairy processing company "Latvijas piens" Ltd.

Object of the research

In order to study the critical limits for non-starter lactic acid bacteria in raw milk, bulk milk samples were analysed (n = 19) in the dairy company. The samples were analysed twice per month over one year period. The samples were taken from raw milk tanks in the dairy company. Bulk milk was kept at 2-4 °C prior treatment.

For better understanding the proliferation of lactic acid bacteria representatives during thermal treatment, raw milk was pasteurized at 74 °C 30 s in the dairy company. Treated milk samples (n = 22) were taken from cheese vats before renneting.

Methods of analyses

Determination of total plate count (TPC) was performed in all analyzed bulk milk samples according to LVS EN ISO 4833-1:2014 using PCA (plate count agar) (OXOID, UK). Sample dilutions were performed according to ISO 6887-5:2010 using salt-peptone solution. The chosen parameters for cultivation of bacteria in PCA agar were 72 hours at 30 °C. The cultivation media were prepared according to LVS CEN ISO/TS 11133-1:2009.

Determination of *Lactobacillus* spp. was performed in all analyzed samples according to LVS ISO 15214:1998 using MRS agar (de Man Rogosa and Sharpe with Tween) media (OXOID, UK). Media were prepared according to LVS CEN ISO/TS 11133-1:2009. Sample dilutions were performed according to ISO 6887-5:2010 using salt-peptone solution.

The chosen parameters for cultivation of lactic acid bacteria in MRS agar were 72 hours at 37 °C, taking as a basis regimes recommended in the scientific literature (Coeuret et al., 2003).

Taking into account the fact that lactic acid bacteria belong to facultatively anaerobic representatives group, anaerobic cultivation of lactic acid bacteria using AnaeroGEN™ Compact system was provided, too.

Identification of *Lactobacillus* spp. colonies was performed taking as a basis the fermentation of carbohydrates using API 50 CHL (BioMerieux, France). The program APILAB Plus version 4.0 (BioMerieux) was used for identification of the isolated colonies up to species.

Data mathematical treatment was performed by using Microsoft Excel programs. The mean and the standard deviation of experimental data were determined.

Results and Discussion

A useful indicator for monitoring the sanitary conditions during the production, collection and handling of raw milk is the total plate count. Its sole value is to indicate changes in the production, collection, handling, and storage environment. Raw milk quality will influence the quality of the processed products. The total plate and lactic acid bacteria count of bulk milk samples is reported in Figure 1.

The microbiological quality of raw milk is strictly related to the management practice, such as equipment and environment hygiene, cow welfare, etc (Little

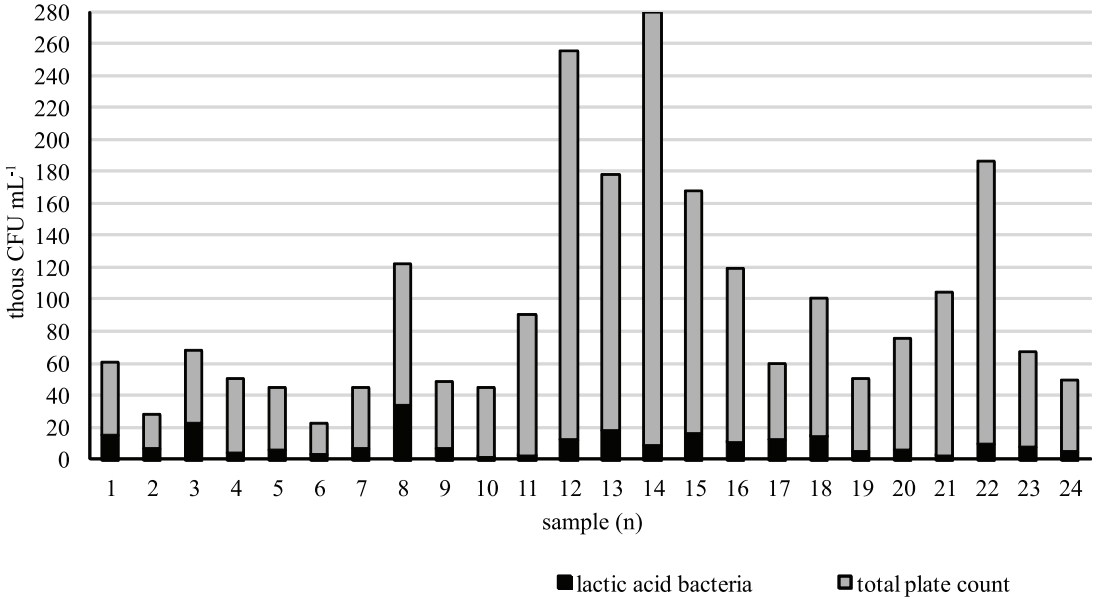

Figure 1. The total plate and lactic acid bacteria count (thous CFU mL^{-1}) in analysed samples.

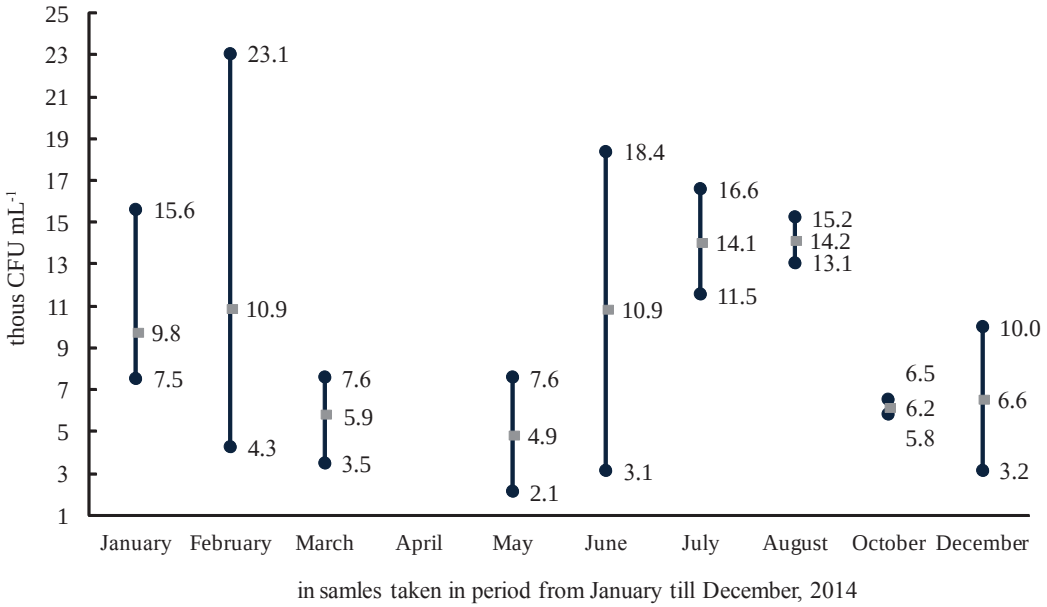

Figure 2. The lactic acid bacteria CFU in analysed raw milk.

et al., 2008). In Latvia, the current regulatory plate count for raw milk is < 100 000 CFU mL^{-1} (at 30 °C) and for raw milk used for dairy products production is less than 300 000 CFU mL^{-1} (30 °C) before processing, as specified in Regulation (EC) 853/004 laying down specific hygiene rules on the hygiene of foodstuffs (Regulation 853/2004). Our results for TPC determined in samples of raw milk indicated that bulk milk samples satisfy official requirements for raw milk hygiene before processing.

According to E. Franciosi et al. (2009) study that LAB/TPC ratio is almost 1, showing LAB dominating role in the bacterial population of raw milk. Our data (it was 0.16) contradicted the E. Franciosi and co-authors study. The microflora of raw milk depends on hygiene of milking, but the cooling rate and temperature, as well as the storage temperature and time, are the main factors taken into account when explaining the results. The microflora of raw milk has changed during the last decades due to milking equipment and storage facilities modernisation. In modern commercial practice, raw milk is normally cooled to 4 °C immediately after milking and may be held at about this temperature for several days at a farm and factory. It means that dominating microflora of raw milk is psychroptropic representatives, such as *Pseudomas* spp., *Alcaligenes* spp., etc. The main differences in ratio between the total plate count and LAB we should explain with the previously mentioned considerations. The min, max and average value of lactic acid bacteria in raw milk samples are showed in Fig. 2.

The analysis carried out in the study showed a seasonal variation in the microbial composition and quantity of the milk. This is an important factor when considering the ultimate use of the milk as, for example, some variations of LAB may affect the flavour development of cheese (Randazzo et al., 2010).

The mean population of LAB was 9.27×10^3 CFU mL^{-1} in the raw milk. Almost all of LAB isolates were lactococci and lactobacilli and the most frequently identified species were *Lactococcus lactis*, *Lactobacillus brevis* and *Lactobacillus fermentum*. According to the findings of E. Franciosi et al. (2010), almost 94% of isolates belong to *E .faecalis*, *E. durans* and *Lactococcus lactis* and only 6% of isolates include *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus plantarum*, etc. in raw milk.

In the present study, microorganisms are identified based on phenotypical criteria, and the obtained results also highlighted the absence of isolates in some analysed raw milk samples, therefore, in Table 1 we summarized all LAB isolates. The microflora of pasteurised milk is primarily of bacterial nature, and bacteria commonly isolated from pasteurised milk are of the same type that is found in raw milk (Table 1).

Table 1

Lactic acid bacteria species isolated from raw and pasteurised milk

Raw milk	Pasteurised milk
Lactobacillus paracasei	*Lactobacillus paracasei*
Lactobacillus brevis	*Lactobacillus brevis*
Lactobacillus fermentum	*Lactobacillus fermentum*
Lactobacillus curvatus	*Lactobacillus curvatus*
Lactococcus lactis	*Lactococcus lactis*
Lactobacillus plantarum	*Lactobacillus acidophilus*
Lactobacillus rhamnosus	*Leuconostoc lactis*

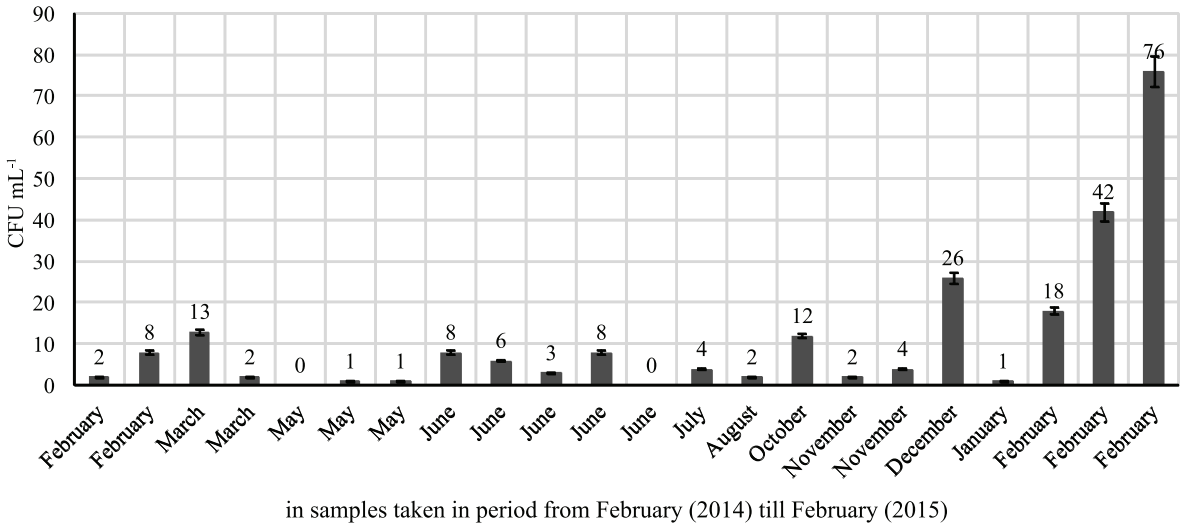

Figure 3. Lactic acid bacteria CFU in analysed pasteurised milk samples during the study period.

The microflora of raw and pasteurised milk is similar to the analysed lactic acid bacteria representatives in the samples. Interestingly, we found the same species in raw milk and pasteurised milk- for example, *Lactobacillus brevis* and *Lactobacillus fermentum* were detected in the same samples of raw milk and pasteurised milk.

Lactococcus lactis was the acidifying bacterium, thus preventing both alteration and growth of potentially pathogenic bacteria. *Lactobacillus plantarum* was rarely detected in milk unlike *Lactobacillus acidophilus*, which was frequently present in raw milk samples. Notably, the species *Lactococcus lactis* and *Leuconostoc lactis* were frequently presented in milk (Casalta and Montel, 2008). Strains of these species have been recognized as an important starter composition in many dairy products and cheeses.

The lactic acid bacteria concentration in pasteurised milk is showed in Figure 3.

Despite the fact that pasteurisation eliminates the most of vegetative cells, some species such as thermoduric microorganisms can survive and subsequently propagate in the final product disturbing quality of dairy products.

Our study showed that lactic acid bacteria concentration was quite low in cheese milk and from this point of view we should not predict potential defects of produced cheeses. According to R. Coppola and co-authors study, mesophilic lactobacilli are present in relatively low numbers in pasteurised milk and in this study it was from 0 to 76 CFU mL^{-1} however, they grow rapidly in cheese during ripening. Therefore in a further study, it would be interesting to determine the impact of lactic acid bacteria on cheese quality starting with raw milk, thermally treated cheese milk and cheese, analysing their influence on the cheese ripening and final product quality.

Conclusions

The most frequently isolated lactic acid bacteria were found at low level in raw milk and the most frequently identified species were *Lactococcus lactis*, *Lactobacillus brevis* and *Lactobacillus fermentum.* The microflora of raw and pasteurised milk is similar to the analysed lactic acid bacteria representatives in the samples. Our study showed that lactic acid bacteria concentration was quite low in pasteurised milk (0-76 CFU mL^{-1}), but they grow rapidly in cheese during ripening; therefore the definition of limits of the non–starter lactic acid bacteria colony forming units in milk should be reasonable for the selection of appropriate raw milk quality for cheesemaking.

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THE POTENTIAL OF FRUCTANS PRODUCING ACETIC ACID BACTERIA IN FERMENTED DAIRY PRODUCTS

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Abstract
In this work the combinations of commercial lactic acid bacteria starters and acetic acid bacteria strain were used for production of fructans in substrate, both with and without sucrose additive, and studied their potential in maintaining technological properties of yoghurt and fermented milk. The objective of this study was to assess the effect of fructans producing starter cultures on milk coagulation technique, the amount of secreted fructans and viscosity of fermented milk samples. An amount of fructans synthesized by starter cultures and *Gluconobacter sp.* B35, pH and viscosity of samples were measured using appropriate standards and analytical methods. Results showed that the addition of acetic acid bacteria did not influence the pH dynamics of fermented milk samples. Increasing sucrose concentration in samples significantly influences fructans production potential. The application of such technology in fermented dairy product production would have potential from microbiological exopolysaccharides increasing position with the aim to promote functionality of dairy foods and to substitute commercial stabilisers etc. The addition of acetic acid bacteria in milk showed negative impact on viscosity of the evaluated samples. The viscosity was liquid in all analyzed samples with acetic acid bacteria, the addition of sucrose helped to make the consistency of yoghurt and fermented milk more liquid. We concluded that the structure of synthesized fructans could not help to improve the textural properties of fermented dairy products. From this point of view, the studied acetic acid bacteria strain should have the potential as prebiotic.
Key words: EPS, fructans, lactic acid bacteria, acetic acid bacteria, fermentation.

Introduction

Exopolysaccharides (EPS) producing lactic acid bacteria (LAB), including *Lactobacillus, Leuconostoc, Lactococcus* and *Streptococcus*, synthesize numerous kinds of homopolysaccharides (HoP) and heteropolysaccharides (HeP), diverse in molecular mass, linkages, solubility, and degree of branching (Patel et al., 2010). *Lactococcus lactis* subsp. *cremoris* produce HeP which consists of rhamnose, glucose, galactose and phosphates or EPS that consist of only glucose and galactose (Marshall et al., 1995). *Streptococcus thermpohilus* produce HeP which contained galactose and rhamnose (Vaningelgem et al., 2004b). *Streptococcus thermophilus* strains are known to produce HoP, too. *Leuconostoc spp.* produces dextrans. *Lactobacillus fermentum* produces 100 mg L^{-1} of EPS in MRS (MRS agar with Tween® 80) broth, some strains of species show high apparent viscosity (Kenji et al., 2010). EPS from *L. delbrueckii* contain glucose equivalents and proteins (Canquil et al., 2007).

A lot of work has been done in the field of isolating and characterising the composition of EPS produced by various strains of lactic acid bacteria (Faber et al., 2002; Grobben et al., 2000; Petry et al., 2000; Ruas-Madiedo et al., 2001; Van Calsteren et al., 2002) and other microorganisms. Recently F.Jakob and co-authors (Jakob et al., 2013) identified several strains of acetic acid bacteria (AAB) as being able to produce high amounts of polysaccharides from sucrose. AAB are involved in food biotechnological processes such as vinegar, kombucha or kefir production for their acetic and gluconic acid production (Dufresne, Farnworth, 2000; Giudici and Pullo, 2008; Gulitz et al., 2011). Fructans synthesis is widely spread among bacteria and was also reported for the AAB species *Gluconobacter oxydans*, *Gluconacetobacter xylinus* and *Gluconacetobacter diazotrophicus* (Velazquez-Hernandez et al., 2009).

At present, there is a great potential for the development of systems for heterologous expression and over-production of exopolysaccharides in different food grade bacteria (O'Connor et al., 2007). They could be exploited to increase the EPS content in foods, thereby improving their technological properties and potentially physiological/health characteristics working as prebiotics.

The objective of this study was to assess the effect of fructans producing starter cultures on milk coagulation technique, the amount of secreted fructans and viscosity of fermented milk samples.

Materials and Methods

Materials

Two types of commercial starter cultures Harmony 1.0 and CHN 22 produced by Chr. Hansen (Denmark) were tested. Harmony 1.0 is a part of Yo-Flex cultures (starter culture containing a mixture of *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus fermentum)* while CHN 22 is a mixed culture used for production of sour cream, buttermilk, kefir, consisting of a combination of mesophilic strains – *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *cremoris, Lactococcus lactis* subsp. *lactis biovar diacetylactis, Leuconostoc spp.* (AD Chr. Hansen, 2006). Additionally, *Gluconobacter*

Table 1

The characteristic of analysed samples, their coding

Code	Starter	Sucrose, %
1 (control)	Harmony 1.0	-
1a	Harmony 1.0 + *Gluconobacter sp.* B35	-
1b		4
1c		8
2 (control)	CHN 22	-
2a	CHN 22 + *Gluconobacter sp.* B35	-
2b		4
2c		8

sp. B35 isolated from kombucha tee (Institute of Microbiology and Biotechnology, Latvia University) was added for increasing of fructans in the fermented substrate.

Preparation of samples

Pasteurized and cooled milk samples were inoculated with Harmony 1.0 starter (as control) and with a mixture of Harmony 1.0 and *Gluconobacter sp.* B35 starters; in similar way CHN 22 starter (as control) and a mixture of CHN 22 and *Gluconobacter sp.* B35 starters were used (Table 1). Taking into account the appropriate substrate for fructans production, different sucrose (granulated sugar EU2 550, Nordic Sugar SC, Sweden) concentrations (4 and 8%) were added. Fermentation was led according to the starter producer's recommendations for each culture and stopped when pH reached 4.5. The fermentation was conducted as follows: in case of Harmony 1.0 starter, the fermentation was carried out at 43 °C for 5 h, in case of CHN 22, at 28 °C for 6 h. The amount of starters was added based on the recommendations of starter manufacturer Chr. Hansen (Denmark), but *Gluconobacter sp.* B35 pure culture – 2 mL to 100 g of milk. Culture consists of at least $5*10^7$ CFU g^{-1} acetic acid bacteria.

Fermented milk samples were stirred, cooled and matured at 4-6 °C for 10-12 hours. Samples were analysed immediately after production taking into account EPS degradation possibilities during storage.

Methods

The amount of fructans synthesized by starter cultures and *Gluconobacter sp.* B35, pH and viscosity of samples were measured.

Fructans were determined in fermented milk samples according to fructans assay procedure for the measurement of fructo-oligosaccharides (FOS) and fructans polysaccharide with recombinant inulinases (Megazyme, Ireland) using AOAC Method 999.03 and AACC Method 32.32 procedures.

The pH of samples was measured using pH-meter Jenway 3520.

The apparent viscosity of samples was measured using DV III Ultra Brookfield viscosimeter with the special spindle SC4-16 at a shear rate of $1min^{-1}$.

Descriptive statistics was carried out to determine the differences of produced fructans concentration in the analysed samples. Correlation analysis was used for determination of the differences between fructans concentration and the apparent viscosity in fermented milk samples.

Results and Discussion

Exopolysaccharides production of LAB is an important attribute for fermented dairy products (Jolly et al., 2002; Welman, Maddox, 2003; Ruas-Madiedo et al., 2010). Our previous experiments (Feldmane et al., 2014) using different Yo-Flex starter cultures (Harmony 1.0, TWIST 1.0 and YF-L902, Chr. Hansen, Denmark) showed that EPS concentration varies from 25.28 to 440.81 mg L^{-1} depending on the fermentation patterns of yoghurt samples. The fermentation temperature significantly contributes to EPS concentration ($p<0.05$) because the increased rate of fermentation is attributed to increased metabolic activity of LAB.

Findings of J.Cerning et al. (1992) and F.Vaningelgem et al. (2004a) showed that optimal temperatures for EPS production were determined as 25 °C for *L. lactis*, 40 °C for *S. thermophilus*, 30 °C for *Leuconostoc spp*. It means that we had chosen appropriate fermentation patterns for fructans synthesis in the present study using lactic acid bacteria starters and lactic acid bacteria starters in the combination with AAB.

For better understanding the influence of fructans producing acetic acid bacteria on the technological properties of fermented milk samples, the pH dynamics of samples during fermentation was studied (see Figure 1 A and B).

The pH dynamics is quite similar during the fermentation of samples. At the end of fermentation, the pH of yoghurt samples ranged from 4.45 to 4.55 and from 4.43 to 4.56 in samples using CHN 22 starter in combination with *Gluconobacter sp.* B35. Results

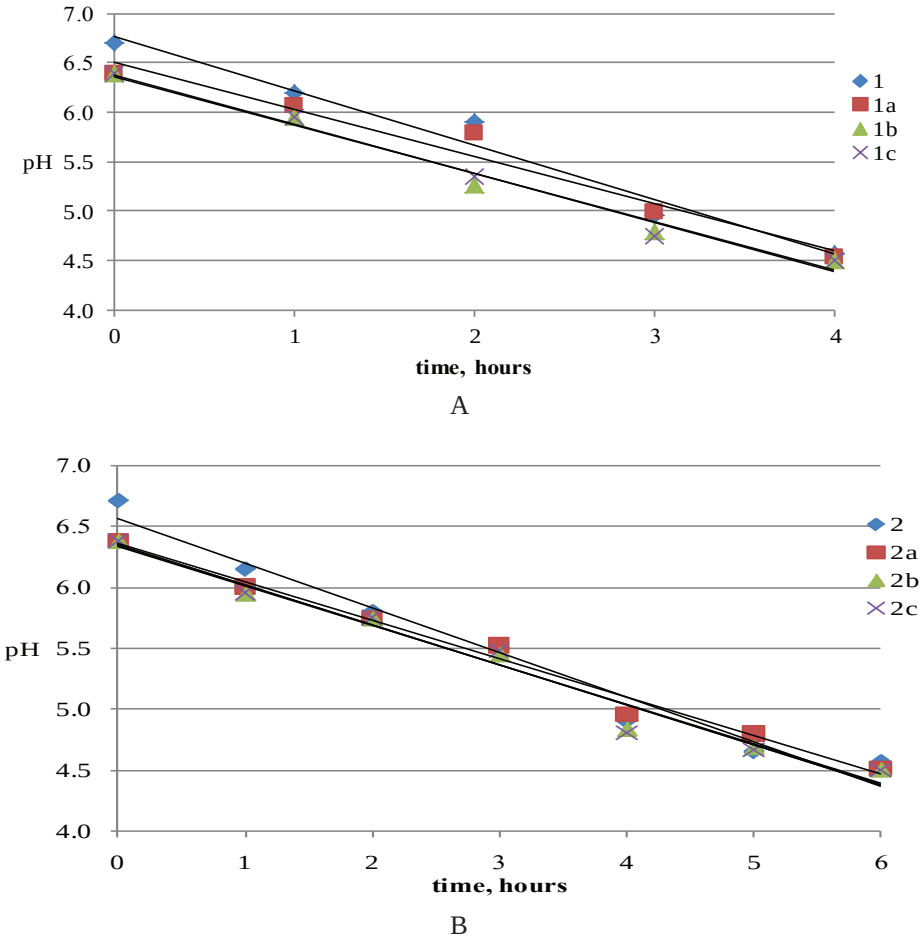

Figure 1. The pH dynamics of samples (A-with Harmony 1.0; B-with CHN 22) during fermentation: 1-Harmony 1.0, 1a-Harmony 1.0+AAB, 1b-Harmony 1.0+AAB+4% sucrose, 1c-Harmony 1.0+AAB+8% sucrose; 2-CHN 22, 2a-CHN 22+AAB, 2b-CHN 22+AAB+4% sucrose, 2c-CHN 22+AAB+8% sucrose.

showed that the addition of acetic acid bacteria had not influenced the pH dynamics of fermented milk samples.

As the aim of present study was to evaluate the potential of acetic acid bacteria on fructans production in milk, we need to clarify that synthesized fructans do not come only from acetic acid bacteria multiplication in fermented substrate. The production of intracellularly synthesized EPS (consisting of glucose and galactose) in yoghurt samples using Harmony 1.0 starter varied roughly from 32.10 to 152.79 mg L^{-1} during the fermentation at temperature interval from 38 to 43 °C (Feldmane et al., 2014), but fructans production potential of experimental samples is summarised in Table 2.

The acquired results show that there is a strong positive linear correlation (r = 0.676) among the amount of added sucrose and fructans content (mg 100 g^{-1}) in fermented milk products. We can declare that the amount of added sucrose increases the fructans production potential in fermented milk products during fermentation.

Table 2

Fructans concentration in analysed fermented milk samples, mg 100 g^{-1}

Sample code	Content of fructans
Harmony 1.0	121
Harmony 1.0+AAB	212
Harmony 1.0+AAB+4% sucrose	241
Harmony 1.0+AAB+8%	256
CHN 22	168
CHN 22+AAB	168
CHN 22+AAB+4% sucrose	184
CHN 22+AAB+8% sucrose	225

Fructans synthesis is catalyzed by fructosyltransferases, which cleave the main substrate sucrose and release glucose in a first step (Jakob et al., 2013). The higher fructans concentrations were observed in 1c, 1b and 2c samples ($p<0.05$). We could explain it with J.Cerning (1990) work conclusions that a mixed culture was characterised with higher

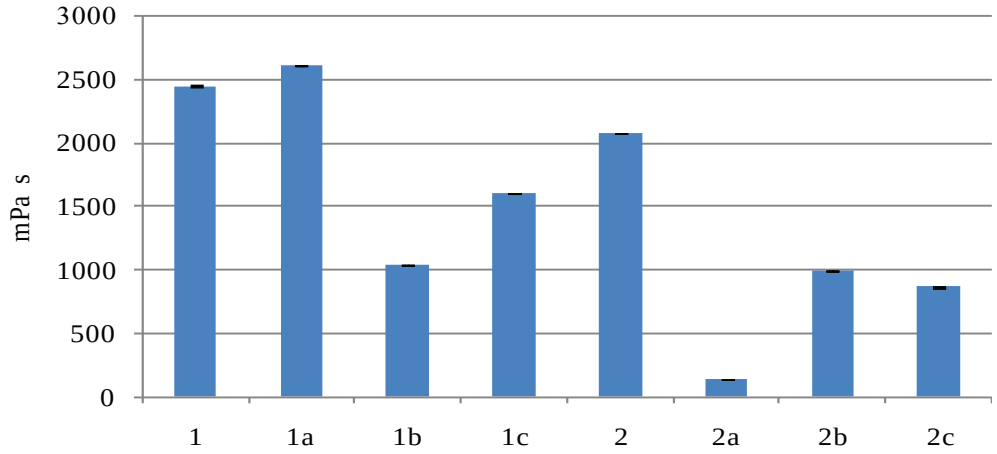

Figure 2. The apparent viscosity of investigated yoghurt and fermented milk samples: 1-Harmony 1.0, 1a-Harmony 1.0+AAB, 1b-Harmony 1.0+AAB+4% sucrose, 1c-Harmony 1.0+AAB+8% sucrose, 2-CHN 22, 2a-CHN 22+AAB, 2b-CHN 22+AAB+4% sucrose, 2c-CHN 22+AAB+8% sucrose.

EPS production potential instead of a single strain. Interestingly, the detected fructans concentrations differed between samples, higher concentrations were observed in yoghurt samples with acetic acid bacteria supplement. These findings were explained by *Streptococcus thermophilus* HeP and HoP production ability and symbioses of starter representatives with AAB during fermentation. Increasing sucrose concentration, significantly influences fructans production potential ($p<0.05$) in yoghurt samples. The application of such technology in fermented dairy products production would have potential from microbiological exopolysaccharides increasing position with the aim to promote functionality of dairy foods and to substitute commercial stabilisers. From negative point of view should be mentioned increased energy value of products.

We know that rheological properties of EPS carry a great importance in their overall characteristics but they are significantly affected by molecular features which need to be understood better (Patel et al., 2010).

The apparent viscosity of samples varied from 131.97 to 2615.58 mPa s (Fig. 2). The highest viscosity was observed in yoghurt samples using Harmony 1.0 starter (1) and Harmony 1.0 starter, and AAB combination (1a). Similar results we found analysing the connection with EPS producing commercial yoghurt starters and viscosity of products (Feldmane, 2013). The presence of *Lactobacillus fermentum* into starter composition helps to increase the apparent viscosity according to information mentioned in the research work of F.Kenji et al. (2010). The 1% (m/V) solution of the purified EPS from *Lactobacillus fermentum* strain showed a high apparent viscosity of 0.88 Pa s at a shear rate of 10 s^{-1}.

We observed a different effect on structural properties of fermented milk samples when acetic acid bacteria and two concentrations (4 and 8%) of the sucrose were added. The addition of acetic acid bacteria in milk showed a negative impact on the viscosity of evaluated samples (1b, 1c, 2a, 2b and 2c). The viscosity was liquid in all investigated samples with AAB with the exception of 1a, also addition of sucrose helps to make more liquid consistency of fermented milk samples. We concluded that the structure of synthesized fructans could not help to improve the textural properties of fermented milk products. Synthesized fructans do not form gels from intermolecular interactions of different polymer chains in milk matrix. We could compare our conclusion with the findings of S. Arvidson et al. (2006) that levan does not form gel from intermolecular interactions of different polymer chains in aqueous solutions and exhibits low intrinsic viscosities even at high molecular weight being typical for spherical particles (Jakob et al., 2013). According to F. Jakob et al. (2013) study, the ability of the isolated levans to bind water should not result from intermolecular interactions of different elongated polymer chains. In fact, intramolecular interactions of individual levan molecules have to be considered to effectively bind water and, therefore, to act as hydrocolloids. The obtained results showed that HeP from LAB play an important role in the rheology of fermented milk products but HoP producers have been evaluated lesser and they are used mainly for fermentation of non-dairy products (Notararigo et al., 2013). From this point of view, the studied fructans of acetic acid bacteria strain should have a higher potential from prebiotic aspect.

Conclusions

This work offers possibilities for further application of AAB (food grade ingredient) strain in dairy products production increasing their functionality and microbiologically synthesized fructans concentration.

Acknowledgement

This study was supported by ERDF grant within activity 2.1.1.1. Contract's No 2014/0037/2DP/2.1.1.1.0/14/APIA/VIAA/108.

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PREVALENCE OF *MYCOPLASMA GALLISEPTICUM* IN THE COMMERCIAL LAYER FLOCK

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Abstract
Avian mycoplasmosis have been considered a severe problem in poultry diseases; *Mycoplasma gallisepticum* being *one of* the most important. This study was conducted in Joint-Stock Company Balticovo, Latvia, to determine the prevalence of *M. gallisepticum* infection in hen's flocks in the farm. A total of 904 serum and 335 swab samples from non-vaccinated birds against *M. gallisepticum* from 65 chicken flocks of different age, from day 1 to 75 weeks old, were all tested. The commercially available enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) tests were used. Results revealed that the seropositive flock rate, based on ELISA tests and, according to ratio that represents the extent to which a light source effectively stimulates the rods: S/P ratios and antibodies titer higher 1.076 were 22/904 (2.43%) and 4/904 (0.44%), respectively, while PCR-positive flock rates were not confirmed. Seroprevalence of *M. gallisepticum* in commercial layer flock in Latvia was more common seen in birds from 17 to 30 weeks of age.
Key words: poultry, *Mycoplasma gallisepticum,* seroprevalence, ELISA, PCR.

Introduction

Outbreaks of infectious disease are a constant risk for the agricultural industry and *Mycoplasma gallisepticum* is the most economically significant mycoplasmal pathogen of gallinaceous and certain non-gallinaceous avian species (Osman et al., 2009). Mycoplasmas are ubiquitous throughout the animal kingdom and virtually every mammal, bird, reptile, amphibian and fish that has been tested for mycoplasmas has revealed unique species (Pitcher and Nicholas, 2005).

Mycoplasma gallisepticum is an avian pathogen most frequently associated with chronic respiratory disease in chickens (*Gallus gallus domesticus*) and infectious sinusitis in turkeys (*Meleagris gallopavo*). It is a major problem in the commercial poultry industry worldwide causing significant economic losses (Levisohn and Kleven, 2000). The most common economic impacts of *M. gallisepticum* are decreased egg production in layers (Mohammed et al., 1987; Levisohn and Kleven, 2000a; Bradbury, 2007).

Transmission of *M. gallisepticum* infection to new hosts can occur vertically in ovo from infected breeders (Levisohn and Kleven, 2000; Bradbury, 2005). Horizontal bird-to-bird transmission occurs within flocks through close contact, probably via respiratory tract excretions. The rate of spread through a flock will be influenced by management systems (e.g., stocking density, type of drinker and feeder). Between flock spread can also occur through fomite carriage (Racicot et al., 2011). *M. gallisepticum* can survive in different reservoirs within a poultry farm and the fact it can weaken the immune system to other diseases, occasionally also respiratory, is a world concern. Among these reservoirs, food, drinking water, feathers, droppings or dust are the most common (Marois et al., 2002).

Mycoplasmosis is one of the most important disease in poultry production nowadays under intensive production conditions and in most countries (Netherlands, Germany and others). Therefore, control programs for *M. gallisepticum* are based on maintaining commercial breeding stock free of infection. There has never been *M. gallisepticum* research in Latvia.

This study was undertaken to determine the prevalence of *M. gallisepticum* infection in hen flocks in Joint-Stock Company Balticovo.

Materials and Methods

Clinical samples

Research was carried out in Joint-Stock Company Balticovo from 2012 to 2014. Number of samples tested (n=1239) are summarized in Table 1.

Blood samples (n=904) from pullets and layers in different ages (1 day to 75 weeks) and two different breeds (Lohman Brown, Hy-Line) were collected aseptically from wing vein of individual birds with 1.5 mL sterilized disposable plastic syringe without anticoagulant and allowed to clot for 1 h in the syringe. Blood containing syringes were kept in the room at 20 °C for 4-5 h. The serum (liquid portion) was decanted in centrifuge tube and centrifuge at 1500 rpm for 10 min to have clear serum. The serum was collected in sterile Eppendorf tube and preserved at -20 °C until further processing for the serological study. Blood was collected to perform sero-analyses to detect antibodies against *M. Gallisepticum* using enzyme-linked immunosorbent assay (ELISA). None of the chickens had been vaccinated with any *M. Gallisepticum* vaccine.

Swab samples (n=335) were taken as described from both clinically healthy and sick birds, both from fallen birds (n=163) (Table 1) to detect

Table 1

Samples from laying hen flock, 2012-2014

Samples	Samples tested
Sera from chickens and layers	**904**
Swab samples from live and fallen birds, including	**163**
Trachea	185
Cloaca	60
Joints	9
Ovarium	6
Air sac	6
Nostrils	1
Swab samples from surroundings, including	**172**
Birds shipping transport	30
Different parts in henhouses (ventilator, cages, floor, food tray, water nipels)	110
Stuff (clothes, shoes, hands)	30
Air filter	2
Total numbers of swab samples	**335**
Total numbers of samples	**1239**

M. gallisepticum using PCR method. In sick and fallen birds the clinical signs of diseases of upper respiratory tract (discharge from nostrils, inflammation of the air sacs and other) was observed. Samples from surroundings (n=172) (Table 1) were taken with sterile transport swab (Sarsted, DE) from birds shipping transport, stuff and henhouses in different parts according to the requirements of standard (LVS ISO 18593:2007).

Serology

Antibodies to *Mycoplasma gallisepticum* were detected with ELISA assay, tested in World's Poultry Science Association Latvia department using commercial kit (BioChek, UK) following the manufacturer's instructions. To read the result, a spectrometer with length of the wave 405 nm was used. In case of the positive reaction in microplates, yellow coloring whose intensity depends directly on presence of anti-MG immunoglobulins forms.

Results were expressed as S/P ratios relative to a standard positive control. Serum samples with S/P ratios equal to or greater than 0.5 were considered positive.

PCR method

Samples were tested in World's Poultry Science Association Latvia department using Polymerase chain reaction (PCR), which was used for detection of *Mycoplasma gallisepticum* in organs (trachea, lungs and air sacs) of infected birds. For isolation of bacterial DNA from tissue, swabs were dipped in PBS for several hours at room temperature (15 – 25 °C), centrifugated of pellet bacteria at 5000 rpm for 10 min and supernatant containing DNA was placed on the QIAcube-shaker (QIAcube Protocol Sheet).

Real-time PCR for identification of *Mycoplasma gallisepticum* the bactotype *Mycoplasma* Mg/Ms PCR Kit (96) (QIAGEN, DE) was used. Amplification was performed in a Rotor-Gene Q.

Cycling parameters were as follows: initial denaturation at 90 °C for 3 min followed by 35 cycles of denaturation at 95 °C for 15 sec, primer annealing at 60 °C for 20 sec, extension at 75 °C for 15 sec, was completed by one cycle of denaturation at 95 °C for 15 sec, primer annealing at 60 °C for 45 sec and extension at 75 °C for 5 min. The amplified products were separated as previously described by C.Marois et al. (2000).

Results and Discussion

In most countries, control programs of the *M. gallisepticum* are based on maintaining commercial breeding stock free of infection. Monitoring programs for the detection of *Mycoplasma spp.* infection are based mainly on serological tests. Regular serological monitoring of commercial poultry is essential for the detection of an infection, provided that representative sample sizes and tests with appropriate sensitivity and specificity are used (Landman, 2014).

The results of the ELISA test in our study (Table 2) showed that 22 from 904 samples of the birds are *M. gallisepticum* serologically positive (2.43%), if we count S/P ratios. The analysis of the results of other scientists testify that the number of hens which are infected with *M. gallisepticum* compared to our results are much higher, for example, in Algeria 69.9% (Heleili et al., 2012), in Bangladesh 64.47% (Zulfekar et al., 2015), in Serbia 19.05% (Kapetanov et al., 2010) positive birds, whereas in France (Dufour-Gesber et al., 2006) and in Netherlands (Landman, 2014) the positive *M. gallisepticum* cases were not detected.

Comparing frequency of infection in different breeds (Table 2), results showed that during the time of monitoring the most of the Hy-Line breed birds were infected (79.17%) with *M. gallisepticum* although other authors (Kapetanov et al., 2010) reported about higher frequency of infection just in Lohman Brown breed hens (76.6%). Our finding testifies that Hy-Line breed hens could be infected already vertically from parents flock.

Assessment of the dynamics of prevalence of *M. gallisepticum* in hen flocks in 3 years' time confirms that the occurrence of *M. gallisepticum* significantly decreased in recent years. Our analysis shows that in 2012 there were 13.51% (20/148) infected birds, in 2013 – 0.49% (2/412), but in 2014 - none (0/342) positive case within examined birds. This beneficial situation in commercial layer flock can be explained by a strong control of the parent's flock and by managing a good biosecurity plan in the hen flock.

During the last years in Europe strong supervision and control plans with the aim to avoid the horizontal and vertical prevalence of MG in the parent's hen flocks and in the commercial poultry flocks were established in accordance with Council Directive 2009/158/EC and Commission Decision 2011/214/EU. Therefore, most of the commercial laying flocks are trying to be free from *M. gallisepticum*, however, frequently the problem with other infectious diseases like Infectious bronchitis virus, Newcastle disease, the positive MG (Landman, 2014) in the flocks could be observed.

Table 2

Anti-MG-ELISA antibody (IgG) status of poultry

Number of samples	year	age	S/P Ratio	Titer	Breed
1	2012	28	0.601	818	Lohman Brown
2	2012	20	0.579	785	Hy-Line
3	2012	20	0.603	821	Hy-Line
4	2012	20	0.722	1001	Hy-Line
5	2012	20	0.563	761	Hy-Line
6	2012	20	0.519	696	Hy-Line
7	2012	20	0.669	920	Hy-Line
8	2012	20	0.545	735	Hy-Line
9	2012	20	0.770	1074	Hy-Line
10	2012	20	0.635	869	Hy-Line
11	2012	20	0.559	755	Hy-Line
12	2012	20	0.561	758	Hy-Line
13	2012	20	0.529	711	Hy-Line
14	2012	20	0.525	705	Hy-Line
15	2012	20	0.888	**1257**	Hy-Line
16	2012	20	0.872	**1232**	Hy-Line
17	2012	20	0.830	**1167**	Hy-Line
18	2012	20	0.780	**1090**	Hy-Line
19	2012	20	0.639	875	Hy-Line
20	2012	20	0.601	818	Hy-Line
21	2013	29	0.767	1070	Lohman Brown
22	2013	25	0.528	709	Lohman Brown

Table 3

***Mycoplasma gallisepticum* in hen flocks**

Age of the birds / Type of sample	ELISA Tested samples / positive samples (%)			PCR Tested samples / positive samples	
	till 16th week	from 17th till 30th week	from 31st till 75th week	till 16th week	from 17th till 30th week
Serum	399 / 0	266 / 4 (1.5%)	239 / 0	NT	NT
Swab samples from live poultries (trachea, cloaca)	NT	NT	NT	76 / 0	62 / 0
Swab samples from died poultries (trachea, articulation, uterine duct, air sacs, nostils)	NT	NT	NT	3 / 0	22 / 0
Samples from environment, personel	NT	NT	NT	140 / 0	32 / 0

NT – not tested

The evaluation of 22 cases with serologically positive *M. gallisepticum* (according to S/P ratio) (Table 2) showed that in our study only four birds had the titer of antibodies above 1076. According to the recommendation of the producer of the ELISA test kit, only *M. gallisepticum* antibodies titer higher 1076 confirms the positive case. Therefore, the results of the current study have shown that *M. gallisepticum* practically (4/904 or 0.44%) was not observed in the hen flock. Findings of sera results with antibodies titer below 1076 we can explain with cross-reaction that can give false positive results (Kemp et al., 1994) because results can be affected by antibodies of other infectious diseases (*M. sinoviae*, Newcastle disease, Infectious Laryngotracheitis) (Adair et al., 1990). Other researchers (Stipkovits, 1993) also have found out that the presence of *M. gallisepticum* infection in flocks could cause cross reacting of the antibodies in serological tests. According to data of A.Ahmad et al. (2008), the sensitivity and specificity of ELISA test for the detection of *M. gallisepticum* was 74.60%.

Screening programs that are only based on seroconversion may be inadequate for diagnostic and control of mycoplasmosis. The authors suggest the adoption of other techniques to confirm the presence of the agent (*M. synoviae*), such as DNA detection by molecular assays (PCR), because antibodies based tests are uninformative about the active infection (Ewing et al., 1996). PCR represents a rapid and sensitive alternative for the traditional mycoplasma culture methods, which require specialized media, reagents for serotyping of the isolates and are time-consuming (Kemp fetal.,1994; Levisohn and Kleven, 2000; Arshad et al., 2013).

The studies of other authors on naturally infected birds the most positive number of samples in air sac 23.3%, trachea 11.6%, lung 8.3% (Reda et al., 2012) were found. Also, M. Rauf with co-authors (Rauf et al., 2013) have reported that the highest detection was in trachea (39.2%) followed by air sac (27.4%) and lowest in lungs (15.92%). In the present study in 2014 for control of prevalence of *M. gallisepticum* 439 samples (Table 3) were taken. Although birds in every age are sensitive to mycoplasmosis, new birds are much more sensitive to infection than grown-up birds (Kleven and Ferguson-Noel, 2008). Therefore, significantly more samples to find the MG we took straight from birds at the age up to 16th week.

Our data without any PCR-positive case (Table 3) confirm the results of strong biosecurity procedures during the rearing of young pullets and MG control in parents flock.

Conclusion

This study confirmed the low seroprevalence of *M. gallinarum* in commercial layer flock in Joint-Stock Company Balticovo and that it was more common diagnosed in birds from 17 to 30 weeks old.

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PROBIOTIC AND PREBIOTIC INFLUENCE ON HEMATOLOGICAL VALUES OF GOAT KIDS

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Abstract
Blood haematology is one of the indicators that shows if a goat (*Capra hircus)* kid gets a sufficient quantity of liquid, has a good supply of oxygen in the body, as well as an indication of inflammatory processes. Our aim of this research was to find out how feed additives (probiotics (*Enterococcus faecium*), prebiotics (Jerusalem artichoke (*Helianthus tuberosus L.*)) and symbiotics (*Enterococcus faecium* + Jerusalem artichoke)) influence on haematological parameters of kids. Research was performed in Latvia in 2014. The blood samples were analyzed for leucocytes (WBC), erythrocyte number (RBC), haemoglobin (HGB) concentration, packed cell volume (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC). Since the blood indicators have not significantly changed in all examination times, we analyzed the results of the age of 4, 8 and 12 weeks. HCT downward trend in blood sampleMRG + PRO, MRG + PRE and MRG + SIM group of kids was observed from the fourth week up to the eighth week and then it gradually increased. The results showed that the HCT, HGB, RBC, MCHC and WBC number was comparable ($p>0.05$) between groups. Our results prove that the use of Jerusalem artichoke powder as an additive to food not only improves digestion and metabolism of ruminant, but also helps to maintain constant haematological values. In the control group and the kids who received probiotics, prebiotics or symbiotics - significant differences are evident in all hematological parameters ($p<0.05$) at the age of 12 weeks.
Key words: goat kid, haematology, probiotic, prebiotic, symbiotic.

Introduction

Latvian goat (*Capra hircus)* industry increases every year. There are two main directions of use - dairy goats and meat goats. Health of animals is very important to increase productivity. Blood haematology is one of the indicators that shows if a kid gets a sufficient quantity of liquid, has a good supply of oxygen in the body, as well as it is an indication of inflammatory processes. There are very few studies about the haematological values in goat kids.

Jerusalem artichoke (*Helianthus tuberosus*) is mostly grown to produce tubers that are further processed and used as diabetic sweeteners and prebiotics. The inulin content of the tubers amounts to 340 mg g^{-1} DM (dry matter). Typically, Jerusalem artichoke flour contains inulin on average 10 g kg^{-1}(%), while the specially developed technology allows to increase the amount of insulin to 48.5 g kg^{-1}(%) - 50.1 g kg^{-1}(%) (Fleming et al., 1979; Hindrichsen et al., 2004; Ārne et al., 2014; Inchuen et al., 2014). Extracts from Jerusalem artichoke tubers have great potential as additives to animal feed due to their bifidogenic effect. The addition of Jerusalem artichoke tuber extracts to diets, for instance, could lead to feed efficiency improvements, improved digestion and reductions in diarrhea (Stanley et al., 2008). Probiotic supplements contain viable bacteria (e.g., *bifidobacteria, enterococcus* and *lactobacilli*) designed to shift the balance of the microflora in the large intestines to the detriment pathogenic bacteria. J.T. Huber (1997) considers that probiotics reduce digestive disturbances, increase weight gain, and increase milk production of dairy cattle (Huber, 1997). Probiotics can contain prebiotics as substrates and then they are called symbiotics (Stanley et al., 2008). T.J. Bunce et al. and E.A. Flickinger consider that symbiotics increase bifidobacteria populations in the colon and may protect against pathogenic *E. coli* (Bunce et al., 1995; Flickinger et al., 2003).

Our aim was to find out how feed additives (probiotics and prebiotics) influence the kid haematological parameters, because these parameters are one of the health status indicators.

Materials and Methods

Research was performed in a farm of Latvia, Zemgale region, from February to April, 2014.

In total, 40 Saanen goat kids were involved in the research. All kids were kept with mothers for two weeks and then separated. Each group was assembled from goat kids of both sexes (n = 10) at of aged 14±2 days. After age of two weeks kids were fed with foregoing calf milk replacer using nipple buckets and lived separately from mothers in a cote. We formed 4 groups for the research. The control group (MRG+C) kids (n=10), probiotic group (MRG+PRO) where kids with milk replacer additionally received *Enterococcus faecium* 0.025 g day^{-1} (n=10), prebiotic group (MRG+PRE) where kids with milk replacer additionally received powder of Jerusalem artichoke (*Helianthus tuberosus L.*) 0.04 g day^{-1} with increased insulin concentrations in flour (n=10), symbiotic group (MRG+SIM) - where kids with milk replacer additionally received *Enterococcus faecium* plus powder of Jerusalem artichoke (n=10).

Drinking water and hay were easy accessible all the time.

The animals were apparently healthy. At the age of two weeks blood analysis of all animals was examined and physiological parameters (heart rate, respiratory rate, temperature) were assessed. In week 4, 8, and 12 we collected blood samples (6 mL) by jugular venapuncture in ethylene diamine tetracetate (EDTA) vacutainer tubes and transported to the laboratory for analysis. The samples were analyzed within two hours after collection. The samples were collected in the morning - two hours after feeding. The blood samples were analyzed for leucocytes (WBC) and erythrocyte (RBC) number, haemoglobin (HGB) concentration, packed cell volume PCV (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) measured by automatic analyzer Mindray BC 2800Vet (Schlamet al., 1975; Egbe-Nwiyi, 2000; Iriadam, 2007; Jawasreh et al., 2009; Elitok, 2012). We investigated the samples in morfofunctional laboratory at Latvian University of Agriculture in Faculty of Veterinary Medicine.

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

Blood haematological values can vary depending on age, gender, goat breed, environmental temperature, feeding type and health status (Daramola et al., 2005; Elitok, 2012). In this study, we wanted to find Jerusalem artichoke concentrate and probiotics *Enterococcus faecium* impact on kid blood haematological values.

Analyzing the results of WBC (Fig. 1), the largest increase in the number of white blood cells is observed directly in MRG + C 15.5 ± 2.90 x$10^{9}L^{-1}$ group of kids, while in MRG + PRO the values are decreasing. The similar trend of WBC was established in the reports of other studies (Daramola et al., 2005; Piccione et al., 2010). From the age of 8 weeks WBC number in MRG+PRO, MRG+SIM and MRG+C decreases and it refers to more stable state of health. Increased WBC number is in response to inflammation or infection diseases or it may also be attributed to physiological phenomena - excitement or strenuous exercise during handling (Daramola et al., 2005; Zamfirescu et al., 2009). We observed that the physiological indicators ranging from 8 weeks became more stable. The most significant changes were observed at the age of 12 weeks. WBC number in MRG+C was significantly higher than in MRG+PRO, MRG+PRE and MRG+SIM ($p<0.05$) ones.

There are studies, in which it is found out that the number of erythrocytes gradually increases, especially in the first three months of life, and reaching the age of 6-9 months, RBC does not change significantly (Egbe-Nwiyi, 2000). In our study, this trend was observed only in MRG+PRE group (Fig. 2). The values ranged from 11.3 ± 1.29 x$10^{12}L^{-1}$ to 14.1 ± 2.17 x$10^{12}L^{-1}$. Also, RBC growth in MRG+PRE kids is observed throughout the study period. In MRG+ C group RBC values decreased from 12.6±1.81 x$10^{12}L^{-1}$ on week 8 till 10.7±0.73 x$10^{12}L^{-1}$ by the end of the research ($p< 0.5$).

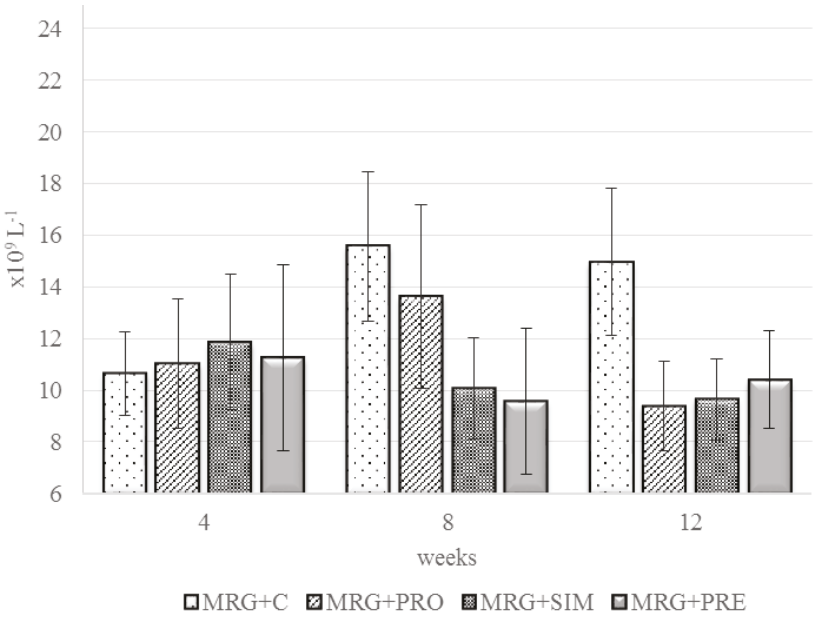

Figure 1. Goat kid leukocyte (WBC) value changes depending on the type of feeding in the first three months of life.

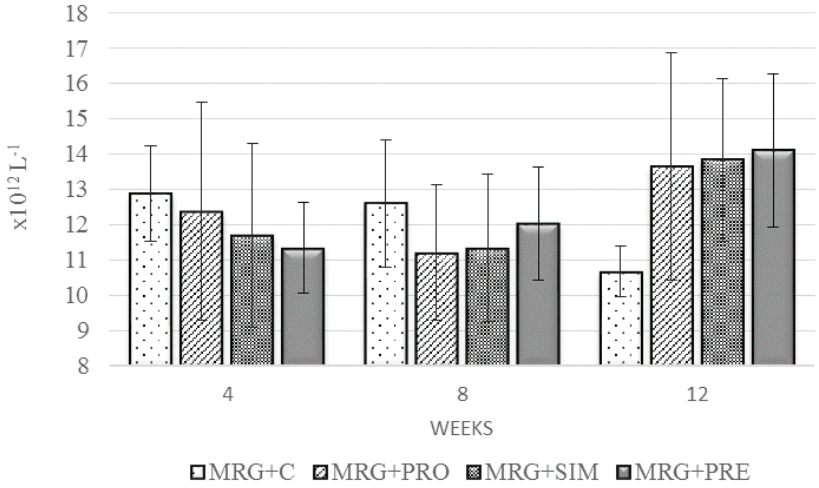

Figure 2. Goat kid Erythrocyte (RBC) value changes depending on the type of feeding in the first three months of life.

Haemoglobin in the kid blood tests is displayed in Figure 3. HGB concentration in all groups fed with additives decreased till the end of the second month and then gradually increased by the end of the research. High HGB values are an advantage in terms of the oxygen carrying capacity of the blood (Daramola et al., 2005). The highest haemoglobin of 8.9 ± 0.71 g dL^{-1} in MRG + C group kids was observed on the fourth week and then it gradually decreased to 6.0 ± 0.65 g dL^{-1} at the age of 12 weeks.

HCT downward trend in blood samples (Fig. 4) in MRG + PRO, MRG + PRE and MRG + SIM groups of kids was observed from the fourth week up to the eighth week, and then it gradually increased. HCT-level of MRG + C kids was falling in the whole research period. In the fourth week HCT was 28.9 ± 2.82% and fell to 18 ± 2.3% at the end of the research. At the age of 8 weeks HCT level in all groups was decreasing below the normal value (22-38%), MRG+PRE was 21.9±2.90%, MRG+C 20.6±3.03% and then increased by the end of the research (Porter et al., 2011; Pugh et al., 2012). There are findings that HCT value varies from breed to breed in goats. Also, J.O. Daramola et al has described that the increase of HCT values in cattle is related to the increase of environmental temperature and this coincides with our findings, since in the course of our investigations the air temperature dropped sharply in mid-March compared to February (Daramola et al., 2005).

Erythrocyte parameters – MCV, MCH and MCHC values in all involved groups are shown below in Table 1. The remaining differences between the groups are

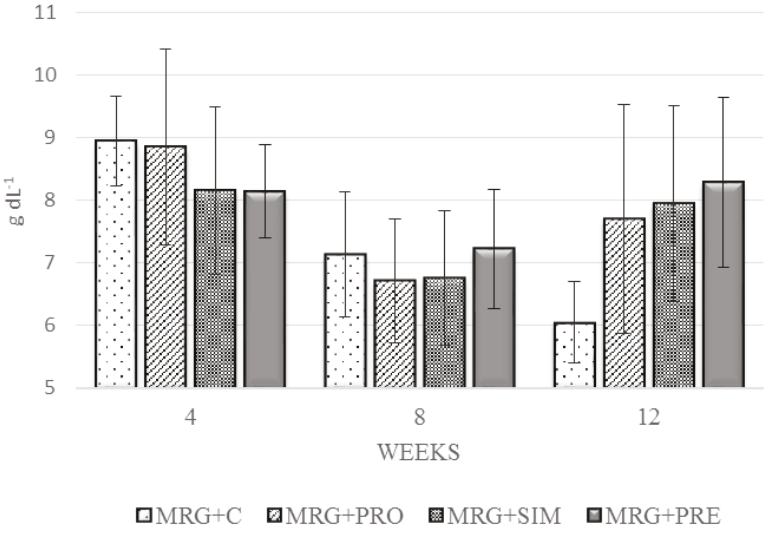

Figure 3. Goat kid haemoglobin (HGB) value changes depending on the type of feeding in the first three months of life.

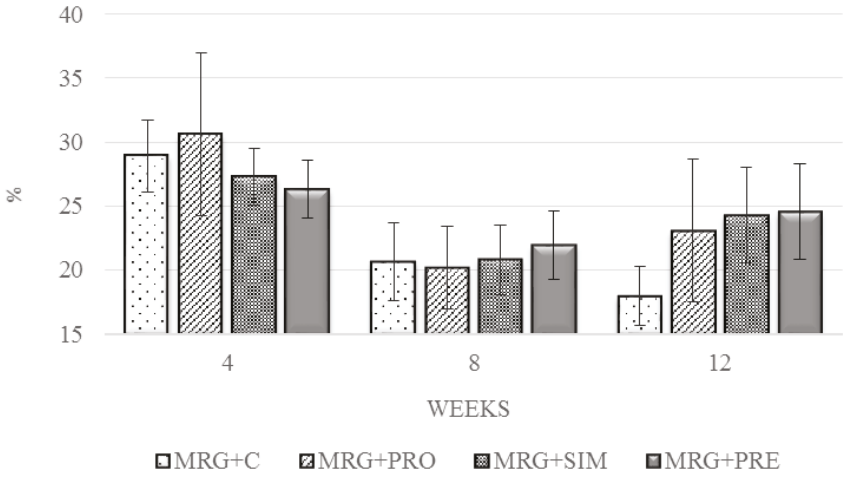

Figure 4. Goat kid packed cell volume (HCT) changes depending on the type of feeding in the first three months of life.

not so significant to evaluate. MCV and MCH amount for all groups during the study dropped alike. At baseline, the amount of MCV in groups ranged from 22.7±2.63 to 25.4±4.78 fl, while at the end of the experiment from 16.1±1 to 1.5± 0.79 fl.

Probiotics, prebiotics or symbiotic additives did not show significant differences between the experimental groups. MCH values between the groups ranged from 6.9 ± 1.54 pg to 7.3 ± 1.54 pg in the fourth week and from 5.6 ± 0.21 pg to 5.8 ± 0.19 pg at the end of the study ($p > 0.05$).

MCHC value gradually increasing trend was observed in MRG + PRE group of kids. In MRG + SIM group the increase was observed up to the age of 12 weeks. The MCHC value decline in MRG + PRO kids was observed from the second to the fourth week, from 30.1 ± 0.85 g dL^{-1} to 29.2 ± 2.73 g dL^{-1}. Till the end of the research MCHC value was increasing.

The results showed that the HCT (PCV), HGB, RBC, MCHC and WBC number was comparable ($p<0.05$) and this coincides with the studies of other authors (Iridiam, 2004; Daramola et al., 2005; Zamfirescu et al., 2009; Shaikat et al., 2013).

Evaluating the above presented results of MCV, MCH and MCHC, it can be observed that haematological parameter values of the control group kids are lower than in other groups. This indicates that probiotics and prebiotics have a positive effect on the digestibility of feed intake and absorption ability of the kid in the body, thus improving blood haematology.

Table 1

Erythrocyte parameters [mean ± S.D] of goat kids which were fed by milk replacer with probiotic or/and prebiotic and kids which were fed by mother milk

Age weeks	MRG+C		MRG+PRO		MRG+PRE		MRG+SIM	
	mean	stdev	mean	stdev	mean	stdev	mean	stdev
MCV fl								
4	22.7	2.63	25.4	4.78	23.3	1.55	24.2	4.07
8	16.4	0.40	18.2	2.42	18.3	0.81	18.5	1.84
12	16.8	1	16.9	0.90	17.4	0.79	17.3	1.43
MCH pg								
4	6.9	0.56	7.3	1.54	7.1	0.30	7.1	1.04
8	5.6	0.18	5.9	0.54	5.9	0.16	5.9	0.41
12	5.6	0.21	5.5	0.21	5.8	0.19	5.6	0.23
MCHC g dL^{-1}								
4	30.9	1.22	29.1	2.73	30.8	0.96	29.8	0.66
8	34.5	0.42	33.3	1.50	32.8	0.92	32.5	1.42
12	33.6	0.65	33.0	1.30	33.6	0.91	33.0	1.71

MCV - mean corpuscular volume, MCH - mean corpuscular haemoglobin, MCHC - mean corpuscular haemoglobin concentrations.

Conclusions

Our results prove that the use of Jerusalem artichoke powder as an additive to food helps to maintain constant haematological values. In the control group and the group where kids received probiotics, prebiotics or symbiotics - significant differences are evident at the age of 12 weeks in all haematological parameters ($p>0.05$).

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CORTICOSTEROID-INDUCED HEPATOPATHY IN DOGS

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Abstract
Corticosteroid therapy is often used on dogs (*Canis lupus familiaris*) to treat different kinds of diseases. One of the most common complications of corticosteroid use is steroid hepatopathy, a specific pathology only in dogs. The objective of this study was to determine whether and how significant the liver functional changes after one administration of different kinds of corticosteroids in standard dosages are. The study took place in private veterinary clinics in Riga, Latvia, during 2013 - 2014, with the permission of dogs' owners. Twenty animals, which received corticosteroids due to present diagnosis, were divided into four groups. To reach the aim such corticosteroids as dexamethasone sodium phosphate, prednisolone acetate, methylprednisolone acetate and hydrocortisone aceponate were used in standard dosage one time to these dogs, respectively. Then, such blood serum enzymes as alaninaminotransferase (ALAT) and alkaline phosphatase (AP) were determined 24, 48 and 96 hours after the use of corticosteroids. It was discovered that the only one administration of dexamethasone sodium phosphate and methylprednisolone acetate in standard dosage can significantly increase ($p<0.05$) ALAT and AP mean values in dogs. The corticosteroid prednisolone acetate was used once in standard dosage and hydrocortisone aceponate spray was used once and did not statistically significantly ($p>0.05$) change the values of alaninaminotransferase (ALAT) and alkaline phosphatase (AP) in dogs' blood serum during this study.
Key words: dogs, corticosteroids, liver, blood serum enzymes.

Introduction

Corticosteroid or glycocorticoid therapy is often used on dogs (*Canis lupus familiaris*) with allergic, anaphylactic, autoimmune and other diseases (Badylak and van Vleet, 1981; Lucena et al., 1999; Abraham et al., 2006). There are a lot of different types of corticosteroids used in veterinary medicine. Between them are local and systemic medications, short and long-lasting types, spray, oral and injectable forms (Feldman and Nelson, 2004). The choice of use depends on a disease form, character and doctor's preference as well.

Corticosteroids have been used in medicine from 1948. At that moment only one type was discovered and it was cortisone, the synthetic form of the adrenal glands cortex hormone. Some authors of that period divided the history of medicine into two parts – 'before cortisol' and 'after cortisol' (Buttgereit et al., 2005). The doctors started to treat almost every disease with the administration of corticosteroids. Unfortunately, during next two years after 1948, there were described a lot of complications due to the corticosteroids usage. It was common to veterinary medicine as well. One of the most common pathologies in dogs that were treated with corticosteroids is a steroid hepatopathy. First time in scientific studies it was mentioned about 45 years ago (Wimberly et al., 1969). Steroid hepatopathy is corticosteroid induced alterations in liver morphofunctional condition; this is a specific pathology only in dogs (Fittschen and Bellamy, 1984, Feldman and Nelson, 2004). Until now this pathology is not sufficiently described and the main question is: why steroid hepatopathy develops so fast in almost all dogs? Recent findings showed us that there might not be only hepatocyte injury but the specific reaction of liver stellate Ito cells producing liver fibrosis as well (Sobczak-Filipiak et al., 2014). The investigations in this direction are ongoing.

It is discovered that increased values of such blood serum enzymes as alaninaminotransferase (ALAT), aspartataminotransferase (ASAT), gammaglutamyl-tranferase (GGT), alkaline phosphatase (AP) and corticosteroid-induced thermostable alkaline phosphatase (cAP) are specific for the steroid hepatopathy (Badylak and van Vleet, 1981; Lucena et al., 1999). Increased ALAT and AP values are common to biochemical indicators in the diagnostics of the liver disease (Center et al., 1992).

It is proved that corticosteroids usually cause changes in 2 – 3 days after the beginning of the therapy (Dillon et al., 1980; Center et al., 2005). Unfortunately, it is not mentioned or the information is not available how many administrations of different types of corticosteroids and dosage a dog needs to develop steroid-induced hepatopathy and how significant the injury is.

The aim of the study was to determine whether and how significant the liver functional changes after one administration of different kind of corticosteroids in standard dosages are.

Materials and Methods

The study took place in private veterinary clinics in Riga, Latvia during 2013 – 2014, with the permission of dogs' owners. We discussed all advantages and risks of this study and made a signed contract with every dog's owner before the study. Twenty dogs participated in the study. Dogs were of different age, conditions, breeds and genders. All of them had different pathologies, and we prescribed

treatment of corticosteroids. They were divided into four groups, five dogs in each group, depending on corticosteroid usage. All chosen medications we used only once on the first day of study. For the first group we used dexamethasone sodium phosphate in intramuscular route in dosage 2.0 mg kg^{-1}, for the second – prednisolone acetate in intramuscular route in dosage 2.0 mg kg^{-1}, for the third – methylprednisolone acetate in intramuscular route in dosage 2.0 mg kg^{-1} and for the fourth – hydrocortisone aceponate spray locally. It was impossible to find out the dosage of hydrocortisone spray per kilogram because it depends on the size of the lesions.

To find out the influence and the impact of different corticosteroids on hepatic function, such blood serum enzymes as alaninaminotransferase (ALAT) and alkaline phosphatase (AP) were determined on the second day of study, 24 hours after the use of corticosteroids, third, 48 hours after the medication use, and the fifth day, 96 hours after the medication use. We determined the same blood serum enzymes one day before the study to be sure that they are within reference ranges. The blood samples were collected from each animal *v. cephalica*. We centrifuged the blood on 1,300 rounds per minute for 10 minutes to separate the blood serum from the erythrocyte mass (Gulbis, 2011). We analyzed the serum within 15 minutes after the separation. ALAT and AP were determined in serum by biochemical analyzer 'MINDRAY BS-120' at the morphofunctional laboratory of Faculty of Veterinary Medicine, Latvia University of Agriculture

It was proven that the concentration of the hepatic enzymes increased because of the hepatotoxic drug influence (Fittschen and Bellamy, 1984). The steroid hepatopathy usually develops in dogs 2 – 3 days after the use of corticosteroids (Dillon et al., 1980; Center et al., 2005). We used corticosteroids only once on the first day of study.

The programs MS Excel and '*RStudio*' were used for data analysis. P-values less than 0.05 were considered to be statistically significant. T-test was used for the comparison of blood serum enzymes values.

Results and Discussion

One day before the study, i.e., one day before the use of corticosteroid (Day 0), we determined alaninaminotransferase and alkaline phosphatase in each dog. It was found that all the enzyme concentrations are within reference limits (see Table 1).

On the second day of the study, 24 hours after the corticosteroid use, such blood serum enzymes as ALAT and AP were determined in all the groups. There was not a significant difference ($p>0.05$) in alaninaminotransferase (ALAT) concentrations between all the groups and in comparison with the values from the day 0 (a day before the use of corticosteroids). It became apparent that after 24 hours of corticosteroids' usage the change of the concentrations of ALAT is not significant. This fact indirectly reflects that at that moment the hepatic function was not changed or was changed insignificantly regardless of chosen corticosteroid. According to the alkaline phosphatase concentration in the blood serum on the second day of study, it was a significant increase ($p<0.05$), 2.4 times higher than on the day 0, in the values of the group 1, which was the group where dexamethasone was used. It was mentioned before that AP increase was noticed because of specific thermostable form of alkaline phosphatase, which was produced by hepatocytes due to corticosteroid injury (Badylak et al., 1981). Other

Table 1

The values of ALAT and AP in dogs during the study

Parameters	Reference limits (Willard and Tvedten, 2012)	Groups**	Day 0	Day 2	Increase from Day 0 (times)	Day 3	Increase from Day 0 (times)	Day 5	Increase from Day 0 (times)
ALAT	10 – 94 U L^{-1*}	1.	51.2±26.3	62.2±27.4	1.2	118.9±18.0	2.3	59.8±24.9	1.2
		2.	60.6±16.6	62.0±18.0	No	61.7±10.4	No	64.5±12.8	No
		3.	52.8±7.4	61.1±6.5	1.2	93.9±30.6	1.8	111.6±16.6	2.1
		4.	42.5±21.3	42.5±16.2	No	41.1±15.4	No	39.9±16.8	No
AP	0 – 90 U L^{-1}	1.	30.4±17.6	72.3±24.9	2.4	94.3±8.4	3.1	73.3±13.1	2.4
		2.	65.2±26.9	67.1±22.3	No	66.9±24.0	No	69.1±12.3	No
		3.	48.8±16.4	55.9±10.7	1.1	97.8±16.1	2.0	117.4±30.0	2.4
		4.	58.2±19.6	52.0±20.5	No	52.3±10.3	No	59.7±11.6	No

* U L^{-1} – units per liter;

** For the first group (1.) – dexamethasone sodium phosphate was used; the second (2.) – prednisolone acetate; the third (3.) – methylprednisolone acetate and the fourth (4.) – hydrocortisone aceponate spray locally.

groups did not show a significant increase ($p>0.05$) on the second day of the study in comparison to the day 0.

On the third day of the study, 48 hours after the corticosteroid use, the same blood serum enzymes were determined. The mean value of ALAT in dogs from the group one and three, which were the groups where dexamethasone and methylprednisolone acetate, respectively, were used, on the third day increased to 118.9±18.0 U L^{-1} and 93.9±30.6 U L^{-1}, which was 2.3 and 1.8 times higher than ALAT value on the day 0; both values were significantly higher ($p<0.05$) than the concentration of ALAT on the day 0 (see Table 1). The mean value of the alkaline phosphatase (AP) in dogs from the group one and three increased to 94.3±8.4 U L^{-1} and 97.8±16.1 U L^{-1}, respectively, both values were significantly higher ($p<0.05$) than the concentration of AP on the day 0 (see Table 1). The mean values of ALAT and AP in dogs from the groups two and four, where prednisolone and hydrocortisone spray were used, did not significantly change ($p>0.05$) in comparison to the day 0 (see Table 1).

On the fifth day of the study, 96 hours after the corticosteroid injection, the last time ALAT and AP were determined. The significant difference ($p<0.05$) from the day 0 in ALAT mean value was discovered only in the group three, where long-lasting methylprednisolone acetate was used once. It was important to mention that the increase was even higher than in the same group on the day 3, 1.8 times and 2.1 times, respectively. The mean value of AP in dogs from the group one and three increased to 73.3±13.1 U L^{-1} and 117.4±30.0 U L^{-1}, respectively, both values were significantly higher ($p<0.05$) than the concentration of AP on the day 0 (see Table 1). The mean values of ALAT and AP in dogs from the groups two and four did not significantly change ($p>0.05$) in comparison to the day 0 even on the fifth day (see Table 1).

It should be noted that ALAT and AP mean values in the group one, where dexamethasone injection was used, had a tendency to decrease on the day 5, but the same values in the group three, where methylprednisolone acetate was used, had a tendency to increase.

The results of this study prove the fact of the negative effect induced by some corticosteroids, such as dexametrasone and methylprednisolone acetate, to the liver functional condition (Badylak and van Vleet, 1981; Lucena et al., 1999; Center et al., 2005; Abraham et al., 2006). These negative effects have been reflected by enzymes ALAT and AP significant increase in blood serum. It should be noted that despite some scientific information about the use of corticosteroids, one injection of prednisolone in standard dosage and hydrocortisone spray did not significantly ($p>0.05$) change liver functional condition during this study.

Conclusions

1. The corticosteroid dexamethasone sodium phosphate and long-lasting methylprednisolone acetate used once in standard dosage statistically significantly increase the values of alkaline phosphatase (AP) after 24 hours from the injection time and the values of both alaninaminotransferase (ALAT) and alkaline phosphatase (AP) in dogs' blood serum after 48 hours from the injection time.
2. The mean values of alaninaminotransferase (ALAT) and alkaline phosphatase (AP) have the tendency to increase after 48 and 96 hours from the injection of methylprednisolone acetate.
3. The corticosteroid prednisolone acetate used once in standard dosage and hydrocortisone aceponate spray used once did not statistically significantly change the values of alaninaminotransferase (ALAT) and alkaline phosphatase (AP) in dogs' blood serum during this study.

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INNOVATIVE WAYS TO GET MILK WITH HIGH SANITARY INDICES

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Abstract
This article presents the results of a comparative evaluation of preventive treatment of udder with probiotic agents: 'Dipal' (manufacturer DeLaval - Sweden), 'Zorka' (manufacturer «NPP Farmaks» - Russia). We have received data on the effects of probiotic preparation on quality of milk and number of somatic cells in milk. Researches have been conducted in two dairy farms of Almaty region of the Republic of Kazakhstan.
Lactobacillus acidophilus 05ch - isolated from shubat (South Kazakhstan region, Turkestan). *Lactococcus lactis 010k* - isolated from three-day kumys (Zhambyl region, Merke). These probiotics are used as a means for sanitary treatment of the udder of cows.
Thus, the procedure of determining the antagonistic activity revealed their high activity against both gram-negative and gram-positive microorganisms, notably *Staphylococcus aureus* (10-13 mm), *Escherichia coli* (12 mm), *Proteus vulgaris* (10-14 mm), *Salmonella abortus ovis* (11-13 mm).
Significant changes in the milk indices can be found in the experimental group: 9.1% rise of lactose, and 7.2% reduction of protein. The level of protein increased by 4.1% , whereas the amount of lactose, on the contrary, went down 1.6%, which may be indicative of the increase in the content of serum protein when the udder is inflamed.
The examination of the milk obtained from the cows of the control and experimental groups showed a nearly double reduction in the number of somatic cells from 488.00 down to 178.000 thousand, which was caused by the daily treatment of cows by probiotic cultures during 2 weeks.
Key words: sanitation, mastitis, probiotics culture, milk quality, somatic cells.

Introduction

Currently, for the dairy farming sector, along with the task of raising the level of milk production, there is an actual problem of obtaining high-quality and safe products. Due to the high level of metabolic processes and increased loads on the organs and systems, highly productive cows are experiencing weakening of the body's defenses and increasing susceptibility to the pathogenic microorganisms, which are present in the environment. Among the most common diseases of the highly productive cows is mastitis (Klimov and Pershin, 2012; Doits and Obritkhauz, 2010). One of the methods for the prevention of mamma's disease is antiseptic treatment of dugs, for this there is a significant amount of products, which contain iodine or chlorhexidine (Kolchina, 2008; Karpova, 2007; Kolchina et al., 2010).

When applying these products, pathogenic and beneficial microorganisms are destroyed; obtained effect is short-lived, as the pathogenic microflora is restored quickly enough. An alternative of chemical method, as per the data of Zimchenko E. I. and Panin A. N., is the use of products on the basis of probiotics (for the treatment of udder before and after milking). In this case, the basis of existing microbiological preparations for the treatment of cows' udder is antagonistic relationships between pathogenic microorganisms and probiotic cultures that are part of the products (Zimchenko and Panin, 2003; Prescott et al., 2008).

Lactobacillus acidophilus 05ch - represent the thin rod cells with the size of 4.7 – 6.2 x 0.6 mkm, with rounded ends, gram-positive, catalase negative, asporogenous, fixed cells. The cell surface is rough, shiny, gray-transparent; the consistency is oily. When grown in a liquid medium, turbidity with sediment is observed.

Tested strain grows very well on hydrolyzed milk, MRS; wort environment is within pH 5.5-6.0. The minimum temperature for the cultivation of these cultures is - 20 °C, optimal – 37-39 °C, maximum – 45 °C. Milk acidifies the mediums with the formation of dense clot without gas release; it has a pleasant milky-sour taste and aroma; it clots within 7-9 hours at an optimum temperature after adding a 1% ferment. The active acidity of *Lactobacillus acidophilus 05ch* is 147 °T, and the final acidity equals 240 °T.

The tested strain grows well in an alkaline environment pH 8.3 with the content of 2.4% NaCl and 20% bile; and on skimmed milk - with the content of 0.4% phenol, 0.01% methylene blue, and with 40% bile it grows slightly. Growth on synthetic medium with mineral nitrogen is good. It acidifies mediums with glucose, lactose, galactose, sucrose; it weakly ferments maltose, arabinose; does not ferment raffinose, mannitol, xylose, starch, glycerol. The culture belongs to the facultative anaerobes; does not liquefy gelatin.

Lactococcus lactis 010k - the strain represents cocci with the size of 0.8 mkm; oval, gram-positive,

catalase-negative, fixed, asporogenous cells. On the surface of the agar medium (when incubated in 28 - 30 °C). It forms a white, round, convex, with smooth edges, shiny colonies with a diameter of 1-2 mm. At depth growth in agar, it forms lenticular colonies of white color; on wort agar with chalk (CAM), the translucent zone is formed around the colonies. In a liquid medium, i.e. in meat-peptone broth (MPB), hydrolyzed milk (HM), wort with chalk gives turbidity with sediment. The minimum temperature for growth is 15°C, optimal – 28 - 30 °C, maximum – 45 °C. In milk at a temperature of 15 - 45 °C it grows well. The final acidity of *Lactococcus lactis 010k* in milk is 108 °T; the active acidity is 62 °T. The strain clots the semi-skimmed milk at a temperature of 30 °C within 16 hours, with the formation of dense uniform clot after adding a 1% ferment. Heating at 60 °C for 30 min has detrimental effect on the strain. The tested strain grows well on hydrolyzed milk with the content of 20 - 40% bile and on MPB at pH 9.2. Growth on synthetic medium with mineral nitrogen is good; it does not grow on a potato medium. It acidifies the mediums with glucose, lactose, sucrose, galactose, fructose, maltose, sorbitol, arabinose, raffinose, salicin and xylose. It does not ferment mannitol, starch and glycerol (Kasenova et al., 2012).

The objective of this work is to study the application of the selected probiotic cultures for sanitary treatment of udders in order to improve individual quality indicators of milk in Kazakhstan.

Materials and Methods

Researches on the effect of products, containing probiotic cultures, on the state of mamma and quality of milk have been performed on the bases of the Agricultural Breeding Cooperative 'Almaty' and in the Research and Production Center 'Bayserke-Agro', which are located in Talgar district (Almaty region). The following probiotic cultures have been used: *Lactobacillus acidophilus 05ch* - separated from shubat (South-Kazakhstan region, Turkestan, 2012), and *Lactococcus lactis 010k* - separated from three-day kumys (Zhambyl region, Merke).

Antagonistic activity of the experienced cultures to the indicative test-strains has been determined by the method of deferred antagonism (Labinskaya, 1978). Test-strains were obtained from the Central Museum of the Republican Collection of Microorganisms.

Researches on the effect of products, containing probiotic cultures, on the state of mamma and quality of milk have been performed on the bases of the Agricultural Breeding Cooperative 'Almaty' and in the Research and Production Center 'Bayserke-Agro', which are located in Talgar district (Almaty region).

On the base 'Bayserke-Agro', the objects of the study were Holstein cows from Canada, with an average annual milk production of 1800 tons. Keeping animals is loose, all-year-around. Milking is carried out around-the-clock, by a robot-milker of Swedish manufacture - DeLaval (installation VMS).

In the breeding plant 'Almaty', Alatau breed cows are selected for the study. Animals are kept tethered; milking is performed three times a day with German milking-machines 'Westfalia Surge'.

More than 24 cows were involved for the purpose of the investigation. Those were 12 Holstein breed cows from Canada and 12 local Alatau breed cows.

Characteristics of Holstein breed cows: the average weight of cattle amounts to 750 kg, they are black-gaudy and have different black markings on their bodies. The height of these cows is on average 143 - 145 cm. The udder of the Holstein breed cows has a rather unusual bowl-like shape and is characterised by a large capacity. Its index equals to an average of 45 - 46%, and it can vary from 38.5 up to 61.3%. Two milking procedures involving these cows give about 65 kg of milk within 24 hours, the maximum speed varying from 3.20 to 3 kg per a minute. The cows are capable of giving 3.3 - 3.8% fat milk. The average annual yield of milk per adult cow stands at 4.000-4.500 kg.

Characteristics of Alataubreed cows: these cows relate to a dairy meat breed. Brownness with different hues is typical of the animals belonging to this breed. The cattle of Alatau breed have a firm body structure: firm bones and well-developed muscles. They also have prominent muscle forms. Their udder has a bowl-like shape with cylindrical teats and with clearly pronounced milk veins. They have medium-sized udders. Their skin is thick, but elastic.

The weight of Alatau cows equals to an average of 500 - 600 kg. Milk productivity of the Alatau cows is quite high. These cows produce 3.8 - 4% fat milk. The average annual yield of milk per adult cow stands at 4.500 kg.

Scientific production experience was carried out on 2 groups of lactating cows. The experimental group included 12 animals, control group - 12 animals. The animals were kept in different bases; the duration of the experience was 2 weeks.

For cleaning the dugs after milking, we used 5% probiotic solutions, applied by spraying immediately after removing the milking machine, upward from the top of the dug within 2-3 seconds. The probiotic solutions were prepared as follows:

the control group carried out sanitary treatment of udder on the technology used in the farm (treatment after robot-milking with the product 'Dipal', and with 'Zorka' - in 'Almaty' Center);

the total microbial contamination of the udder skin (before and after probiotic preparation) has been studied by taking washes/samples from 100 cm^2. A total of 96 samples was studied.

For the test we made a series of sequential decimal dilutions of the sample (wash). We took 1.0 cm^3 of the sample from each tube and separately brought to the tubes with 9 cm^3 sterile distilled water, receiving the first dilution of wash; then 1 cm^3 was transferred to the 2nd tube with 9 cm^3 sterile distilled water, obtaining dilution 10^{-2}. The operation is repeated until dilution 10^{-6}. After thorough mixing, 1 cm^3 of diluted sample from each tube is sterilely transferred to the microbiological dishes, in which then 15 cm^3 of melted and cooled to 45 °C meat-peptone agar is poured. The dishes are placed into a thermostat for 24 hours at the temperature of 37 °C. Interpretation of results is produced in 24 hours, by counting the grown colonies for the number of viable cells (mln cm^{-3}), and is determined by the formula:

$$X=Nx10p,$$

where N- the number of grown colonies;

p- ordinal number of decimal dilutions.

To determine the amount of Escherichia coli, we made sequential decimal dilutions, and then, from each tube we transferred 1 cm^3 of washes/samples for Kessler's medium with lactose; wherein we put the swab into a test tube with the medium and transferred the remaining washing fluid.

Inoculation on the Kessler's mediums is incubated at 37 °C. After 18-24 hours, from the Kessler's medium, we made seeding on the dense differential medium Endo (seeding from the Code's medium is produced in the case of a color change or the medium turbidity).

Obtained materials are placed in an incubator at 37 °C for 24 hours, and then are reviewed / studied. From the colonies, suspicious or typical for Coliform bacteria, we prepared smears, Gram stained and studied with a microscope. Detection of Gram-negative rods indicates the presence of Coliform bacteria (State Standard 30726-2001).

To identify Staphilococcus aureus, seeding is carried out analogously, using as nutrient medium - 6.5% vitelline-salt agar. Dishes with seedings are incubated at a temperature of (37 ± 1) °C within 24-48 hours. After incubation, seedings are studied for growth of typical colonies.

On vitelline-salt agar, colonies of Staphylococcus aureus have the form of flat discs with diameters of 2-4 mm; of white, yellow, cream, lemon, golden color with smooth edges; around the colonies there formed a rainbow ring and zone of the medium's turbidity.

From each Petri dish, we select at least five typical colonies and transfer on the surface of the chamfered nutrient agar, but without the addition of sodium chloride and vitelline emulsion.

Seedings are incubated at a temperature of (37 ± 1) °C within 24 hrs. On the grown colonies, we determine the ratio of color to Gram (State Standard 30347-97).

To determine the lactobacteria, we use laktobak-agar. Seedings are incubated at a temperature of 37 °C within 24 hrs. Interpretation of results is carried out by counting the grown colonies by the formula above.

The animals were under constant surveillance for 2 weeks. We performed control milking for the selection of milk from cows of experimental and control groups in order to perform the laboratory analysis, which included the determination of somatic cells in milk (after using preparations), as well as determination of fat, protein, density and MSNF in milk, using milk analyzer 'MilkosanFT+', 'FossomaticFT+'. Test conditions are: air temperature - 20 °C ± 2 °C, humidity - 71%. The milk samples were taken to a sterile container for collecting biological fluids.

Sampling of milk was carried out on the site of its acceptance as per State Standard 13928-84 and State Standard 26809-86.

There were studied 48 samples of milk: 24 samples were taken before the start of the experiment, and other 24 - after experiment.

Results and Discussion

Results of the study of strains of tested probiotic cultures on antagonistic activity

The research work was aimed at studying the probiotic properties and selection of microorganisms, promising to create bacterial preparations.

Antagonistic properties have a particular importance in the study of lactic acid bacteria. As test-strains, we used the following microorganisms: *Sarcina flava, Bacillus mycoides, Staphylococcus aureus, Esherichia coli, Proteus vulgaris, Dyplococcus septicus, Salmonella choleraesuis (str.177), Salmonella abortus equi (str.841), Salmonella abortus ovis, Salmonella typhimurium, Salmonella dublin, Salmonella gallinarum.*

When studying the antagonistic activity of strains, we proved (Table 1) that both tested cultures have a more or less pronounced degree of antagonist activity.

Thus, when studying the antagonistic activity of the test-strains, we noted their high bactericidal activity, both for gram-negative and gram-positive organisms, in particular in relation to *Staphylococcus aureus (10-13 mm), Escherichia coli (12 mm), Proteus vulgaris* (10-14 mm), *Salmonella abortus ovis* (11-13 mm). In conclusion, we can say that *Lactobacillus acidophilus 05ch and Lactococcus lactis 010k* have

Table 1

Antagonistic activity microorganism strains

Strains	*Sarcina flava*	*Bacillus mycoides*	*Staphylococcus aureus*	*Escherichia coli*	*Proteus vulgaris*	*Dyplococcus septicus*	*Salmonella choleraesuis (str.177)*	*Salmonella abortus equi (str.841)*	*Salmonella abortus ovis*	*Salmonella typhimurium*	*Salmonella dublin*	*Salmonella gallinarum*
Lactobacillus acidophilus 05ch	12	10	13	12	10	10	9	9	11	9	9 9	10
Lactococcus lactis 010k	8	8	10	12	14	10	14	13	13	10	10	10

antagonistic activity to a number of pathogenic and conditionally pathogenic microorganisms.

The main stage of our research is to determine the effectiveness of probiotic agents at sanitary treatment of the udder in a production environment. Analysis of the obtained results of the study of total microbial contamination of the udder skin shows that this indicator of the cows' udder skin is approximately equal to the control group (Table 2). Subsequently, after treatment of these areas of the udder with probiotic cultures, total contamination by microorganisms has increased significantly (4 times). It was determined, that the increase in total bacterial contamination of the cows from experienced group was mainly due to the prevalence of bacteria of the genus *Lactobacillus acidophilus 05ch* and *Lactococcus lactis 010k*.

Thus, in the experimental group, the number of conditionally pathogenic microflora (compared to the control group) is significantly reduced. In particular, the amount of bacteria of Staphilococcus (by treatment with probiotic agents (1 and 2)) decreased by 73.1 - 79.3%; and by treatment with 'Zorka' and 'Dipal' – the reduced number of bacteria was 51.9% and 76.5% (see Fig. 1). It should be pointed that probiotic preparations and product 'Dipal' have higher bactericidal effect than 'Zorka'.

The same pattern is observed (Fig. 2) with respect to Escherichia coli. During treatment with probiotic agents, number of E. coli decreased by 68.4- 77.2%; and by treatment with 'Zorka' and 'Dipal' - the reduced number of bacteria was 66.2% and 79.5%.

According to the obtained results, we need to emphasize that the tested probiotic preparations on bactericidal activity, with respect to conditionally pathogenic microorganisms, do not yield to the product 'Dipal' (DeLaval), which is widely used in the advanced farms of the Republic of Kazakhstan and is one of the most effective means/products for udders' treatment. However, advantage of probiotic agents is their ecological safety, low cost, positive biological effect on the skin of dugs and udder at various injuries.

The next stage of our work was to investigate the effect of probiotic agents on quality of milk. Under adverse conditions (related to disorders in technology of milking or due to udder infection), there is, primarily, increase of the level of somatic cells;

Table 2

The results of the comparative analysis of microbial contamination for the cows' udder skin when treatment with probiotic preparations and preparations 'Zorka' and 'Dipal'

Name of the Drugs tested		Total Contamination of Teat Skin CFU* 10^6	Staphylococcus aureus, CFU* 10^6	Escherichia coli, CFU* 10^6
Lactococcus lactis 010k	Before treatment	298±23.12	5.9±0.36	6.6±0.41
	After treatment	86.4±8.51	1.2±0.07	1.5±0.08
Lb.acidophilus 05ch	Before treatment	302±21.82	6.8±0.38	5.7±0.47
	After treatment	91.2±7.82	1.9±0.06	1.8±0.40
'Zorka'	Before treatment	277±12.34	7.3±0.42	8.2±0.56
	After treatment	19.1±5.21	3.6±0.08	2.9±0.14
'Dipal'	Before treatment	202±10.33	7.8±0.51	8.6±0.68
	After treatment	17.3±8.31	1.83±0.06	1.76±0.09

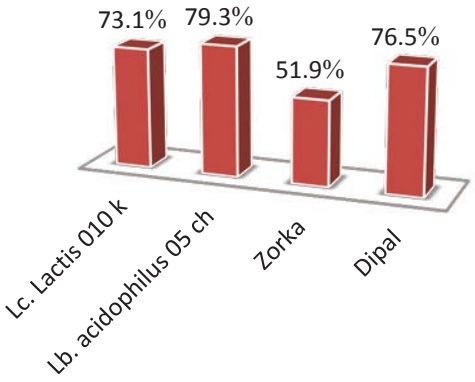

Figure 1. Effectivity of the bactericidal effect on Staphylococcus aureus.

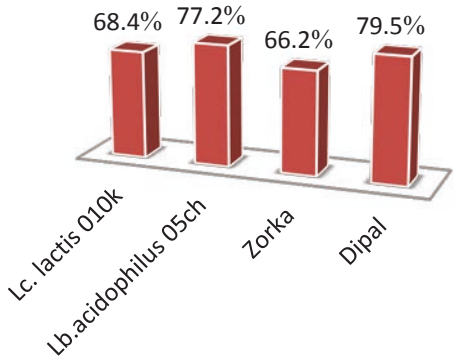

Figure 2. Effectivity of the bactericidal effect on Esherichia Coli.

Table 3

The dynamics of the changing indices of milk after the treatment of udder with the application of probiotic drugs

Milk Quality Indicators	Experimental Group				Control Group			
	Lc. lactis 010k		Lb.acidophilus 05ch		'Zorka'		'Dipal'	
	the start of the experiment	the end of the experiment	the start of the experiment	the end of the experiment	the start of the experiment	the end of the experiment	the start of the experiment	the end of the experiment
Fat, %	3.62±0.04	3.96±0.1.41	3.73±0.23	4.01±0.16	3.0±0.56	3.44±0.38	2.1±0.09	2.3±0.14
Protein, %	3.21±0.12	2.98 ±0.61	4.01±0.38	3.27±0.29	3.58±0.34	3.73±0.54	3.6±0.07	3.01±0.05
Somatic cells, thousands/ cm^3	482.21±19.1	221.19±17.2	488±24.9	178±15.1	628±25.2	367.18±23.6	253±12.5	173±11.9
Casein, %	2.39±0.2	2.33±0.3	2.48 ±0.1	2.33±0.2	2.54±0.5	2.97±0.01	2.29±0.08	2.35±0.25
Lactose, %	4.32±0.05	4.75±0.05	4.11±0.31	4.86±0.25	3.42±0.18	3.01±0.09	4.58±0.08	5.0±0.11
Dry Substances, %	13.13±0.59	12.29±1.1	15.01±1.23	13.52±0.98	11.6±1.56	12.38±0.84	11.56±0.71	11.04±0.12
MSNF, %	8.53±0.89	9.33±0.57	8.46±0.39	9.93±0.42	8.03±0.81	9.43±0.63	8.32±0.57	8.94±0.82
Urea, mg %	21.25±0.87	23.25±0.54	23.2±0.71	23.75±0.49	17.25±0.56	19.5±0.61	18.2±0.27	14.8±0.43

along with this, other changes in milk are observed: in particular, decreased content of fat and lactose, increased content of protein and chlorine, decreased density and acidity of material, etc. In this regard, due to changes of such indicators as fat, protein, lactose, somatic cells, we can determine the quality of milk (Table 3).

Analysis for studying the composition of milk from cows of the experimental group showed the presence of positive changes, indicating improvement in the qualitative composition of milk. There was a significant increase in content of fat, lactose - 9.1% and decrease in the amount of protein - 7.2%. In the control group of animals, protein level was increased by 4.1% and the amount of lactose was decreased by 1.6% that may indicate the elevated levels of serum proteins on a background of inflammatory processes in the udder.

Research of collected milk from the experimental and control cows (using somatic cell counter 'FossomaticFT+'") has shown, that after 2 weeks of daily application of complex probiotic preparations, there was a reduction in the number of somatic cells from 488 to 178 th. ml^{-1} (2 times).

Thus, based on the study results, we can conclude that tested probiotic products have a positive impact on the mamma and milk quality, and that shows prospects of their further studies and implementation to the technology of industrial production of milk.

Conclusions

1. When using probiotic agents, there is a marked increase in bacterial contamination of cows' udder skin (the prevalence of bacteria of the genus Lactococcus lactis 010k and Lb.acidophilus 05ch) and reduced number of conditionally pathogenic microflora (*Staphylococcus aureus*, *Esherichia coli)*, compared with the control group.
2. The probiotic agents do not deteriorate the quality of milk. The number of somatic cells dropped by

63.5%, and the content of fat increased significantly (by 9%), lactose – by 9.1% and the amount of protein reduced by 7.2%, the other indices were at a physiological level.

3. Based on the results of the study, we can recommend the use of the above materials as starter cultures for the creation of biological products for sanitary treatment of the udder before and after milking, since they do not have any chemical action, cause reproduction of beneficial microflora on the surface of the udder, which may have a beneficial effect on the whole organism of the animals.

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MORPHOLOGICAL CHANGES IN ARTIFICIALLY REARED ONE YEAR OLD SEA TROUT (*SALMO TRUTTA* L.) DURING SPRING

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Abstract
Morphological parameters and their changes common for artificially reared one year old sea trout were examined from January to May the year 2013 to determine whether these parameters indicated the smoltification in one year old sea trout and whether these fish achieved smolts stage. Fish were reared in flow-through and recirculation systems in hatcheries based on three different rivers (Brasla, Daugava, Venta basin). Sea trout were examined in the Latvia University of Agriculture, Faculty of Veterinary Medicine, Institute of Food and Environmental Hygiene and in the Institute of Food Safety, Animal Health and Environment BIOR, Laboratory of Aquaculture and Fish Pathology. To appreciate fish growth stage condition index, hepatosomatic index, spleen index was calculated and silvering level was evaluated. The fish condition index decreased in all hatcheries and flow-through and recirculation rearing systems from January to April and increased in May. Spleen index was the most stable parameter and did not change a lot showing that fish did not have migratory stress in April and May. Silvering level increased from January to April but suddenly the increase became slower in May without reaching the top level. These results made us to consider that one year old sea trout parrs released in May 2013 probably did not become smolts before release and they had to stay in river for one additional year until reaching pronounced smolt stage.
Key words: smoltification, condition index, silvering, hepatosomatic index, spleen somatic index.

Introduction

Spring is the time when juvenile salmonids such as sea trout start parr-smolt transformation called smoltification. The main purpose of smoltification for salmonids is to perform physiological adaptation to be ready for environment change from fresh water to salt sea water. It is very important for future survival and growth in salt water (McCormick, 2013). There are several physiological factors which can be taken into account for determination of smoltification – different blood parameters, gill Na+ K+-ATPase activity (Zaugg and McLain, 1972) and several morphological parameters such as fish length, weight, condition index, silvering etc. (Quigley et al., 2006; McCormick et al., 2007).

The most visible morphological sign of smoltification is silvering which is caused by deposition of guanine in derma and under fish scales. However, there are some salmonid species, which migrate to sea without any visible signs. It is detected that certain level of weight and age has to be reached for several species before migration to sea and adaptation to salt water (Stoskopf, 1993). It was assumed that it is better to release bigger smolts because they are more adult to protect themselves from predators (Salminen and Kuikka, 1995); however, it has been studied that purposeful raising of temperature in fish farm to promote growing of fish parr, can negatively affect smoltification – inhibit raising of gill Na+ K+-ATPase activity (Zaugg and Wagner, 1973; Ewing et al., 1979; Handeland et al., 2000). During smolitification different important physiological changes perform in the body of fish, for instance, reserves of body lipids and especially level of triglycerides rapidly decrease. A decrease in lipid content is a reason for reduction of condition index (CI) (Sheridan, 1989). Spleen somatic index (SSI) used to determine stress level in fish, shrinks due to contraction of spleen smooth muscles in stress conditions (Gerwick et al., 1999). Hepatosomatic index (HSI) changes according to season and fish nutritional status. It is affected by parasite infections and water pollution (Montenegro, González, 2012).

Fish growth depends on feeding and water quality. The most important factors of water quality for fish rearing are water temperature, oxygen saturation and pH. Optimal water pH for sea trout rearing is 6.0, but not higher than 8.5, and oxygen concentration should be above 7 mgL^{-1} (Peterson et al., 1972; Маликова и Иозепсон, 1987). Unfortunately, additional warming of water, which would be necessary for promotion of fish growing is not economically viable in flow-through rearing systems (Mitāns, 2001).

Physiological appreciation of smoltification is usually quite expensive and time-consuming; therefore, morphological evaluation is more applicable, because it is cheaper and simplier to perform.

The aim of the sea trout restocking in Latvia is improvement of natural salmonid resources mainly to compensate the negative effect of hydroelectric power station cascade on river Daugava and other anthropogenic factors. Till now in Latvia smoltification is determined just by fish weight, and other parameters are not taken into account. Besides, there is insufficient amount of studies about sea trout growth, development and smoltification in conditions

common for the environment of Latvia. Many theories are based on theoretical presumptions and not on scientifically approved facts. The aim of the research was to start improve current situation and to appreciate the morphological changes of one year old sea trout in spring to assess their physiological readiness for migration to sea.

Materials and Methods

The study took place in the Latvia University of Agriculture, Faculty of Veterinary Medicine, Institute of Food and Environmental Hygiene and in the Institute of Food Safety, Animal Health and Environment BIOR, Laboratory of Aquaculture and Fish Pathology in 2013. In total of 1482 one year old sea trouts were examined in the investigation. Fish were reared in different hatcheries based on three rivers of Latvia – hatchery A (river Venta basin), hatchery B (river Brasla) and hatchery C (river Daugava). In all these hatcheries fish are reared in flow-through systems, but in the hatchery C fish are reared also in recirculation system. Fish were examined once every two weeks from January to February but from March to May once a week. In May, fish with appropriate weight were released in natural environment. Morphological factors were determined for fish individuals of different weight and size. We measured fish fork length (length from anterior-most part of the fish to the end of the median caudal fin rays) and fish were weighed. Then section was performed and fish liver and spleen were weighed. We visually evaluated sea trout body silvering and calculated CI (Formula 1), HSI (Formula 2) and SSI (Formula 3).

CI was calculated by the following formula (Berrill et al., 2006):

$$CI = \frac{W}{L^3} \cdot 100, \quad (1)$$

where
CI -condition index;
W-fish weight, g;
L- fork length, cm.

HIS was calculated by the formula (Nikolsky, 1963):

$$HSI = \frac{W_L}{W_F} \cdot 100, \quad (2)$$

where
HSI – hepatosomatic index;
W_L – liver weight, g;
W_F – fish weight, g.

SSI was calculated by the formula (Nikolsky, 1963):

$$SSI = \frac{W_S}{W_F} \cdot 100, \quad (3)$$

where
SSI – spleen somatic index;
W_S – spleen weight, g;
W_F – fish weight, g.

Silvering was evaluated using scale from 0 to 4 (Birt and Green, 1986, modified by Rutkovska) (Table 1).

Table 1

Evaluation of silvering level

Scale	Description
0	Parr. No signs of silvering, clearly visible parr marks.
1	Parr. Slightly silvery colour. Visible parr marks, some signs of silvering.
2	Smolt like Parr. (50% silvering). Silvery colour and visible parr marks.
3	Smolt like Parr. (75% silvering). Silvery colour and only slightly visible parr marks.
4	Smolt. Silvery colour, black fin margins. No visible parr marks.

The water temperature data were obtained from hatcheries daily temperature measurements.

Average and standard deviation (SD) was calculated for the data obtained. To compare parameters' changes from month to month, differences between hatcheries of different rivers and differences between flow-through system and recirculation system in hatchery C, T-test for comparison of two separate samples was used (Sokal and Rohlf, 2000).

Results and Discussion

The water temperature data obtained from hatcheries showed that in the beginning of the study water temperature in hatchery A was 0.2 °C, and it started to increase from the beginning of April. On May 1, water temperature reached 9.5 °C and at the end of the research it was 16.0 °C. In the hatchery B water temperature was 2.5 °C in January and did not change significantly until April 24 when it started to increase. On May 1, it reached 6.4 °C and on May 10, it was 8.2 °C. In the hatchery C flow-through system water temperature was 2.0 °C in January, then it lowered to 1.0 °C in February. Temperature started to increase from April 19 and on May 1 it reached 7.5 °C, and in the middle of May it was 16.5 °C. In the hatcheries C recirculation system water temperature was 8.0 °C in January and it started to increase in April reaching 14.0 °C at the end of the research.

Table 2

One year old sea trout condition index in spring

Fish farm/ rearing system	Month				
	January	February	March	April	May
A/ flow-through	1.08 ± 0.08	1.03 ± 0.09[a]	1.03 ± 0.10	1.06 ± 0.12[b]	1.08 ± 0.08
B/ flow-through	1.16 ± 0.09	1.08 ± 0.10[a]	1.05 ± 0.10	1.06 ± 0.11	1.08 ± 0.06
C/ flow-through	1.17 ± 0.13[c]	1.15 ± 0.11[c]	1.04 ± 0.10[ac]	1.07± 0.14[c]	1.14± 0.07[ac]
C/ recirculation	1.24± 0.12[c]	1.22 ± 0.12[c]	1.16± 0.12[ac]	1.15 ± 0.14[c]	1.32 ± 0.11[ac]

[a] significant difference p<0.01 in comparison with previous month
[b] significant difference p<0.05 in comparison with previous month
[c] significant difference p<0.01 between rearing systems of hatchery C

Table 3

One year old sea trout hepatosomatic index in spring

Fish farm/ rearing system	Month				
	January	February	March	April	May
A/ flow-through	1.68 ± 0.32	1.59 ± 0.71	1.36 ± 0.28[b]	1.30 ± 0.30	1.63 ± 0.21[a]
B/ flow-through	1.61 ± 0.31	1.65 ± 0.32	1.62 ± 0.29	1.63 ± 0.31	1.38 ± 0.21[a]
C/ flow-through	2.08 ± 0.37[c]	1.91 ± 0.42[bc]	1.82 ± 0.28[c]	1.70 ± 0.35 [ac]	1.37 ± 0.14[a]
C/ recirculation	1.66 ± 0.36[c]	1.63 ± 0.28[c]	1.47 ± 0.28[ac]	1.41 ± 0.23[c]	1.27 ± 0.25[b]

[a] significant difference p<0.01in comparison with previous month
[b] significant difference p<0.05in comparison with previous month
[c] significant difference p<0.01 between rearing systems

When analyzing results obtained in the spring of 2013, it can be seen that CI varied between fish farms and months (Table 2). From January it gradually decreased reaching its lowest level in March and then increased again. This could be seen in all fish farms. It can be explained by the water temperature, which affects a feed consumption. In winter water temperature is low; therefore, fish feed less than in warm water but still they have their lipid reserves which gradually are being spent. Accordingly, fish had the poorest condition in March. Then, water temperature increased and fish fed more and fish condition improved again. It can be seen that fish from recirculation system had steadily significantly higher CI than fish from flow-through systems but still CI slightly decreased from January to even April and then sharply increased in May. Water temperature in this system in winter was higher than in flow-through systems.

In our study it could be seen that fish did not lose their body lipids in May; on the contrary, CI increased. In other studies with Atlantic salmon (*Salmo salar* L.) situation was different - CI decreased during smoltification (Sheridan, 1989; Quigley et al., 2006), but there are other studies with sea trout where CI increased during parr-smolt transformation (Tanguy et al., 1994; Quigley et al., 2006). D.T.G. Quigley et al. (2006) suggests that increase in CI can be connected with non-proportional growth of the caudal peduncle or with not so intensive lipolysis.

A different situation was observed with HSI, where no common tendencies could be seen (Table 3). In every fish farm tendencies were different. In the fish farm A, HSI decreased from January to April and then sharply increased in May. In the fish farm B, HSI was more or less stable until it rapidly decreased in May. In the fish farm C, HSI decreased from January to May. Moreover, it happened in both recirculation and flow-through rearing systems. There was significant difference between HSI of both rearing systems of the fish farm C from January to April. It was not so easy to find the reason for these fluctuations of HSI. There were no signs of seasonal changes in flow-through systems or we could not find connection with changes of CI. Studies of other researchers have shown that HSI changes according to season and fish nutritional status. It is affected by parasite infections and water pollution (Montenegro and González, 2012). We could consider the possibility of water pollution in flow-through systems, but we have no clear evidence of that because water quality was not controlled at that time. We could see that there was significant difference between HSI of fish from flow-through and recirculation systems of the hatchery C. The water quality in recirculation system is good with no fluctuations of pollutants. Underground water was used in this system and all tests made have indicated a good water quality. Unfortunately, during this study water quality analysis had not been made in hatcheries and we can just rely on previous data of water quality

Table 4

One year old sea trout spleen somatic index in spring

Fish farm/ rearing system	Month				
	January	February	March	April	May
A/ flow-through	0.14 ± 0.05	0.17 ±0.06	0.14± 0.06	0.13± 0.05[a]	0.12 ±0.04
B/ flow-through	0.20± 0.08	0.20 ±0.08	0.21± 0.08	0.20± 0.07	0.19± 0.07
C/ flow-through	0.15± 0.06	0.16 ±0.06	0.14± 0.07[ac]	0.13± 0.04[c]	0.14± 0.04
C/ recirculation	0.15± 0.06	0.17 ±0.07	0.16± 0.05[c]	0.16± 0.05[c]	0.16 ±0.05

[a] significant difference p<0.01in comparison with previous month

[c] significant difference p<0.01 between rearing systems

Table 5

One year old sea trout silvering level in spring

Fish farm/ rearing system	Month				
	January	February	March	April	May
A/ flow-through	1.55	2.11	2.56	2.70	2.67
B/ flow-through	1.36	1.68	2.35	2.60	2.70
C/ flow-through	1.54	2.14	2.37	2.64	2.96
C/ recirculation	1.96	2.36	2.79	2.74	2.95

provided by hatcheries. Therefore, in the hatchery C the reason of HSI decrease could be seasonal changes – the raise of water temperature. The temperature increase initiated HSI decrease.

More stable situation was detected for SSI (Table 4). There was not significant fluctuation of SSI in fish farms B and C (recirculation system). Some changes can be seen in the fish farm A from March to April and in the fish farm C flow-through system from February to March.

In fish organisms changes caused by stress are coordinated through the hypothalamus which leads to contraction of smooth muscle in the spleen (Thomas, 1990). Analyses revealed that fish did not have migratory or other stress in any of studied hatcheries during April and May. These months are the time of year when smoltification has to appear. Studied sea trout did not have stress during smoltification or they had not become smolts yet, as smoltification is associated with stress.

In all fish farms a level of silvering slightly increased during spring period, but it didn't reach a maximum level – 4 (Table 5).

Our results coincide with previous studies of other researchers, showing that there are not as visible changes in sea trout as it is detected for salmon during smoltification (Soivo et al., 1989; Debowski et al., 1999). Some researchers think that it may be connected with very intensive growth of sea trout during spring (Fahy, 1990; Bohlin et al., 1993; Tanguy et al., 1994).

Conclusions

CI of one-year old sea trout decreased from January to April and then started to increase. SSI was quite stable and did not decrease in April and May. There could be seen silvering in one year old sea trout in spring, but it is not clear whether these fish managed to become smolts in the end of first year of life. The low silvering level and absence of migratory stress indicated that most part of one year old sea trout did not reach smolt stage and it would be necessary to continue rearing for one more year until fish become two years old. More investigations have to be done to evaluate the possible HSI connection with smoltification.

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CHANGES OF BALTIC SEA COAST DURING THE PERIOD BETWEEN 2008 - 2015

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Abstract
The article presents the comparative, descriptive statistics analysis of the changes of the Baltic Sea coast in the territory of the Republic of Lithuania. This paper analyzes the Baltic Sea coast measurements taken during the period between 2008 and 2015. The formation of the strip of the Northern breakwater - Giruliai during this period was influenced by the Hurricane Felix on January 10 of 2015. Describing the Baltic Sea coast strip dynamics trends, the Baltic Sea coastline change during the period between 2008 and 2015 was selected and calculated on the basis of the measurements results. Analysis of the erosive and accumulation processes of the 4 km long strip from the Northern breakwater to Giruliai strip was provided.
The carried out data of the Baltic Sea coast changes analysis show that seacoast limits are constantly changing. The replenishment of the Baltic seacoast spatial data set during the period between 2008 and 2013 with the revised spatial data of the period between 2014 and 2015 showed that during the months of January during the period between 2008 and 2015 the 4 km long strip of the Baltic Sea coast decreased by 3.7075 ha, in the Ist Melnrage area, the 0.7 km long strip of coastline has moved more than 30 m inland. It was found that in the southern half of the researched section erosional processes prevailed, while in the northern part – both erosional and accumulative ones.
Key words: accumulation, spatial data set (SDS), erosion, coastal zone, coastline, shore storage.

Introduction

Article relevance. At present, a broad scientific attention and concern is paid to the state of sea coasts, at the same time and to the Baltic Sea coast, because coasts are strongly influenced by erosion and the situation is growing worse every year.

Lithuanian sea coast line is very short - 90 kilometers, therefore it is particularly important to not only protect the sea from pollution and other negative human activities, but also from the decline of coasts resulting from the natural conditions. Evaluation of the current situation of coasts, change tracking, analysis and trendsetting as well as the finding of problem solutions – is a hot topic.

The Baltic Sea has changed considerably during the recent decades, which is partly due to human influence (Loptien and Meier, 2011).

Human activities, both on the Baltic Sea itself and especially throughout its drainage area, have over the last centuries put considerable pressure on its marine ecosystem (Backer et al., 2010).

The Baltic Sea is highly dynamic and strongly influenced by large-scale atmospheric circulation, river runoff and by the restricted water exchange due to its narrow entrance area (Omstedt et al., 2004).

The Baltic Sea is a small intra-continental, shallow coastal sea under severe human-induced pressures, such as global climate change, excess nutrient release, pollution, ammunition dumping, overfishing, and various engineering-based modifications, including the strong growth of coastal settlement, hydro- and nuclear power plants, massive wind farms, gas pipelines, and various bridge and tunnel crossings (Omstedt et al., 2014).

The anthropogenic impacts are substantial and include extensive nutrient emissions, pollution from toxic substances, fishing pressure and heavy ship traffic. The Baltic Sea hosts a unique ecology, as relatively few species have had the ability to adapt to this low-salinity environment (Jutterstrom et al., 2014).

The Baltic Sea coastal erosion is accelerated by the crosswinds hurricanes and storms occuring at the Lithuanian coasts from time to time as well as by the marine indusry and human-induced factors.

The main reasons for the changes of coasts: reasons of accumulation / abrasion processes of more active coasts - natural processes (water level rise, storms) and human economic activities (urbanization, recreation, hydraulic structures) (Bagdanavičiūtė and Kelpšaitė, 2012). Intensification of abrasion processes primarily affects beaches. These processes narrow their width and decline stocks of sand, beaches become wet and little suitable for recreation. Disappearing beaches fail to protect dunes during storms, coastal sand dunes from waves as well as cliff from offensive flow. As a result, dune ridge or coastal dunes begin to degrade, cliff begins to retreat, deflation areas start to expand. In weaker places of coastal sand dunes or dune ridge breachings begin to form through which the sea water during storms can break into the rear of the coast (Butkutė, 2012).

It was found that the Ist Melnrage at the northern pier is one of the most vulnerable, the most affected by the negative effects of the hurricane. This zone is not only influenced by climate, large undulation, but by the port gate as well. The zone is constantly washed out. Scientists believe that the problem should

be solved otherwise than in the Lithuanian seaside resort of Palanga, where coast scour takes place, and the measure coast rescue – i.e. sand replenishment is applied. The above measure requires a significant investment, and the addition of sand to natural coasts is temporary, as it is done every year. For the Ist Melnrage zone at the northern breakwater scientists suggest solid measures - such as breakwaters, dikes or reinforced concrete and asphalt coast, sand while widenening the coast and designing and installing recreational, active recreation area, cafes and so on (Lietuvos pajūrio, 2015).

There are suggestions not only to deepen the port, but also to change and prolong the gates. Thus, the port will change the dynamics so that erosion will increase. The dredging of shipping channel and the prolonging of breakwaters will allow waves to freely destroy the shores.

The number of hurricanes in the Lithuanian seaside does not increase, however, it is observed that the power of hurricanes and duration are increasing, and therefore the negative effects become more and more difficult to control.

The object of the research is the strip of the Baltic Sea coast from the Northern breakwater to Giruliai.

The aim of the research is to determine changes of the Baltic Sea coast (from the Northern breakwater to Giruliai) during the period between 2008 and 2015, while clarifying and supplementing the spatial data sets of the Baltic Sea coast.

Tasks of the research:

1. To perform the adjustment of the Baltic Sea coast limits of the years between 2014 and 2015 in the strip from the Northern breakwater to Giruliai coast and to make the plan of the Baltic Sea coast limits.

2. To supplement the spatial data set of the Baltic sea coast of the years 2008 and 2013 with the revised set of spatial data of the years 2014 and 2015 as well as to determine the Baltic Sea coast limit changes from the northern breakwater to Giruliai.

3. To analyze and compare the accumulation and erosive processes of the object.

Materials and Methods

Comparative, analytical as well as statistical and logical analysis methods were used for the research.

For the research the following methods were used: field measurements results and information sources analysis, data visualization, and the Baltic Sea coast (from the Northern breakwater to Giruliai) special plan vectorization using a computer program GeoMap 2007.

In determining the Baltic Sea coast strip (from the Northern breakwater to Giruliai) dynamics trends, measurements included the period between 2008 and 2015. The formation of the strip Northern breakwater - Giruliai was influenced by the Hurricane Felix, which raged on January 10 of 2015. Describing the Baltic Sea coast strip (from the Northern breakwater to Giruliai) dynamics trends, selected from the period between 2008 and 2015, the Baltic Sea coastline change was calculated on the basis of results of measurements. This article analyzes erosive and accumulative processes occuring in the area of 4 km from the Northern breakwater to Giruliai Strip.

The prepared spatial data set of the Baltic sea coast is realized for the speed up of the decision-making processes, data collection and analysis convenience as well as visual presentation possibilities of wider range of uses.

Results and Discussion

Characteristics of the Baltic Sea coast

The general Baltic Sea coastline length makes up 8.000 km, of which 2.626 km stretches throughout Lithuania, Latvia, Estonia and Poland. Due to the dynamic formation, the coastal diversity in each Baltic Sea country is different - and here one can find the shifting dunes and sandy beaches, rocky shores, limestone and moraine scars (Ruskule et al., 2009).

Lithuania has the shortest coastline – only about 90 km, which is characteristic for sand beaches and accumulative dunes. Exclusive Lithuanian coast is the Curonian Spit - 97 km (51 km belong to Lithuania) and 3.8 km wide curved peninsula with the highest drifting white dunes in Europe (Ruskule et al., 2009).

In 2001-2002 during the reconstruction works of Klaipeda Seaport breakwaters were extended (the northern breakwater – by 205 meters, the southern breakwater – by 278 meters). It was found that in the closest to the coast breakwater section of the continent, where the coast was relatively stable during the last decade (till the port breakwater extension), after the reconstruction the coast erosion tendencies started to prevail. In the coast of the Curonian Spit, where during the last decade (till the port breakwater extension) in the closest to the coast breakwater section intensive erosion tendencies prevailed, after the reconstruction of breakwaters the coast first stabilized and then silt accumulation trends started to prevail (Jarmalavičius et al., 2011).

Changing nature and climate conditions, strong winds cause significant damage throughout the world and along the European coast, too. For example, the dunes and beaches of Palanga are being increasingly eroded by repeated battering, such as was inflicted during the January 2007 hurricane "Ervin" and this bringing down process has been dragging on for every year up to now (Vitkienė, 2007).

In the coast of the mainland, erosion processes predominated during the period between 2002 and 2007. In the dynamics of the coastline position change, the amount of deposits on the beach and coast negative trends were determined. During the period between 2007 and 2013 accumulation trends already dominated - coastline shifted more to the sea, higher silt content accumulated in the beach and protective beach dune ridges (hereinafter - the dune ridges). During the period between 2002 and 2013 the mainland coast remained relatively stable (except in the strips of moraine cliffs, south Melnrage and coast strips situated north from Palanga Bridge), but accumulation in some coast sections occurred at the expense of the erosion of other coast sections.

The highest accumulation was found on the south side of the Šventoji recreational zone, in Palanga recreational zone and in the southern coast strip as well as in Giruliai – the IInd Melnrage recreational zones. Accumulation processes in Palanga recreational zone occurred only due to the coast replenishment with the sand (Lietuvos pajūrio, 2015).

Spatial data creation of the Baltic sea coast

For the implementation of the research and the determination of the object changes, the measurements of the Baltic Sea coast section were arried out using GPNS receiver *DAP Technologies M9000* with GPNS antenna *SATLAB iSURVEY SL500*. GPNS receiver and antenna were configured by means of the *Bluetooth* connection. RTK mode was used to switch to LitPOS network. 4 km strip of the beach, which is situated from the Northern breakwater to Giruliai, was measured twice: in August of 2014 and in January of 2015 aftermath of Hurricane Felix. The strip of the researched object was divided into 8 sections of 0.5 km each.

The Baltic Sea coastline turning points were coordinated using GPNS receiver *DAP Technologies M9000* with antenna GPNS *SATLAB iSURVEY SL500*. 25S-1522 geodetic point was also recorded, spatial coordinates are as follows: x = 6181097.313; y = 316,799.583; z = 4.55 m (in the LKS-94 coordinate system). The spatial data set of the coast limits was supplemented by the indications of GPNS receiver: the coordinated points of the Baltic Sea coast and dunes.

Table 1

The data of the Baltic Sea coast changes (from the Northern breakwater to Giruliai) from the period of the months of January of the years between 2008 and 2015

Plot No.	Process	Period of 2008 and 2010; area, m^2	Period of 2010 and 2012; area, m^2	Period of 2012 and 2014; area, m^2	Period of January of 2014 and 2015; area, m^2	Period of January of 2008 and 2015; area, m^2
1.	erosion	5599	5862	1093	3408	14644
	accumulation	437	-	761	120	-
2.	erosion	2587	4568	642	3298	8610
	accumulation	1062	-	1241	182	-
3.	erosion	956	4163	147	516	2986
	accumulation	771	-	2022	250	247
4.	erosion	794	4643	-	1757	3908
	accumulation	2007	-	2237	1163	2121
5.	erosion	5957	3289	-	3845	6489
	accumulation	2633	417	5309	131	1888
6.	erosion	1465	4518	73	4254	2866
	accumulation	4854	13	5285	184	2892
7.	erosion	1475	2991	544	4815	2858
	accumulation	3652	395	3083	370	533
8.	erosion	552	3095	674	3394	3758
	accumulation	1837	189	2843	451	1363
In the strip of 4 km, area, m^2	erosion	19385	33129	3173	25287	46119
	accumulation	17253	1014	22781	2851	9044
In the strip of 4 km, the coast area, m^2; increased (+) or decreased (-)		- 2132	- 32115	+ 19608	- 22436	- 37075
Most erodible strip		Plot No. 5	Plot No. 1	Plot No. 1	Plot No. 7	Plot No. 1
Most accumulated strip		Plot No. 7	Plot No. 7	Plot No. 5	Plot No. 4	Plot No. 6

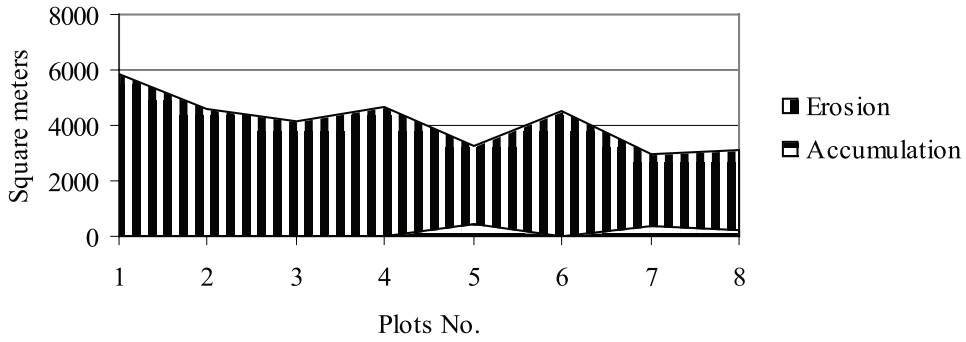


Figure 1. The Baltic Sea coast change during the period between 2010 and 2012, area in square meters.

In August of 2014, in the Baltic coast strip Ist Melnrage – IInd Melnrage – Giruliai, 115 points were coordinated that were received in coordinating the coast limits.

In January of 2015, aftermath the Hurricane Felix in the analysed strip: Ist Melnrage – IInd Melnrage – Giruliai, 438 points were coordinated that were received in coordinating the coast and dunes limits.

For the identification of the coastline the software GeoMap 2007 was used. Using this program ortophoto images were uploaded in the program and the Baltic Sea coastlines of 2014 and 2015 were identified.

For the evaluation of the most damaged and problematic areas the coastline turning points coordinates were determined.

Using the data obtained, the plans of the Baltic Sea coast changes (from the Northern breakwater to Giruliai) of the months of January of the years between 2008 and 2010, 2010 and 2012, 2012 and 2014, 2014 and 2015 as well as between 2008 and 2015, were drawn.

According to the drawn plans, the summary of the Baltic Sea coast changes of the years between 2008 and 2015 (from the Northern breakwater to Giruliai) was made (Table 1).

After calculations, specific areas of the Baltic Sea coast, which are exposed to erosion and accumulation processes, have been determined.

Analysis of Baltic sea coast changes

Based on the above mentioned data of the Baltic Sea coast (from the Northern breakwater to Giruliai), the coast changes diagrams from the period of the months of January of the years from 2008 to 2010, 2010 to 2012, 2012 to 2014, 2014 to 2015 as well as from 2008 to 2015 were created.

It was found that in the period between 2008 and 2010 the Baltic Sea coast erosion was at its maximum in the areas of plots No.1; No.5, the maximum accumulation was in the strips of plots No.6 and No.7.

After analysis, it appears that in the period between 2008 and 2010 the Baltic Sea coast (from the Northern breakwater to Giruliai) erosion and accumulation processes are almost the same: the erosion process made up 53 percent, and accumulation - 47 percent.

During the period between 2010 and 2012 the Baltic Sea coast (from the Northern breakwater to Giruliai) witnessed the rapid erosion throughout the area, especially in the strips of the plots No.1, No.4 and No.6, and a slight accumulation ranged in the the strip from the plot No.4 up to the plot No.8 (Fig. 1).

During the period between 2010 and 2012 from the Northern breakwater to Giruliai dominated erosive processes: erosion process made up 97 percent and accumulation – 3 percent.

During the period between 2012 and 2014 the Baltic

Sea coast (from Northern breakwater to Giruliai) witnessed the rapid accumulation throughout the area, and particularly in the strip from the plot No.4 up to the plot No.7, and erosive processes, as usual, showed up only in the strip of the plot No.1 (Fig. 2).

During the period between 2012 and 2014 accumulation processes dominated in the coast of the Baltic Sea (from the Northern breakwater to Giruliai): erosion process made up 12%, and accumulation - 88%. In January of the years 2014 and 2015, similar erosion prevailed throughout the coast in the analyzed object, except the strip of the plot No.3. During the period of January of 2014 - 2015 erosive processes were strongly influenced by Hurricane Felix. Blurry accumulative processes were observed in the area from the plot No.2 to the plot No. 8. The maximum accumulation was observed only in the coast strip of the plot No.4 (Fig. 3).

In January of 2014 and 2015, erosion processes prevailed in the coast of the Baltic Sea: the erosion process made up 90 percent, and accumulation – 10 percent.

It can be said that during the periods of the months of January between 2008 and 2010, 2010 and 2012, 2014 and 2015 rapid erosion took place, and the 2012 - 2014 year period was characteristic for accumulation processes.

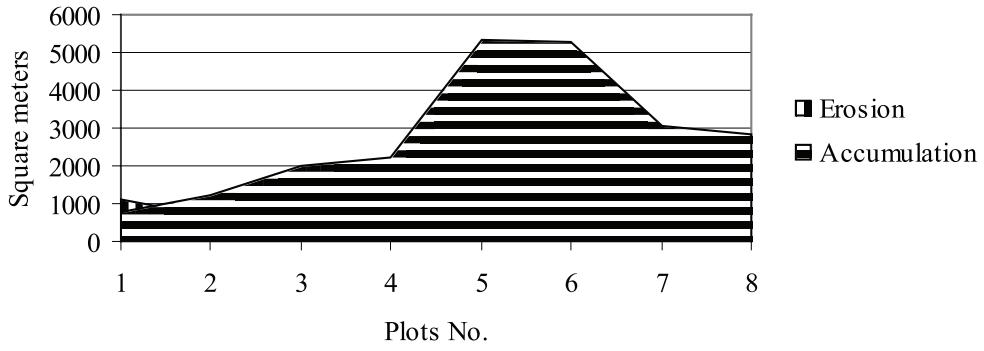


Figure 2. The Baltic Sea coast (from the Northern breakwater to Giruliai) change during the period between 2012 and 2014, area in square meters.

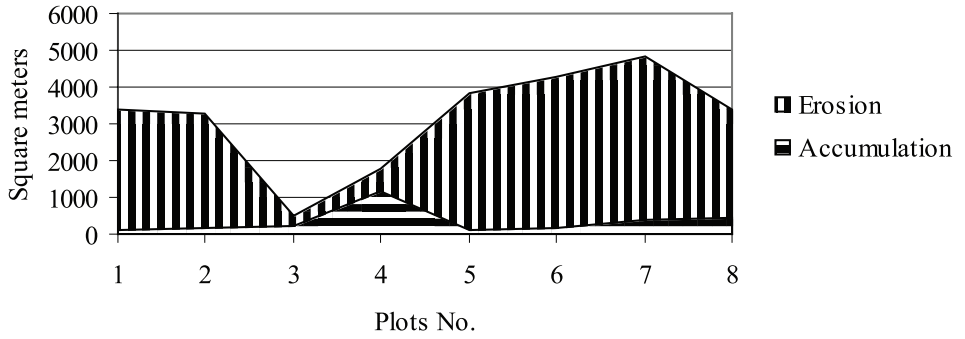


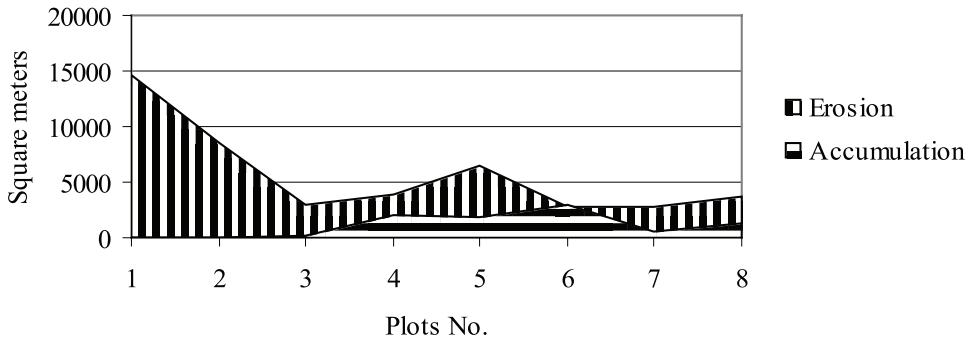
Figure 3. The Baltic Sea coast (from the Northern breakwater to Giruliai) change during the period between 2014 and 2015, area in square meters.

After setting up and analysing the data of all periods of erosion and accumulation processes, the results obtained from the period of January months between 2008 and 2015 showed that coastal erosion (from the Northern breakwater to Giruliai) took place throughout the strip, especially in the plots No.1 and No.2; i.e. in the area of the Ist Melnrage. Accumulative processes were observed in the strip from the plot No.3 to the plot No.8, the maximum accumulation was observed in the area of the plot No.6 (Fig. 4).

After analysis, it was found that during the seven-year period (from the period of the months of January between 2008 and 2015) erosional processes prevailed in the Baltic Sea coast (from the Northern breakwater to Giruliai): erosion process made up 83 percent, and accumulation - 17 percent.

During the months of January of the period between 2008 and 2015, the Baltic Sea washed 2.8194 ha of the Ist Melnrage beach area, in some places of this strip the coast beach area increased by 0.1307 ha. This means that the coast limit moved to the land thus reducing beach area.

In the 0.7 km strip of the Ist Melnrage area coastline has moved more than 30 m inland, and in the 0.4 km strip the coastline shifted from 4 to 14 m into the sea. Comparing the Baltic coast limits from the



Fiure 4. The Baltic Sea coast (from the Northern breakwater to Giruliai) change during the period between 2008 and 2015, area in square meter.

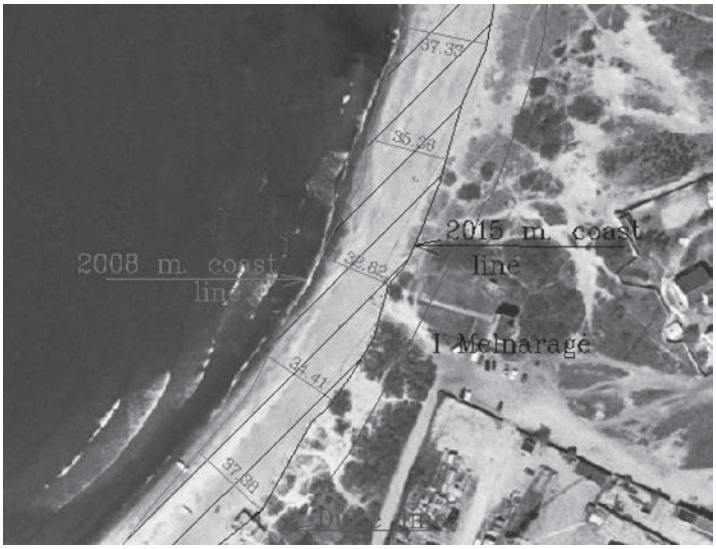


Figure 5. The shift of the Baltic Sea coast during the months of January in the period between 2008 and 2015.

period between 2008 and 2015, it was observed that in 2015 the Baltic Sea coast rapidly shifted towards land (Fig. 5).

With reference to the coast measurements data of the period between 2008 and 2015, it was found that during the period between 2008 and 2015 in the area of the IInd Melnrage beach 1.1 km long strip decreased by 1.9567 ha and the Baltic Sea coast limit in some places has moved 25 m inland, and in 0.6 km long strip in some places of the IInd Melnrage beach was supplemented with sand in 0.6374 ha and the coast limit has moved about 30 m into the sea.

Comparing the Baltic Sea coast limits of the years between 2008 and 2015 in the area of Giruliai, it was noticed that in 2015 the Baltic Sea moved 27 m inland, and up to 14 m – into the sea. In 2015, the area of Giruliai beach decreased by 0.3758 ha in some places, in other places it has increased by 0.1363 ha.

When analyzing the coast measurements data of the period between 2008 and 2015, it was determined that during the months of January of the period between 2008 and 2015 the 4 kilometer long strip of the Baltic Sea coast (the Northern breakwater – Giruliai) has decreased by 3.7075 ha. It can be argued that Melnrage beaches are particularly vulnerable, especially the area of the Ist Melnrage, where there almost no accumulation processes occur.

Conclusions

1. The measurements of the Baltic Sea coast limits from the Northern breakwater up to Giruliai Strip were performed with the help of GPNS receiver "DAP Technologies M9000" with GPNS antenna "SATLAB iSURVEY SL500" in the LKS-94 coordinate system. In the strip of the Baltic coast in August of 2014, 115 points were coordinated, which had been received during the coordination of coast limits and in January of 2015, 438 points were coordinated during the coordination of the coast and dune limits. The Northern breakwater - Giruliai Strip plan was made by means of GeoMap 2007 software in the LKS-94 coordinate system, in the Baltic height system.
2. During the period of the months of January between 2008 and 2015, the 4 km strip of the Baltic Sea coast (Northern breakwater - Giruliai) decreased by 3.7075 ha, in the 0.7 km strip of the Ist Melnrage area coastline has moved more than 30 m inland.
3. During the seven-year period (during the months of January between 2008 and 2015) erosive processes dominated in the Baltic Sea coast (from the Northern breakwater to Giruliai): erosion process made up 83 percent, and accumulation process made up 17 percent.

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LAND CONSOLIDATION IN SLOVAKIA, WHERE IT HANGS?

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Abstract
Land consolidations in Slovakia are regarded as an instrument for solution of ownership fragmentation in accordance to rural development. In the introduction of this paper, we describe problems in Slovakia associated with the ownership fragmentation. Country, rural areas were significantly influenced by the period of collectivization. The benefits of land consolidation project are shown in a case study area for Kanianka cadastre. We compare ownership relations before and after the land consolidation. Statistical values as number of resolved ownerships by LC, number of plots and average size of plots are shown. New infrastructure, water management and ecological elements are discussed. In conclusion, we give the reason why activities related to land consolidation in Slovakia stagnates despite positive response.
Key words: land consolidation, ownership fragmentation, land consolidation project, rural development, Slovakia.

Introduction

If we want to evaluate the process of land consolidation, we need to consider two major problems. The first is a specific problem of ownership fragmentation in Slovakia. The second problem is related to the landscape development, which was significantly influenced by the course of history.

Problems with land fragmentation

High number of land co-owners is typical for Slovakia. Average number of co-owners' shares per one plot is 11.11 (Urban, 2013). In some cases, plots are only 2 m wide, but 700 m long. Individual owners have their land scattered throughout the whole cadastral area and extreme fragmentation of land is very common. Meaningful use of these plots is very difficult, as they are not accessible, often located in the center of large agricultural units and high number of co-owners prevents selling or renting.

High fragmentation of land is due to the inheritance laws from the time of the Austro-Hungarian Empire. Generally, all children inherited equal shares of land. Constructions of technical projects such as railways, roads, water flow regulations also had significant impact on land fragmentation (Dumbrovský et al., 2004). Narrower and more elongated shapes of plots are caused by inheritance and dividing. Problems with ownership are characterized mainly by the following features: dispersion and fragmentation of plots, improper shape and inaccessibility of the plots. Many authors abroad also indicate problems related to the inaccessibility of plots (Hartvigsen, 2015; Sky, 2014; Parsova, 2014).

Statistical overview of the average land ownership in Slovakia is as follows: average number of parcels of one owner – 20.6; average number of co-owners per parcel shares – 11.1; number of proprietary relations in SR – 98 million; number of parcels – 8.8 million; the average area of plot– 0.55 ha; number of landowners – 4.1 million (Ministry of Agriculture and Rural Development of the Slovak Republic, 2013).

Problems in the landscape

Agricultural land in Slovakia frequently looks monotonous. Gigantic arable units are erosion predominant. This kind of condition, however, entails a large number of environmental problems, such as washing away of top soil, degradation of fertile soil, sudden local floods, pollution of streams, damage on public structures and buildings. The large units of arable land paradoxically hide a large number of original plots with high number of co-ownership relations.

Ecological imbalances persist and continue since the period 1948-1989, when land use was oriented in completely different direction – towards establishing and maintaining large scale agriculture (collectivization). With the new organization of territory, in the form of economic-technical adjustment of land (ETAL, HTÚP in Slovak), all natural barriers in the landscape were plowed (barriers, roads between plots, etc.) and plots were further consolidated to gigantic proportions. Joint agricultural cooperatives (JAC, JRD in Slovak) have been created (Muchová and Konc, 2010). The owners of these plots could not cultivate their land. Discrepancy between ownership records in the Slovak Real Estate Cadastre and the actual state, with large-scale units, inaccessible landscape, inaccessible plots which are often located in the center of large arable unit, high water and wind erosion, decreased soil quality, lack of natural ecological barriers, reduced ecological stability of landscape and biodiversity, etc. (Bažík et al., 2014) is still prevailing.

Land consolidation – a possible solution

Issues mentioned above, e.g. the high fragmentation of ownership and natural imbalance can be solved

through the land consolidation. It returns natural barriers into the landscape such as erosion control measures, environmental elements, road networks, flood control measures. Purchase of land from unsettled ownership is problematic. If municipality, state or other public entity cannot obtain the land at the required location from the owners, good projects are not feasible.

Land consolidation (Varga and Bažík, 2013) includes rearrangement of ownership and ownership relations (consolidation, inheritance, etc.) and technical, biological, economic and legal measures related to new reorganization of legal relations. Land consolidation returns natural barriers to the landscape which had been disturbed due to historical/political reasons. Proposals of these elements rearrange ownership and after their implementation, they change the landscape character and improve its functioning.

The basic legislative regulation in the area of land consolidation in Slovakia is Act No. 330/1991 Coll. on land arrangements, settlement of land ownership rights, district land offices, the land fund and land associations as amended (land consolidation law).

Land consolidation is generally carried out for whole cadastral area, mostly in rural areas, which form perimeter of land consolidation. It is composed of surveying and project activities that are compiled into stages due to time and content. These stages are linked to each other and may also overlap in time. The time sequence of individual steps during the LC project according to the land consolidation law is as follow (Muchová and Antal, 2013):

Initial documentation contains: area of land consolidation, updating of the soil-ecological units (SEU) and land-value maps, initial state registry, general principles of functional organization of the local territorial system of ecological stability.

Proposals for a new arrangement of plots in the land consolidation perimeter contains: principles of the placement of new parcels, plan of shared facilities and measures and plan of public facilities and measures, partitioning plan in the form of placement and marking plan.

Implementation of the land consolidation project contains: demarcation and marking/labelling of break points at the borders of new plots, updating of both the registry of initial state and the partitioning plans in the form of placement and demarcation plan, partitioning plan in the form of geometric plan.

Aim of this paper is to describe problems in Slovakia associated with land consolidation and also to show the benefits of this process. Problems are described through the ownership fragmentation. The benefits are shown through comparison of ownership relations before and after the land consolidation. Also new infrastructure, water management and ecological elements can be beneficial. Final task is to describe reason why activities related to land consolidation in Slovakia stagnates despite positive elements.

Materials and Methods

Slovak Republic (SR) with total area of 49036 km^2 and population of 5415949 is one of the smallest countries on the European continent. Agricultural land covers 49.7%, forest areas 41%, water bodies/flows 2%, built-up areas 5% and other area 3% of the total area. Population density is 110 people per square kilometer. Landscape of Slovakia is much diversified; highlands and mountains cover 60% and lowlands 40% of the territory. Elevation ranges between 94–2655 m. Slovakia has 9115 m^2 of land per capita, from which agricultural land is 4518 m^2 (2653 m^2 arable land), forest area 3731 m^2, water areas 173 m^2, built-up and other areas 692 m^2.

Case study

To demonstrate the importance of land consolidation in Slovakia, cadastral area of Kanianka was picked as a case study area. Land consolidation in cadastral area of Kanianka, in Trenčiansky region, has been completed and registered in the Slovak Real Estate Cadastre in January 2011. Intensive agricultural production is concentrated mainly in the eastern part of the cadastre. In the western part of the cadastre, mainly grassland, pastures and forests are located. Some parts of the area were endangered by water erosion and therefore relevant measures were proposed. Agricultural land accounts for about 17% of the total area of the cadastre (Muchová et al., 2008).

Indicators of changes in rural areas

Indicators of changes in land use consist of elements that are divided by main categories: arable land, forest land, vineyards, gardens, orchards, grasslands, water areas and other areas. On this basis, we evaluate the tendency of changes in land use in 100 years period.

Three time horizons were used for evaluating of changes in land use: historical landscape structure (second military mapping), current landscape structure and proposed landscape structure.

Second military mapping survey of Austrian empire (Zeman, 2012) took place in 1806–1869. Unlike the first military mapping, the geodetic bases have been already made, which served not only for topographic but also for land surveying. These maps show the historic character of the environment, which either completely disappeared from landscape or is gradually disappearing. These materials can be and should be a guideline for revitalizing river systems, the restoration of the original road network etc.

Planimetric mappings for land consolidation project were selected for purpose of interpretation current landscape structure. These mappings are focused on the current situation in landscape and identify changes between actual and registered state in the Slovak Real Estate Cadastre. They are performed in the 3rd class of accuracy (0.14 m) for scale 1:10000. All features are the object of planimetric mapping.

New organization of road network, new system of erosion control and flood protection, system of ecological stability, etc. are the results of land consolidation. Proposed landscape structure is defined within general principles of functional organization of the territory and it defines new organization and skeleton of existing and proposed measures.

Ownership structure of case study

Changes in the ownership structure were evaluated before and after land consolidation using the following indicators: number of plots of common property, number of owners, number of ownership relations, number of plots with one owner 1/1, number of plots in co-ownership, average number of co-owners of one plot, maximum number of co-owners of one plot, average size of one plot (m^2), average number of co-owners of one plot, average number of plots per one owner, average size of property per one owner (m^2).

Results and Discussion

Results

On the basis of input and output parameters of land consolidation in cadastral area Kanianka the process of LC with our active participation (Muchová et al., 2008) is demonstrated. The main reasons of land consolidation in this cadastre were arrangement of ownership relations mostly due to historical development, access to plots and functional and spatial rearrangement of agricultural land.

Land fragmentation

Considering the size of cadastre (794 ha) ownership fragmentation was very high. Figure 1 (left panel) presents current land ownership according to the Slovak Real Estate Cadastre. These very narrow plots contain multiple shares of several co-owners. Figure 1 (right panel) presents the state after the land consolidation, where even in this complex ownership 'chaos' the problems of individual owners were resolved.

Comparing input and output values of LC (Table1) shows that the number of plots decreases more than three-fold and average size of plot increased more than three times. An important benefit is the reduction of ownership relations by nearly half. Plots in co-ownership decreased from 997 to 157. Owners who own only one plot increased from 81 to 157. Moreover, the numbers of plots in co-ownership with 2-5 co-owners decreased from 659 to 102 and co-ownership with 6-10 co-owners from 243 to 28.

Landscape

Historical landscape structure of Kanianka was different than today. Location in Strážovské Mountains intensively influenced the use of the cadastre back in

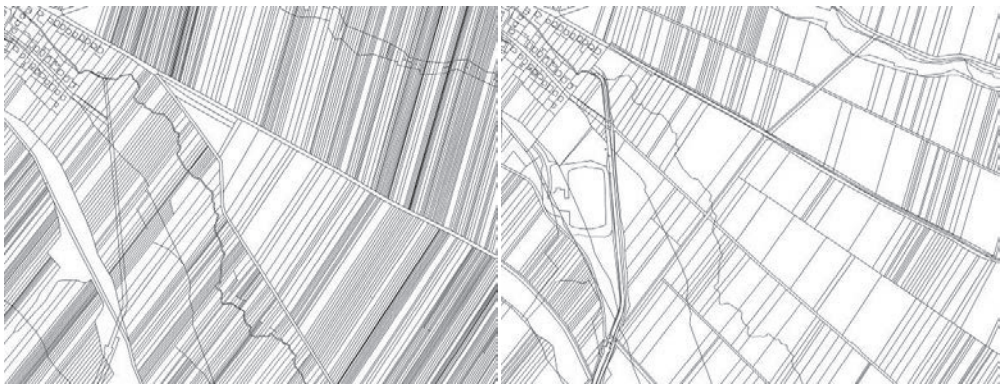

Figure1. Map of ownership fragmentation before (left) and after (right) the land consolidation.

Table1

Land fragmentation before and after land consolidation

Parameters	Before LC	After LC
Number of owners	565	565
Number of ownership relations	12596	7351
Number of plots	1711	951
Average size of plot	0.40 ha	0.74 ha

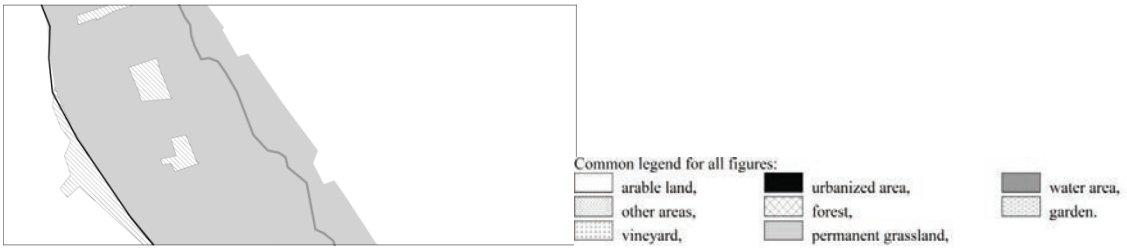

Figure 2. Historical landscape structure.

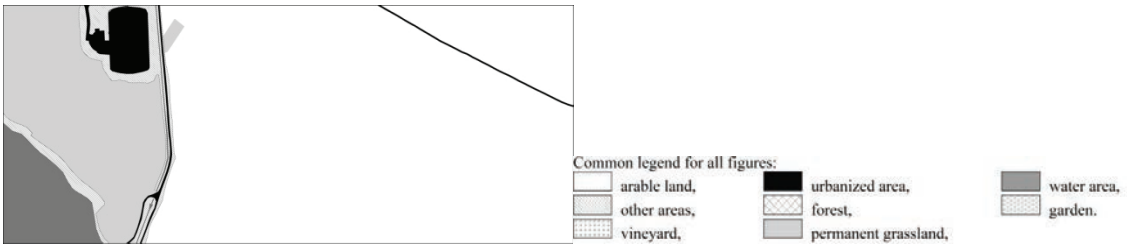

Figure 3. Current landscape structure.

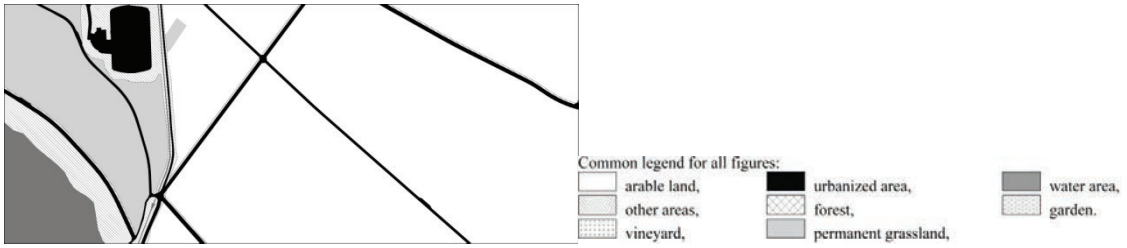

Figure 4. Proposed landscape structure.

1845. A relatively compact complex of mixed forest covered almost 57% of the total area. The second largest landscape element on almost 28% was arable land, while the grassland took 11% surrounding the stream Kanianka in south part of cadastre. Road network directed mainly to neighboring communities. Figure 2 shows parts of historical landscape structure, which are, subsequently, compared with same parts in other time horizons.

Current landscape structure (Figure 3) shows that cadastre is mostly covered by forest (70%). Representation of arable land is 17% and permanent grassland is 6%. It means that this area is intensively used for forestry and agricultural use is only complementary. In the area of about 9 ha the reservoir Kanianka was built for irrigation as a regulatory reservoir. It is now also used for recreation. Ecological stability of the area is positive and it is very significant.

Land consolidation in cadastral area of Kanianka was initiated in 2007. Proposed activities were focused on comprehensive rearrangement of the rural landscape, whose main goals were to protect and ensure renewable resources (water, soil), plant and animal species and their communities and new land use. The main goals of proposed measures were (Figure 4):

a) accessing of plots and buildings on them,
b) slowing down the degradation process on the agricultural land, preserving and promoting the natural productive soil functions,
c) protection and management of the environment, increasing ecological stability,
d) preservation and creation of the landscape (support of structural elements of the landscape and aesthetic values, uniqueness and multiplicity of the landscape).

Because of bad slope conditions, the cadastre is less suitable for agriculture and the forest is dominant type of land structure. This also determined the owners to preserve arable land as much as possible, because the negative environmental factors, according to their opinion, do not affect the territory.

Changes in land use

Table 2 shows changes in spatial representation of land use in all three time horizons. It is obvious that the current landscape structure was significantly

Table 2

Representation of landscape structure in different time horizons

Land type	Historical landscape structure		Current landscape structure		Proposed landscape structure	
	ha	%	ha	%	ha	%
Arable land	221	27.84	122	17.23	118	16.63
Garden	7	0.94	0.1	0.02	0.1	0.02
Permanent grassland	54	12.44	47	6.62	42	5.97
Water area	2	0.52	10	1.46	10	1.46
Built-up area	3	0.63	9	1.28	14	1.97
Other area	3	2.34	17	2.47	28	3.99
Forest	459	57.80	500	70.92	498	69.96

Table 3

Landscape parameters

Parameters	Before LC	After LC
Agricultural land	197 ha	161 ha
- Arable land	122 ha	118 ha
Forest land	500 ha	498 ha
Length of field roads	4.32 km	13.79 km
Length of forest roads	17.05 km	36.17 km
Area of erosion control measures	0 ha	3.48 ha
Area of water management measures	11.92 ha	11.92 ha
Area of ecological measures	13.66 ha	30.80 ha

influenced by large-scale production during the period from 1948 to 1989. This period clearly brought to the landscape large, up to 200-300 ha, land units. These were created at the expense of permanent grassland and non-forest wood vegetation. Proposed landscape structure, through the land consolidation, reintroduces the green area in the landscape and gives the opportunity to create the conditions for rural development.

Land consolidations are not performed only for the sake of owners or users but also for the whole country as well. Ecological measures are priority, together with erosion control and water management (Table 3). More than half of the area share for common facilities and measures were used for these measures. The rest of share was used for road network. The ecological character of land consolidation is more evident when there is vulnerability to anthropogenic impacts and intense agricultural use.

Land consolidation projects are a tool, which significantly affects the creation of landscape. Realization of common facilities and measures as they were proposed allows for significant positive changes. In our case study area many common facilities and measures have been proposed (Table 3.) They are going to be gradually implemented.

Discussion

Despite the benefits from land consolidations, these processes are not continuing as expected. Figure 5 shows number of projects started since 1991. Currently (31.12.2014) land consolidations are carried

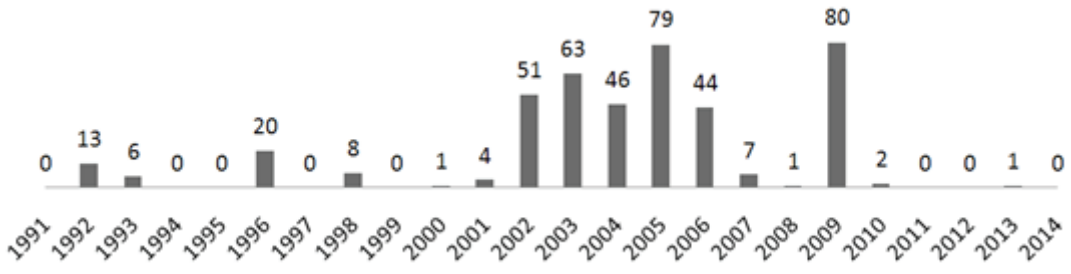

Figure 5. Number of land consolidation projects assigned by the years.

out in 426 cadastral areas which cover approximately 12% of Slovak Republic territory. 261 projects are finished and 165 are in progress.

As figure 5 shows, land consolidation projects are assigned very unequally. Reasons for that are mainly because cycles of programming period regarding the EU funds, political priorities, deformed business environment (low demand), deformation of prices and obstructions in the process of evaluating public tenders etc.

Many projects were assigned in the early years, when there was a hope for successful completion based on a new land consolidation law. Based on 'concept of ownership organization' in 1993, most projects were completed to the elaboration stage of 'initial state registry'. This happened because of extreme ownership fragmentation. In period of 1991–1995, the initial state registry methodology was prepared. Based on this methodology the 'Register of Renewed Real Estate Cadastre' (ROEP in Slovak) must be performed before LC project. The transparency of ownership registry is improved by ROEP.

From the original 52 projects, only 12 have been completed and entered into the Slovak Real Estate Cadastre but behind schedule. In the years 1996–2003 more complex projects were entered especially in environmentally degraded areas, mainly in the Vysoké Tatry and Žiarska basin. In the period of 2002–2006 (based on the EU pre accession programme SAPARD, the Sectoral Operational Programme and the Rural Development Plan) many projects were started. Unfortunately, there are also years when no projects were started. Despite good methodology, bad period for land consolidation occurred since 2010. This challenging period is a result of wrong political decisions evaluating their importance. There were also problems with transparency and efficiency of implementation of the proposed measures, etc. Some of the land consolidations have even been accelerated so that projects became more simplified and cheaper. New technological process and the price list were proposed, but these activities did not bring recovery of land consolidation.

For the purpose of land consolidation, 80 million euros allocated from the EU funds in the new programming period 2014-2020. So far, however, all activities related to land consolidation are in the hands of politicians. There are no known aims, how and for what purposes the funds will be used.

Conclusions

Land consolidations have been instrumental in promoting rural development in Slovakia. They have the potential to make significant contributions towards improving the quality of rural life and also to solve ownership fragmentation. New approaches and solutions through land consolidation are able to solve fragmentation, social, cultural, economic, legal, administrative and political environment with financial and other resources mostly from EU funds. In our case study area of Kanianka the main goal was to consolidate ownership of the land. The number of plots decreased more than three-fold and average size of plot increased more than three times. An important benefit is the reduction of ownership relations by nearly half. Plots in co-ownership decreased from 997 to 157. Numbers of owners who own only one plot increased from 81 to 157. Ecological measures became priority together with erosion control and water management. More than one half of the area share for common facilities and measures (of totally 40.09 ha) has been used for these measures. The rest of the share was used for road and forest network. Despite the benefits from land consolidation projects, these processes are not developing as expected. This is a result of wrong political decisions about importance of land consolidation and there are also problems with transparency and efficiency of implementation of the proposed measures.

Acknowledgement

Results obtained in the research project VEGA (Scientific Grant Agency of the Ministry of Education of the Slovak Republic and of Slovak Academy of Science) 'Creation of a data foundation/base for the implementation of an information system on land consolidation' no. 1/0656/12 have been presented in this paper.

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DETERMINANTS OF TOURISM DEVELOPMENT IN AREAS OF HIGH NATURAL VALUE

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Abstract
Development of tourism, understood as the result of human activity aimed at adapting space to the needs of tourism, requires a variety of specific spatial determinants recognized from the viewpoint of multi-dimensionality of tourist space. One of the major determinants of tourism intensity in an area are landscape values, including natural values. Natural elements of the environment (including the terrain, water reservoirs, natural forms of land cover), sustainable landscape, or the unique cultural objects are the basis for long-term development of tourist activities. These values are characteristic of the so-called naturally valuable areas, including areas under the national and international law of different forms of nature protection. The paper aims at examining the relationship between the existing natural determinants affecting the development of tourism and the intensity of tourist movement as well as tourism intensity and the level of development of tourist facilities in the gminas of the Warmińsko-Mazurskie voivodship.
The overall objective will be implemented in three stages. The first one will be to identify the existing natural determinants for the development of tourism in the Warmińsko-Mazurskie voivodship. The second phase will consist of an examination of the intensity of tourist movement and the level of the development of tourism by designating Schneider's, Baretje's and Defert's indicators and the share of recreational areas. The final step will be to identify relationships between the variables obtained in the process of obtained in the two previous stages.
Key words: areas of high natural value, tourism development, natural values.

Introduction

The development of tourism as a phenomenon with great economic potential has been accompanied by the appearance of the notion of tourism management understood as the result of activities whose main goal is the adaptation of space to tourist function. The formation of so-called 'tourist space', i.e. the space in which human tourist activity is observed, is the result of 'the co-operation and the co-existence' of varied geographic sub-spaces: social, ecological, cultural, technological, and economic. One of the most important sub-spaces is the ecological one. Tourism uses the following elements of ecological space: terrain, water reservoirs, climate, biological diversity, and landscape. These often unique natural creations are at the same time the natural values of a tourist space. The use of ecological space for tourist purposes is a desired phenomenon but only under one condition: the use of natural elements must be responsible and reasonable. In other words, natural resources should be used in a way that guarantees their durability of nature and minimizes irreversible environmental changes caused by anthropopressure.

Naturally valuable areas are one of the most significant components of ecological space. In Poland, in the previous two decades the concentration of tourist movement has gradually progressed. Hence, extending the range of activity on naturally valuable areas is connected with the expectations and needs of increasingly demanding tourists (Michałowski, 2008). Moreover, the development of tourism is a chance for socio-economic stimulation. It often happens that tourism is a privileged form of activity in naturally valuable areas. Sometimes it is the only form of economic activities allowed within their borders (Schubert, 2010).

In the literature, there exist various, sometimes imprecise definitions of a 'naturally valuable area' (Ryszkowski, 1985). In the most general sense this area can be understood as a space of high values determined by the presence of different, unique natural resources (Dobrzańska, 2005). Taking into account only the ecological aspect, there are no doubts that space described as a naturally valuable area should be characterized by high degree of biodiversity -the diversity of organisms and ecosystems (Dobrzańska, 2005; Szczepanowski, 2007). These areas should be distinguished by low degree of anthropogenic transformation (from natural, unchanged forms to forms changed in small degree) (Dobrzańska, 2005). Moreover, they should perform various functions: environmental (regulating), social, cultural, aesthetic, recreational, scientific, and educational (Łuszczyk, 2010).

Definitions of naturally valuable areas should take into account not only the ecological aspect, but also the socio-economic one. Land with high biological diversity often is or may be the dominant factor determining economic activation (Dobrzańska, 2005). The management of such areas should reflect the principles of sustainable development where the process of socio-economic development should not disturb the environmental balance and the durability of natural processes so as to ensure the opportunity to fulfill the needs of the present and future generations (*Act of 27 April 2001 on the protection of nature*).

In light of these assumptions, any economic activity in naturally valuable areas should be conducted in a way which guarantees adequate protection (Łuszczyk, 2010). Effective protection of valuable areas should be regulated (Łuszczyk, 2010).

Development of tourism is mainly determined by the presence of the so-called tourist values – unique features of space, attracting the interest of potential tourists (Gołembski, 2002). Taking into account tourist motives, we may distinguish three main groups of values: recreational, landscape (including natural, cultural and advantages connected with contemporary human achievements) and specialist (Kiryluk, 2005). On the basis of the definition of naturally valuable areas in which high degree of biodiversity is noted, we can state that the areas are characterized by significant natural values. Besides the presence of rare species of fauna and flora in the areas there are additional values, such as varied terrain, natural streams, rivers and water reservoirs, underground forms of erosion (caves, vaucluse springs, ravines), favorable microclimate, and clean air. A large part of naturally valuable areas is legally protected land, which points to the fact that such areas constitute significant natural value. The use of natural conditions is conducive to the formation of additional values with natural features (botanical parks, wildlife parks, etc.).

The existence of naturally valuable areas is a guarantee of the presence of recreational values. The higher the quality of particular components of natural environment, the greater the attractiveness of leisure (Kiryluk, 2005). Recreational values are the set of features of space providing optimal conditions for leisure and recreation (Płocka, 2009). Natural conditions and features of naturally valuable areas determine specialist values. The latter create the opportunity to develop various forms of qualified tourism, e.g. canoeing, sailing, fishing (Łuszczyk, 2010). In such areas there often appear products of material and non-material culture which are the results of human activity and which constitute the so called cultural values.

Although naturally valuable areas are characterized by a variety of tourist attractions, their presence alone may not guarantee tourism development. From the point of view of a tourist, it is also important whether the area has a system of functionally connected devices and services aimed at satisfying their basic requirements (Kowalczyk and Derek, 2010).

The development of tourism in naturally valuable areas should be realized in a sustainable way in the social, ecological and economic dimensions (Gałązka, 2009; Niezgoda, 2008). Such development is determined by a rational distribution of elements of tourist infrastructure in the area. The implementation of tourist infrastructure puts an increasing burden on the natural ecosystem. Tourist investments should be realized in a way which will not lead to exceeding the resistance of the environments and to irreversible loss of natural values (Szczapanowski, 2007). In accordance with the trends in contemporary tourist management of naturally valuable areas, three concepts can be used: spatial zoning, concentration-dispersion and restriction of accessibility (Kowalczyk and Derek, 2010).

Functional zoning of space is recommended in order to reduce, on the one hand, spatial conflicts within the biodiversity of naturally valuable areas and, on the other, the intensity of tourist infrastructure. The main rule here is to plan tourist movement in a way which will not contribute to damaging areas of great importance for the protection of environment (Kowalczyk and Derek, 2010). The implementation of functional zoning of space is connected with dividing an area into zones with varied tourist accessibility depending on the specificity of the naturally valuable area. In each zone there is allowed an appropriate composition of elements of tourist infrastructure.

Another concept which may be useful in tourist management of naturally valuable areas is concentration-dispersion of tourist movement. There are two alternative ways of its implementation. The most common one is to concentrate tourist movement in selected places with greater resistance to negative human impact on the environment. In these places there are usually main elements of tourist infrastructure (car parks, campsites, tourist information, catering objects, etc.). An alternative way of implementing the concept includes actions aimed at the dispersion of tourists. As a result, there are visits of small groups of tourists in particular areas (Kowalczyk and Derek, 2010).

The third strategy aimed at controlling tourist use of naturally valuable areas is associated with limiting accessibility. Unlike the above-presented methods, this one depends on the use of administrative regulations which may decrease tourist movement. The most common way of implementing this strategy is to determine the limit of the number of tourists or to set an entrance fee (Kowalczyk and Derek, 2010).

The selection of the strategy for tourist use of naturally valuable areas depends on the character of an area, the forms of its protection, resistance to anthropogenic factors and the current intensity of tourist exploitation. The use of appropriate plan may determine adequate strategies for socio-economic activation of an area in accordance with the principles of sustainable development.

Materials and Methods

The study area is located in the administrative borders of the Warmińsko-Mazurskie voivodship[1], in north-eastern part of Poland (Fig. 1). A large part

1 Voivodship – administrative region of the 1st order in Poland (NUTS 2).

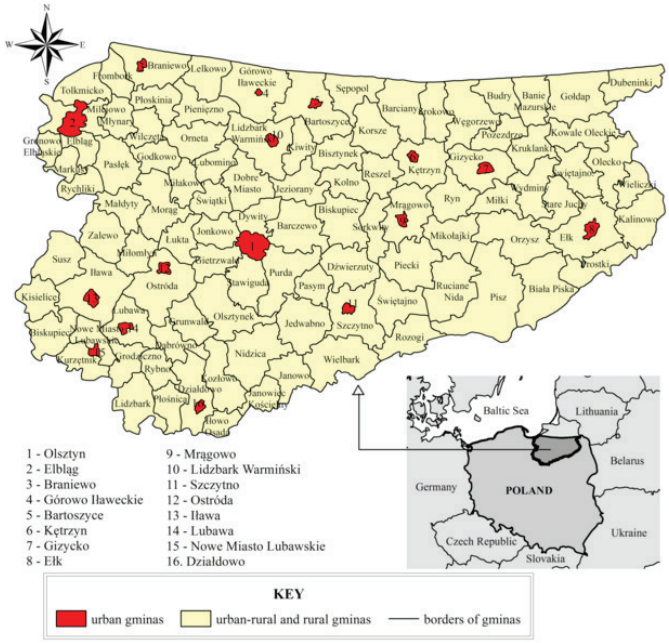

Figure 1. Administrative division of the Region of Warmia and Mazury.

Source: Own elaboration.

of the voivodship belongs to the macro-region of the Masurian Lake District and a small northern part to the Gdańsk Shoreland. In the West, it is located in the Iława Lake District and in the Chełmińsko-Dobrzyńskie Lake District. Due to the fact that the terrain has been shaped by the activity of ice sheet and fluvioglacial water, the voivodship is characterized by richness of natural values (varied terrain, numerous lakes, a developed river network) (Cabaj and Kruczek, 2010). The terrain, high share of forest cover (30.9%), of water area (6%) and of areas under legal protection (more than 4%) determine the tourist attractiveness of the land.

Almost the entire area of the Warmińsko-Mazurskie voivodship (except for Kisielice gmina[2]) is located within the borders of the Green Lungs of Poland (in Polish *Zielone Płuca Polski*, ZPP).

Warmińsko-Mazurskie voivodship, with the total area of 24,173 km^2 and population amounting to 1 446 915, has one of the lowest population density in the country (59 persons per 1 sq. km; average population density in Poland amounts to 122 persons per sq. km). The voivodship includes 116 gminas (16 urban, 33 urban-rural and 67 rural gminas).

In order to determine the natural conditions important for the development of tourism in the Warmińsko-Mazurskie voivodship, we have conducted spatial analyses aimed at determining the share of naturally valuable areas in the total area of individual gminas.

2 Gmina – administrative region of the 3rd order in Poland (NUTS 5).

It is really very difficult to classify naturally valuable areas due to the lack of precise indicators that would allow us to compare the natural diversity of objects (Dobrzańska, 2005). According to the Act of 16 April 2004 on the protection of nature, naturally valuable areas include protected land. Because of the above-mentioned reason, in this classification we have taken into account only protected areas.

In the research we have considered the legal, national forms of the protection of nature: landscape parks, nature reserves, areas of protected landscape, landscape nature protected complexes, ecological lands and documentation sites (as total). The source data on the forms of the protection of nature was derived from the Central Statistical Office (http://stat.gov.pl/, DoA: 23.05.2014). When we obtained the data, we checked different forms of protection and eliminated the problem of the duplication of areas.

According to the Central Statistical Office, data on the legally protected land does not contain information on the areas comprising Natura 2000. Therefore, we collected data on the Natura 2000 areas as they are a form of the protection of nature within the EU legal system, operating independently of the national system. Spatial data on Natura 2000 areas was obtained in the process of the digitalization of their borders available at the Geoservice of the General Directorate for Environmental Protection (http://geoserwis.gdos.gov.pl/mapy/). Analyzing Natura 2000 areas we considered the fact that the Special Areas of the Protection of Sites (in Polish *Specjalne Obszary Ochrony Siedlisk* - SOOS) and the Areas of Special Protection of Birds (in Polish

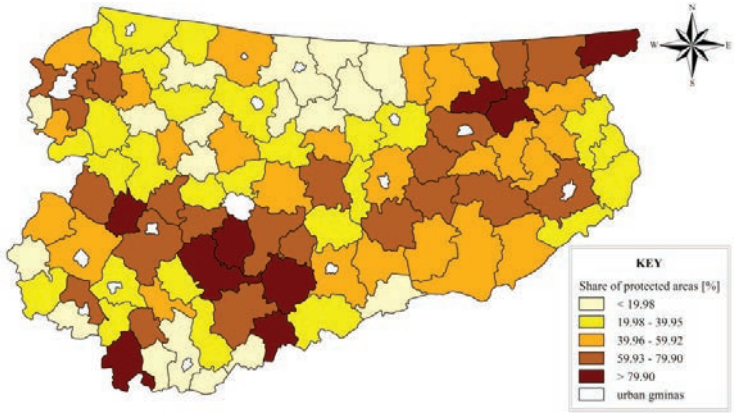

Figure 2. Share of protected areas in the total area of particular municipalities of the Region of Warmia and Mazury.

Source: Own elaboration.

Obszary Specjalnej Ochrony Ptaków -SOO) may be partly the same.

In order to determine the intensity of tourist movement and the level of development of tourist facilities in gminas (NTS 5) of the Warmińsko-Mazurskie voivodship we have prepared spatial analyses including:

- Schneider's indicator – describing the intensity of tourist movement and calculated as the number of tourists using accommodation per 100 permanent inhabitants,
- Baretje's and Defert's indicator – calculated as the number of tourist beds per 100 permanent inhabitants,
- the share of recreational areas in the total area of individual units[3].

Statistical data necessary to calculate first two indicators was derived from the Central Statistical Office. Information on the recreational area was obtained from the register of land and buildings (state as of 1 January 2013) provided by the Regional Center of Geodetic and Cartographic Documentation in Olsztyn.

The results of spatial analysis with regard to the natural conditions determining tourism development, intensity of tourist movement and the level of development of tourist facilities in the gminas of the Warmińsko-Mazurskie voivodship were presented as maps (made by the usage of ArcGIS 10.2.2. software). The final stage included the calculation of linear correlations between natural conditions determining tourism development and the intensity of tourist movement as well as the level of development of tourist facilities in particular gminas. The results were presented in a table. The calculations were conducted by the usage of Statistica software.

3 According to the Regulation of the Ministry of Regional Development and Construction of 29 March 2001 on land and buildings registers: recreational areas comprise land not occupied by buildings belonging to recreational centers; land occupied by children playgrounds; squares; arranged parks; green areas (out of streets); land with historic objects: ruins of castles; fortified settlements; barrows; natural monuments, etc.; sport grounds: stadiums; sports grounds; ski jumps; toboggan runs; shooting ranges; swimming pools; etc., areas performing recreational functions: amusement parks, etc.; zoological and botanical gardens; non-arranged green areas including forests; wooded and bushed land).

Results and Discussion

The spatial distribution of natural determinants of tourism was presented as the share of protected areas (total: landscape parks, nature reserves, areas of protected landscape, landscape nature protected complexes, ecological lands, documentation sites) in the total area of individual gminas (Fig. 2).

The most favorable conditions occur in the gminas located to the south of the capital of the voivodship. It is a relatively compact area including the following gminas: Gietrzwałd, Stawiguda, Olsztynek, Purda, Pasym, Jedwabno, Nidzica, Janowo. The Protected Area of Napiwodzko-Ramucka Forest is located within the area of the gimnas.The area is connected through Gietrzwałd gmina with another group of gminas characterized by high share of protected land. This area consists of the following gminas: Łukta, Ostróda, Miłomłyn, Zalewo. Within its borders there are areas of protected landscape including, among others, the Elbląg Canal, the Tyborskie Forest, the Dylewskie Hills, and the landscape parks of, among others, the Iławskie Lake District and of the Dylewskie Hills. Moreover, there are two units of high natural value, i.e. the group of gminas forming the Land of Great Masurian Lakes and the area of so-called Masuria Garbate including the following gminas: Dubieninki, Gołdap, Banie Mazurskie, Kruklanki, and Pozezdrze. The weakest natural determinants for the development of tourism are noticed in the group of six gminas located in the north of the voivodship: Barciany, Sępopol, Bartoszyce, Korsze, Bisztynek and Kiwity.

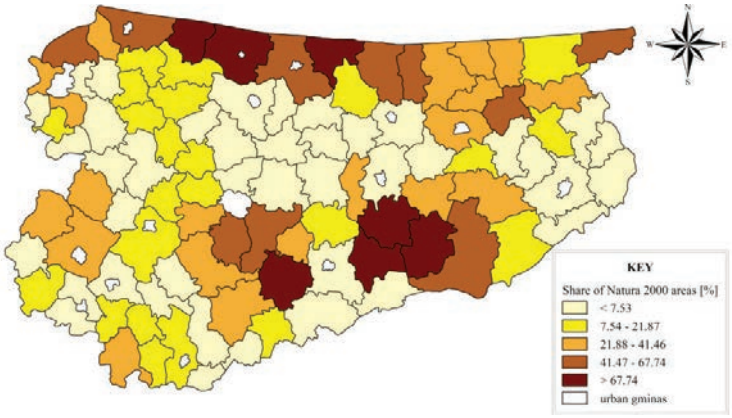

Figure 3. Share of Nature 2000 areas in the total area of gminas of Warmińsko-Mazurskie voivodship.

Source: Own elaboration.

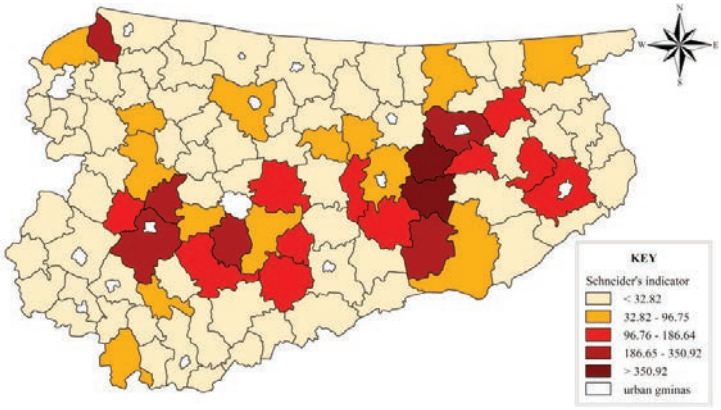

Figure 4. Schneider's indicator in the gminas of Warmińsko-Mazurskie voivodship.

Source: Own elaboration.

In order to complete the research results concerning natural conditions affecting tourism, we have also analyzed the spatial distribution of the Natura 2000 areas in the total area of individual gminas (Fig. 3). We have found that some parts of these areas are also the areas with the most favorable conditions. These are areas located in the south of Olsztyn and the group of gminas forming the Land of Great Masurian Lakes. The whole area of two gminas, Ruciane Nida and Piecki, is included in Natura 2000. A completely different situation has been observed in the gminas located in the north of the voivodship: from Tolkmicko gmina to Srokowo gmina. There is an area of bird protection called the Ostoja Warmińska.

The gminas where Natura 2000 areas have a very small share are located to the north and east-north of the capital and on the eastern border of the voivodship.

The intensity of tourist movement in the individual gminas of the voivodship has been measured by Schneider's indicator (Fig. 4). The highest intensity of tourist movement is observed in the group of nine gminas located in the Land of Great Masurian Lakes: Sorkwity, Mrągowo, Ryn, Giżycko, Pozezdrze, Mikołajki, Piecki, Ruciane Nida, and Pisz. One of the main conditions affecting the tourist movement in this part of the voivodship is natural richness (wooded areas, lakes and microclimate). The second group of gminas (Purda, Pasym, Jedwabno, Stawiguda, Olsztynek, Gietrzwałd, Łukta, Ostróda, Miłomłyn and Barczewo) intensively visited by tourists is located south of Olsztyn. Besides natural values, the tourist attractions in this area include cultural values. Additionally, the areas where the intensity of tourist movement is slightly higher in the voivodships are located near Ełk and in the gminas near the Vistula Lagoon.

The spatial distribution of Baretje's and Defert's indicator presenting tourist function (Fig. 5) is analogous to the results of Schneider's indicator. Gminas with the most developed tourist accommodations are located in the center of historic lands: Warmia (south of Olsztyn) and Masuria (the Land of Great Masurian Lakes). In the remaining part of the voivodship tourist accommodations are not well-developed.

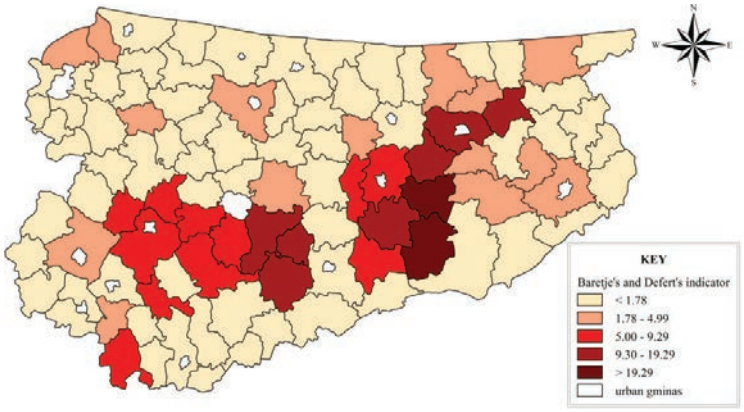

Figure 5. Baretje's and Defert's indicator in the gminas of Warmińsko-Mazurskie voivodship.
Source: Own elaboration.

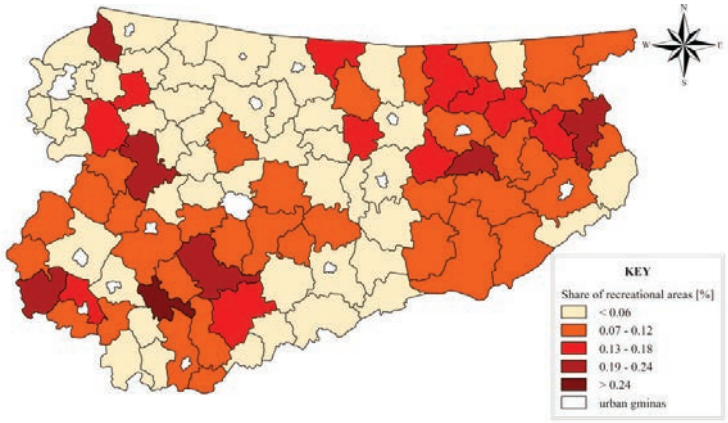

Figure 6. Share of recreational areas in the total area of gminas of Warmińsko-Mazurskie voivodship.
Source: Own elaboration.

Table 1

Linear correlation between analyzed variables

Variable	Statistically relevant values, p < .05000, N = 100		
	Schneider's indicator	Baretje's and Defert's indicator	Share of recreational areas [%]
Share of protected areas [%]	0.33	0.48	0.13
Share of Natura 2000 areas [%]	0.09	0.17	0.08

Source: Own elaboration

The spatial distribution of the intensity of tourist infrastructure is presented as the share of recreational area in total area of individual gminas (Fig. 6). It has been noticed that the most developed recreational area is observed in the north and in the group of gminas located to the south and to the west of the capital of the voivodship. The least favorable conditions for tourism are noted to the north of Olsztyn.

The correlation between natural determinants of tourism and the intensity of tourist movement as well as the level of development of tourist facilities (Schneider's indicator and Baretje's and Defert's indicator) in the gminas of the Warmińsko-Mazurskie voivodship is presented in Table 1. It shows that there is an average relationship between the considered variables. It means that the increase in the share of protected areas is connected with average increase in tourist intensity and tourist facilities. A similar trend is observed in the relationship between the share of protected area and the share of recreational areas but the correlation is stronger.

The values of Person's correlation indicator showing the strength of the relationship between the share of the Natura 2000 areas and the indicators of the intensity of tourist movement as well as the level of development of tourist facilities are very low. The

results of this analysis and comparison cartogram of the share of Nature 2000 areas in the total area of gminas of Warmińsko-Mazurskie voivodship (Fig. 3) with cartograms showing the intensity of tourist movement and the level of development of tourist facilities in gminas of the Warmińsko-Mazurskie voivodship (Fig. 4, Fig. 5, Fig. 6), mean that the Natura 2000 areas located in the voivodship, especially those in the northern part of the region, are not used for tourist purposes too intensively in the analyzed period.

Conclusions

Warmińsko-Mazurskie voivodship is characterized by high values of natural environment. Naturally valuable areas are mainly located in the central part of Warmia (south of Olsztyn) and in the Land of Great Masurian Lakes. In the same areas there is the highest intensity of tourist movement. Moreover, these areas are well-equipped in tourist accommodations and tourist recreational facilities. There is also observed a high level of the use of unique components of ecological space for tourist purposes, especially with regard to terrain, water reservoirs, climate, biodiversity, and landscape.

It is recommended that the study area should be developed according to the principles of sustainable development aimed at achieving balance between social, ecological and economic dimensions. The main determinant of sustainable development in the naturally valuable areas of Warmia and Masuria is adequate distribution of tourist facilities. The optimal concept of management may determine socio-economic activation in accordance with sustainable development. The desired forms of tourist activity in the study area should include the so-called light tourism, such as spa tourism, educational tourism, tourism connected with horse riding, fishing, canoeing, walking, eco-tourism, and agro-tourism.

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ENERGY AGRICULTURE AS AN EXAMPLE OF MULTIFUNCTIONAL DEVELOPMENT OF AGRICULTURE AND RURAL AREAS IN POLAND

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Abstract
The paper aims at analyzing agricultural production for energy generation purposes, including the production of agricultural biogas, as an opportunity for functional diversification of agriculture and for multifunctional rural development in Poland. There have been many changes observed in Polish agriculture. New directions of crop production and of the use of agricultural products have emerged. One of the changes is the increasing significance of the production of agricultural biogas and energy from biogas. There have been built both small-scale and large-scale biogas plants. Most of the agricultural biogas plants are located in northern, western and south-western Poland, i.e. in the areas where there are relatively large farms which can provide a supply of substrates necessary for the production of agricultural biogas. The formation of biogas plants and the use of agricultural production for generating energy, are an eagerly anticipated trend contributing to the dissemination of renewable energy sources, the functional diversification of agriculture, the development of additional economic activities in rural areas, and the increase in the energy security of particular regions and the entire country.
Key words: Poland, diversification of agriculture, multifunctional rural development, agricultural biogas plants.

Introduction

Rural areas and agriculture are integrally connected. Agricultural activity is visible in social, economic, and cultural rural space. The relation between agriculture and rural areas is bidirectional: changes within one cause transformations within the other. Hence, a stable and multifunctional development of rural areas mainly requires the diversification of agriculture and its multifunctional development.

The diversification of agriculture approaches the state in which, except for food production, agriculture plays additional functions, i.e. economic, ecological, social, and cultural (van Ploeg et al., 2000; Adamowicz, 2005; Czarnecki, 2005; van Huylenbroeck et al., 2007; Halamska and Śpiewak, 2008; Kłodziński, 2008; Wilkin, 2008; Fleskens et al., 2009). In order to achieve this goal, it is necessary to extend agricultural activities and diversify the use of farm resources. New economic activities based on agricultural production, including those testifying to the small scale of rural industrialisation (cf. Kłodziński, 2010), contribute to the creation of new jobs and new sources of additional income not only for farmers, but also for all inhabitants of rural areas. T. Marsden and R. Sonnino (2009) argue that multifunctionality in agriculture aims at: (i) increasing revenues, (ii) constructing new agriculture that meets the requirements of the entire population, (iii) using viable rural resources (human, natural, technical, and organizational) in farms or elsewhere. Local governments have an important role to play in such projects because the prerequisite for success, besides initiatives from below, is institutional support. It is really very important in the context of the implementation of economic projects which give an impulse to the creation of new functions. Such actions require the flow of information, access to knowledge and skills to use the knowledge in practice. The latter are mainly determined by appropriate institutions acting on behalf of local governments (Floriańczyk et al., 2012).

An example of non-consumptive use of crop production is its use in the process of energy generation and the formation of energy agriculture. These changes concern not only the agricultural sector, but rural areas in general. The use of agricultural production to generate energy implies successive economic projects connected with both the production and the distribution of achieved energy products such as centers purchasing energy stock and producing briquettes from agricultural waste. Considering the fact that in Poland the most important and most prospective renewable energy sources are wind energy, biomass and biogas, including agricultural, whose use is closely related to agriculture and rural areas (Chodkowska-Miszczuk, 2014), it should be stated that the development of renewable energy is a chance for functional diversification and multifunctional development of rural areas in Poland. A good example here is the use of agricultural production such as organic substrates or crop production for energy purposes, i.e. for the production of biogas in agricultural biogas plants: large ones with installed capacity above 200 kW (average app. 1 MW) and small-scale ones with installed capacity below 200 kW. The formation of agricultural biogas plants is the effect not only of the search for non-consumptive use of agricultural products but also of the diversification of energy sources and the shift towards renewable,

locally available energy sources. The use of agricultural stocks and substrates for the production of agricultural biogas and energy also brings plenty of benefits for the environment (Dyrektywa 2009/28/WE).

The paper aims at analyzing agricultural production for purposes of generating energy, including the production of agricultural biogas, as a chance for a functional diversification of agriculture and for a multifunctional rural development in Poland. The issue has been discussed in the context of changes observed in the energy sector, in agriculture and in rural areas. It should be emphasized that projects realized at the interface of agriculture and power industry create opportunities for the implementation of multifunctional agriculture and non-agricultural functions in rural areas.

Materials and Methods

The following research has been conducted on the basis of data derived from the Agricultural Market Agency (in Polish: *Agencja Rynku Rolnego*, ARR), The Agency for Restructuring and Modernization of Agriculture (in Polish: *Agencja Restrukturyzacji i Modernizacji Rolnictwa*, ARiMR), Energy Regulatory Office (in Polish: *Urząd Regulacji Energetyki*, URE), The Local Data Bank from the Central Statistical Office in Poland (in Polish: *Bank Danych Lokalnych Głównego Urzędu Statystycznego*, BDL GUS) and The European Funds Portal (PFE).

We have considered the years 2005–2013 and 2005–2014. We have employed methods of data aggregation and modification, including mathematical-statistical analyses (regression and correlation), and methods of data presentation (maps, maps with diagrams, charts).

All analyses have been made in relation to the first level of administrative division in Poland – i.e. the level of regions (we have considered 16 voivodships) and to the third level of administrative division in Poland, i.e. in all gminas (57) where agricultural biogas plants were registered as of the end of 2014.

Results and Discussion

Renewable energy sources in electricity generation

In Poland, the role of renewable energy sources in energy production, including electric, is becoming more and more important. In 2012, the share of electricity generated from renewable sources amounted to 4%, in 2013, it increased to 6% (URE, 2015). This trend is more evident when we consider the installed capacity of power plants using renewable energy sources. In the years 2005–2014, there was an over five-fold growth in the total capacity installed in power plants using renewable energy sources. Analyzing the structure of renewable energy sources, it should be emphasized that in the years 2005–2014, the largest (almost 45-fold) growth of installed capacity was noticed in wind plants. At the end of 2014, total installed capacity in wind plants amounted to 63.6% of total installed capacity in all technologies of renewable energy sources in Poland. Moreover, in the analyzed period, a five-fold increase in installed capacity was also observed in biomas plants. The latter, with total installed capacity of over 1 GW, are the second, after wind energy (3.8 GW), power among all renewable energy sources in Poland.

Presently, biogas, including agricultural biogas, is becoming more and more important. In the years 2005–2014, the total installed capacity in biogas plants in Poland increased approximately six times. The growing number of agricultural biogas plants and their growing installed capacity play a crucial role here. In the year 2011, the total installed capacity in agricultural biogas plants accounted for 24%, in 2012 – for more than 26%, at the end of 2014 – for 35% of total installed capacity in all biogas plants in Poland (URE, 2015; ARR, 2014).

Changes in crop production and the development of energy agriculture

Analyzing the changes in Polish agriculture related to the evolution of energy agriculture, it is worth noting that the directions of crop productions have been modified. It is apparent, for example, in the increase of the production of oilseed rape used for energy purposes, which is mainly used for the production of biocomponents and biofuels. In 2006, 66% of the production of oilseed rape was allocated for consumption. In 2009, it was only 40%. As E. Rosiak (2006) notes, this trend has been strenghtening.

In 2013, compared to the state as of 2005, the area of oilseed rape and turnip rape in private farms doubled (according to the Regulation of the European Parliament and Council No. 1166/2008 of 19 November 2008, together with data on the cultivation of oilseed rape, the data on the cultivation of turnip rape is announced – as one variable describing land use). In 2005, half of the national crop of these plants was located in four voivodships: Dolnośląskie, Kujawsko-Pomorskie, Wielkopolskie, and Zachodniopomorskie, and in 2013, in five voivodships: the four mentioned above and in the Lubelskie voivodship (Fig. 1). The increase in energy use of oilseed/turnip rape was, among others, the result of legal regulations and of financial instruments, including direct payment for energy crops in the years 2007–2009. In 2009, the total area of the crop of oilseed/turnip rape accounted for 70% of the total area of all plants (annual and perennial plants) cultivated for energy purposes (Chodkowska-Miszczuk and Szymańska, 2011).

A significant increase has also been noted in the area of maize allocated for silage use as, among

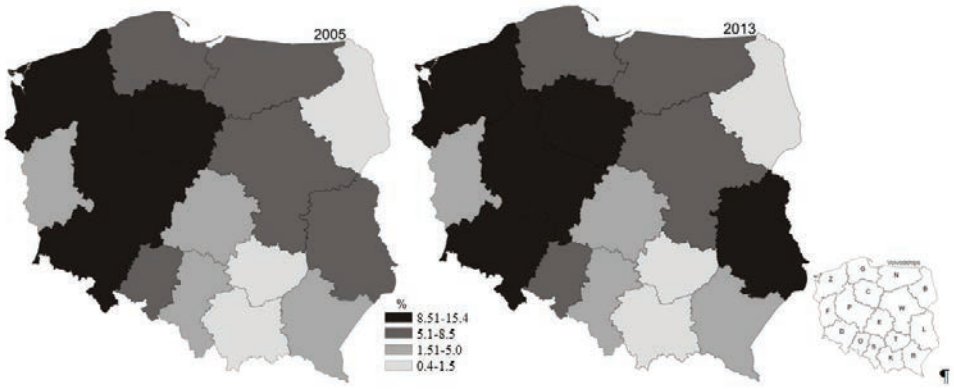

Figure 1. Share (%) of the oilseed rape and turnip rape area in private farms in voivodships in the oilseed rape and turnip rape total area in private farms in Poland in 2005 and 2013; Voivodships: B – Podlaskie; C – Kujawsko-Pomorskie; D – Dolnośląskie; E – Łódzkie; F – Lubuskie; G – Pomorskie; K – Małopolskie; L – Lubelskie; N – Warmińsko-Mazurskie; O – Opolskie; P – Wielkopolskie; R – Podkarpackie; S – Śląskie; T – Świętokrzyskie; W – Mazowieckie; Z – Zachodniopomorskie.

Source: developed by the authors based on the data from BDL GUS.

others, substrate for the production of agricultural biogas. In the years 2005–2013, the area of maize in private farms grew by more than half and, as the results of regression show, this trend will continue (Fig. 2). The increasing importance of this crop is also reflected in the higher number of voivodships which are the main regions producing maize in Poland. In 2005, more than half of national crops was produced by three voivodships: Mazowieckie, Podlaskie and Wielkopolskie. In 2013, a significant share of maize

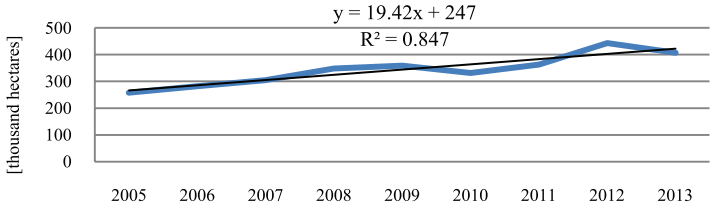

Figure 2. Changes from year to year in the area of maize (for silage) in private farms in Poland with a trend line and a regression equitation.

Source: developed by the authors based on the data collected from BDL GUS.

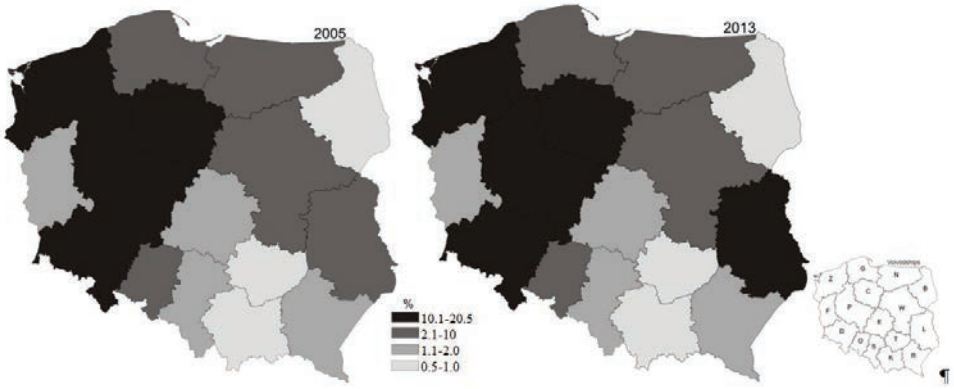

Figure 3. Share (%) of the maize silage area in private farms in voivodships in the maize silage total area in private farms in Poland in 2005 and 2013; Voivodships: B – Podlaskie; C – Kuiawsko-Pomorskie; D – Dolnośląskie; E – Łódzkie; F – Lubuskie; G – Pomorskie; K – Małopolskie; L – Lubelskie; N – Warmińsko-Mazurskie; O – Opolskie; P – Wielkopolskie; R – Podkarpackie; S – Śląskie; T – Świętokrzyskie; W – Mazowieckie; Z – Zachodniopomorskie.

Source: developed by the authors based on the data collected from BDL GUS.

area in its total area in Poland was registered in these three above-mentioned voivodships and in the Kujawsko-Pomorskie voivodship (Fig. 3).

The increasing importance of energy crops is not only the result of available financial incentives. It is also the result of low investing costs connected with the production, processing and energy use of crop production as well as the opportunity to store and convert them to different types of energy substrates (Machan, 2001; Sims et al., 2006; Panoutsou, 2007). A great role is also ascribed to environmental effects, including the possibilities to use agricultural land of lower quality (Scholz and Ellerbrock 2002; Jasiulewicz, 2010).

Agricultural biogas plants

Projects implemented at the interface of agriculture and power industry, including also biogas plants, create new chances for functional diversification of agriculture and dissemination of non-agricultural functions in rural areas. On the one hand, they involve agricultural production; on the other hand, they contribute to undertaking new actions in rural areas and to creating new jobs.

The determinants affecting the market of agricultural biogas in Poland include endogenous factors concerning the agricultural sector, i.e. the directions of agricultural production, mainly crop production, and agrarian structure. Among the most important exogenous factors are legal regulations, including (i) the Directive of the European Parliament and the Council no. 2009/28/WE, (ii) "The directions of development of agricultural biogas plants in Poland in the years 2010–2020" as well as available financial support.

Considering endogenous factors, we should emphasize that the formation of agricultural biogas plants in Poland requires an appropriate supply of substrates – intentional crops and/or wastes of agricultural production from farms. The larger the biogas plants, the larger is the amount of substrates necessary for their functioning. Only relatively large farms are able to provide the optimum amount of stock in some areas. Generally, farms with an average area of 10–15 ha determine the formation of biogas plants having larger installed capacity. Therefore, the higher the share of private farms of 10–15 ha in the total number of farms in the gmina in which the biogas plant works, the higher is the installed capacity in this biogas plant ($r=0.328$, $p<0.500$).

At the end of 2014 in Poland there were 57 large (average installed capacity above 1 MW) agricultural biogas plants. In comparison, in 2011, there were only 22 of them, and at the end of 2012 – 29. The spatial distribution of agricultural biogas plants is not even in the country. They are located mainly in northern, western and south-western Poland (Fig. 4).

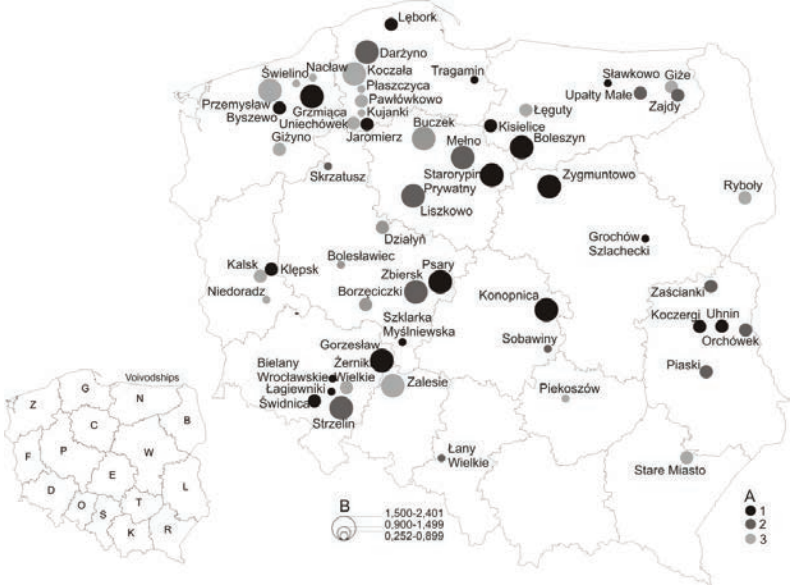

Figure 4. Agricultural biogas plants distribution in Poland, in the end of 2014
Explanation: A – basic feedstock for agricultural biogas production: 1 – liquid manure, 2 – other agricultural and food industry waste, 3 – maize silage; B – total installed electric capacity of agricultural biogas plants [MW]; Voivodships: B – Podlaskie; C – Kuiawsko-Pomorskie; D – Dolnośląskie; E – Łódzkie; F – Lubuskie; G – Pomorskie; K – Małopolskie; L – Lubelskie; N – Warmińsko-Mazurskie; O – Opolskie; P – Wielkopolskie; R – Podkarpackie; S – Śląskie; T – Świętokrzyskie; W – Mazowieckie; Z – Zachodniopomorskie.

Source: developed by the authors based on the data collected from the ARR, 2014.

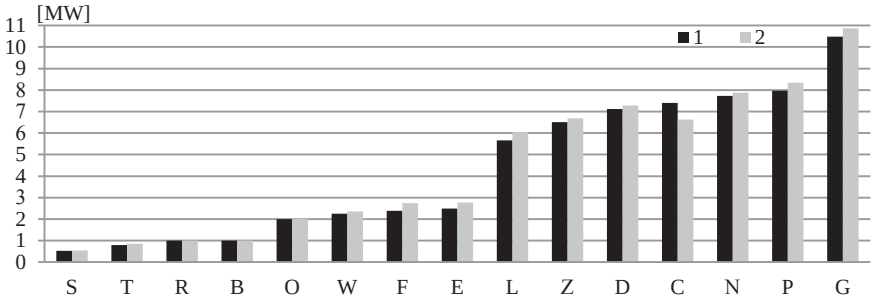

Figure 5. Total installed capacity of agricultural biogas plants in Poland, in September 2014
Explanation: 1 – installed electric capacity, 2 – installed thermal capacity; Voivodships: B – Podlaskie; C – Kuiawsko-Pomorskie; D – Dolnośląskie; E – Łódzkie; F – Lubuskie; G – Pomorskie; K – Małopolskie; L – Lubelskie; N – Warmińsko-Mazurskie; O – Opolskie; P – Wielkopolskie; R – Podkarpackie; S – Śląskie; T – Świętokrzyskie; W – Mazowieckie; Z – Zachodniopomorskie.
Source: developed by the authors based on the data collected from the ARR, 2014.

In Poland, total electric capacity installed in agricultural biogas plants amounts to above 65 MW, almost the same value is reached by thermal capacity (above 67 MW). The largest agricultural biogas plants are noticed in Pomorskie, Wielkopolskie, Warmińsko-Mazurskie, Kujawsko-Pomorskie, and Wielkopolskie voivodships. The total power installed in biogas plants in the above-mentioned voivodships accounts for 60% of the total power installed in all agricultural biogas plants in Poland (Fig. 5).

Considering biogas plants from the point of view of substrates for the production of biogas acquired from agricultural production, we can note the division of all installations into three groups. In the first group, there are biogas plants using liquid manure (basic organic waste for the production of agricultural biogas). In the second group, there are biogas plants based on other than liquid manure waste of agri-food production. In the third group, there are biogas plants whose work depends on intentional crops, mainly silage from maize. Biogas plants using organic waste from agri-food production (liquid manure and others) are mainly located in such voivodships as Zachodniopomorskie, Pomorskie and Wielkopolskie. These voivodships are characterized by outstanding animal husbandry in all agricultural production against the background of the entire country. In central and eastern Poland, there is a domination of biogas plants whose functioning is connected with the supply of maize.

Due to a relatively short history of the production of agricultural biogas in Poland, high investment costs and long and complicated documentation stage, most of the agricultural biogas plants have been built with the participation of external investors (outside the place where the biogas plant is constructed). Among the projects of biogas plants co-financed from EU funds in the programming period 2007–2013 note the dominance of, external firms investing in large (average capacity installed 1 MW) biogas installations in, among others Zachodniopomorskie, Pomorskie, Warmińsko-Mazurskie, and Podlaskie voivodships (PFE, 2015). Agricultural biogas plants often appear next to existing manufacturing facilities specializing in, for example, pig husbandry or food processing, in order to use production waste. In this way in Poland most of the biogas plants in which the production of agricultural biogas is based on organic waste were built.

Small-scale and micro-scale agricultural biogas plants

In Poland, there are investments in small-scale biogas plants with installed capacity up to 200 kW and micro-scale biogas plants with capacity up to 40 kW. The formation of small-scale biogas plants and solar energy follow one of two ways (besides small hydropower) of the development of small-scale renewable energy sources installations in Poland (Chodkowska-Miszczuk, 2014a). The development of the installations, including small-scale agricultural biogas plants, cannot be properly conducted without effective system solutions (Barry and Chapman, 2009). Hence, the formation of small plants, including biogas plants, was included and accepted in the regulation called "The Energy Three Pack" in July 2013. According to the regulation, one of the most crucial legislative instruments was the support for micro-installations (up to 40 kW). Moreover, the owners of micro-installations do not have to have business. The legislature enables the owners to acquire preferential conditions to join the national grid system and to sell the electricity generated in micro-installations (Act on 26 July 2013). It is supposed that further support for prosumer power generation will be reflected in the Act on renewable energy sources which is being created by the Polish Parliament at this moment (http://www.mg.gov.pl/).

The building of a small plant using renewable energy sources, including a biogas plant, is connected with relatively high investment costs, especially from the point of view of individual investors, e.g. farmers. Therefore, it is crucial to obtain external funds. An example of subsidizing small-scale renewable energy sources investments is the system of co-financing agricultural biogas plants from Rural Development, EU Program 2007–2013. The financial support acquired from Rural Development is allocated for building small-scale agricultural biogas plants. Due to an arduous investment process, the projects of biogas plants co-financed from these financial means are currently in the phase of implementation. In Poland, 176 farmers applied for financial support for building micro-scale biogas plants (data from ARiMR, state as of 18.11.2011). Most applications prepared by farmers were from the Wielkopolskie voivodship (every third application). A higher than average number of applications was made also by farmers from Kujawsko-Pomorskie, Lubelskie, and Mazowieckie voivodships.

It seems that the existing financial and legislative instruments supporting the diversification of agriculture and energy use of agricultural production available in the previous EU programming period (2007–2013), as well as those provided in the current programming period (2014–2020) have intensified the development of the market of agricultural biogas in Poland, especially with regard to the formation of small-scale biogas plants. Taking into account the much smaller scale of such a project, with regard to both the financial and substrate requirements, small-scale and mainly micro-scale agricultural biogas plants (with installed capacity up to 40 kW) have a chance to be built in the areas with fragmented agrarian structure, where there is a dominance of small farms of on average 5 ha in eastern and south-eastern Poland, e.g. in Lubelskie, Podkarpackie, Świetokrzyskie, Malopolskie voivodships. Agriculture and rural areas in the above-mentioned voivodships especially require new, non-agricultural directions of development, additional sources of income and new jobs.

Conclusions

In Polish agriculture, there are observed many changes, including new directions of crop production and use of agricultural products. Such elements as the production of agricultural biogas and energy from biogas are becoming more and more significant. Large- and small-scale biogas plants have been built. Most of the agricultural biogas plants have been erected in northern and south-western Poland. Only there are relatively large farms of 10–15 ha which are able to provide an optimal supply of substrates necessary to the production of agricultural biogas, mainly from maize silage, but also from organic waste. In this part of Poland there are being built large (with installed capacity app. 1 MW) agricultural biogas plants. Biogas plants based on waste from agricultural production are formed mainly as investments within existing firms from agri-food industry, as for example biogas plants in Pomorskie, Zachodniopomorskie and Wielkopolskie voivodships.

Due to the fact that a great number of biogas plants, including those with the largest capacity installed, use intentional crops, the significance of crop production for energetic purposes (i.e. of maize or of oilseed rape) is becoming more and more important. These crops play a crucial role in such voivodships as Zachodniopomorskie, Wielkopolskie, Kujawsko-Pomorskie, Dolnośląskie, and Lubelskie in terms of oilseed rape cultivation, and in Wielkopolskie, Kujawsko-Pomorskie, Mazowieckie and Podlaskie in terms of maize cultivation. It is predicted that this trend connected with increasing significance of energy crops (especially the increase in the area of maize crops) will be maintained in the following years.

The development of agricultural biogas plants is reflected in changes occurring in agriculture. New functions have appeared and energy agriculture has been more and more common. Agriculture has access to new production fields, and becomes the basis for actions directly associated with the energy security of the country. In this context, the formation of small-scale installations, especially micro-scale agricultural biogas plants, is important. They provide an opportunity for agricultural diversification and multifunctional rural development in areas where the dominance of small farms is noticed, mainly in eastern and south-eastern Poland. This trend has been eagerly anticipated and it contributes to the dissemination of renewable energy sources, the development of additional economic activities, new jobs connected with investment process, servicing of installations in rural areas, and the growth in the energy security of particular regions and the entire country. It is worth noting that the production of agricultural biogas plants and/or substrates can be significantly based on the current infrastructure and the facilities of farms. An additional advantage is also the opportunity to use the produced energy for the purposes of a farm or for sale (the development of prosumer power generation). Therefore, there is a special need to support legislatively, administratively and financially projects aiming at developing dispersed energy generation, including small-scale biogas installations in Poland.

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THE CONCEPT STUDIES OF RURAL AREAS EXPOSED TO EXTREME WEATHER EVENTS

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Abstract
Each year, natural disasters affect various regions of the world. This is a profound problem, which leads to growing financial and human losses. It is believed that natural catastrophes are caused mainly by greenhouse gases, ozone depletion, deforestation, desertification, urbanization and land use. In rural areas, losses associated with natural disasters can also be exacerbated by local factors. These factors have caused the division of the area of research on subpopulations that showed homogeneous groups of factors. Areas covered by extensive forests, farmland, meadows, marshes and water bodies are more susceptible to financial losses in agriculture than territories with average share of those land features. Spatial attributes that are important determinants of agricultural production, including soil quality, climate, water availability and land relief, do not alleviate the negative consequences of extreme weather events. Spatial planning systems should be developed for managing high-risk areas in a way that minimizes the resulting losses.
Key words: climate change, extreme events, attributes of non-urbanized areas, spatial planning.

Introduction

Natural disasters and other calamities generate massive financial and human losses. Natural disaster is a natural event with catastrophic consequences for living things in the vicinity (Sivakumar et al., 2005). Andreson (1990) defines natural disasters as temporary events triggered by natural hazards that overwhelm local response capacity and seriously affect the social and economic development of a region. Susman et al. (1983) describe disasters as the interface between an extreme physical environment and a valuable human population. Natural disaster is also defined as "a serious disruption of the functioning of society, causing widespread human, material or environmental losses which exceed the capacity of the affected society parts" (Sivakumar et al., 2005). Humans have to learn to cope with natural disasters by adapting to their negative consequences or preventing such events whenever possible. According to the United Nations Office for Disaster Risk Reduction (UNISDR), 357 natural disasters were registered in 2002-2011 (Table 1), leaving behind 124.52 million victims and causing damages worth USD 157.34 billion.

The number of reported disasters in Europe (65) was above the annual average disaster occurrence between 2002 and 2011 (56). This is largely due to cold waves and extreme winter conditions, which affected most European countries in the beginning of the year. The number of such climatological disasters (45) is almost three times superior to its annual average for 2002-2011 (17). Inversely, the year 2012 shows a strong decrease in the number of hydrological disasters (16) compared to the annual average of 23. Such a decrease is still more pronounced for meteorological disasters: only one was reported in 2012 – the lowest number ever reported since the 1990s in comparison with the annual average of 14 in 2002-2011. Therefore,

Table 1

Natural disasters in Europe and global – occurrence and impacts

Type of natural disaster	No. of natural disasters	Global	Victims (millions)	Global	Damages (USD $ bn)	Global
Climatological 2012	45	85	0.45	36.65	4.15	26.63
Average 2002-11	17	59	0.27	102.29	2.76	9.49
Geophysical 2012	3	32	0.03	2.91	15.80	18.62
Average 2002-11	2	36	0.01	8.12	0.53	44.36
Hydrological 2012	16	150	0.10	64.74	4.24	25.61
Average 2002-11	23	197	0.28	117.71	4.73	23.66
Meteorological 2012	1	90	0.00	20.22	0.01	86.48
Average 2002-11	14	102	0.11	39.75	3.64	51.81
Total 2012	65	357	0.58	124.52	24.20	157.34
Average 2002-11	56	394	0.66	267.88	11.60	129.33

Source: own study based on Guha-Sapir et al., 2013.

compared to the previous decade, 2012 appears as an atypical year in Europe, with disasters occurring in proportions distinctly different from those of previous years. In 2012, the decrease in the number of victims (0.58 million) compared to their 2002-2011 annual average (0.66 million) is largely explained by the decrease in the number of victims of meteorological disasters (-99.7% in 2012 compared to the 2002-2011 annual average) and hydrological disasters (-62.2%). In contrast, the number of victims of geophysical disasters increased by 76.6% and climatological disasters – by 68.2% in comparison with the annual average for 2002-2011. In Europe, damages caused by natural disasters in 2012 (USD 24.2 billion) were the highest in the decade at more than twice the annual average for 2002-2011 (USD 11.7 billion). Damages from climatological disasters (USD 4.2 billion) were 1.5-times higher than the annual average for 2002-2011. Damages from hydrological disasters (USD 4.2 billion) were close to their 2002-2011 annual average (USD 4.7 billion) (Guha-Sapir et al., 2013).

Natural disasters and human-caused threats to space and the environment have social and economic impacts. Floods, draughts, landslides, tornados, fires and ground frost can lead to crop failure, loss of safety, property or life, migration and economic losses. The adverse consequences of extreme weather events are accumulating in agriculture and forestry. New standards for communicating information about extreme events and evaluating their consequences should be developed. Advancements in technology and science contribute to the accuracy of disaster forecasts and estimations of probable losses. This vital information can be used to effectively mitigate and alleviate the consequences of extreme weather. According to the UNISDR, disaster risk reduction is "the conceptual framework of elements considered with the possibilities to minimize vulnerabilities and disaster risks throughout a society, to avoid (prevention) or to limit (mitigation and preparedness) the adverse impacts of hazards, within the broad context of sustainable development". Many studies focus on the covariate losses generated by extreme events, including partial or total loss of household assets, loss of income or productivity. In recent years, researchers have also emphasized the linkages between rapid urbanization and disasters (Sanchez-Rodriguez et al., 2005). Urbanization has become the dominant feature of human settlement patterns in the past century. The linkages between rapid urbanization and disasters were sometimes described as reflexive: cities create their own risks by causing degradation of local, regional, and global environments (Hardoy et al., 2001; Sanchez-Rodriguez et al., 2005).

Many studies have explored the financial and economic impacts of extreme events, such as hurricanes, floods, earthquakes, heat waves and wild fires, at the local and regional level (Pielke, 2008; Zhang et al., 2008). This study generally focuses on aggregate impacts of natural disasters, including business interruption costs, infrastructure damage, loss of business structures and productive capital, as well as the available measures for minimizing economic risks, such extended insurance cover, enforcement of construction code standards and development of disaster preparedness plans. Vulnerability is not evenly distributed across society, and some individuals, households or groups are likely to be disproportionately affected by climate change or disasters. This is also relevant to extreme events related to climate change. It should also be noted that the context in which climate extremes and hazards occur is constantly changing as the result of many factors, including rates of economic development, resource exploitation, urbanization, deforestation and land use changes (O'Brien et al., 2008; Kocur-Bera and Dudzińska, 2014b).

A spatial planning system should identify areas characterized by high risk of natural and human-made hazards and should propose land management methods that would minimize damage resulting from extreme events (Olsen and Bindi, 2002; Falloon and Betts, 2010). The main goal of the study is to identify local factors that reinforce the negative effects of extreme events because this is an important part of every risk reduction strategy. The aim of the research was carried out with the help of such tasks as identifying areas where natural disasters have occurred, a description of local and regional conditions, the use of cluster analysis and discussion of analytical results. Historical data concerning natural disasters and similarities in their geographic, geophysical and environmental determinants (Kocur-Bera and Dudzińska, 2014a) can be used to create groups of homogeneous objects with specific features. This approach is adopted to determine a given area's susceptibility to extreme events on local scale and to identify high-risk locations. According to O'Brien (2008), lowland areas, coastal areas and small areas with high population density and high concentration of physical capital are most susceptible to natural disasters.

Groups of objects susceptible to extreme weather events on local scale can be identified, and the resulting knowledge can be used to develop planning documents, introduce adaptive measures (Olsen et al., 2011; Dudzińska et al., 2014) and teach people how to cope in a changed environment. At present, we are unable to forecast the location of extreme weather events with high accuracy. The gap between climate forecasts and local needs calls for effective natural disaster risk reduction measures. There is a great demand for local research into the determinants and

attributes of locations threatened by extreme weather events and climate change.

Materials and Methods

Study Area

The study covered the Region of Warmia and Mazury in Poland. The region spans the distance of 146 km along the north-south axis (1°18′44″) and 240 km along the east-west axis (3°39′28″). Warmia and Mazury has a total area of 24 173.47 km^2 (7.7% of Poland's territory) and the population of 1.45 million. Its population density of 60.06 persons per km^2 is one of the lowest in the country (the national average is 123.24 persons per km²), and its rural population density is estimated at only 25 persons per km². Forests occupy nearly 30% of the region's territory. Farmland spans the area of 1.3 million ha and covers 55% of the region's territory (the national average is 61%). The remaining land-use types in Warmia and Mazury include the land covered by trees and shrubs (32%), water bodies (6%), developed land (3.5%) and other land types (3.5%). Rural areas occupy 2,359.600 hectares, i.e. 97.5% of the region's territory, and have the highest share in the country. Warmia and Mazury comprise 21 counties which are divided into 116 municipalities, including 16 urban municipalities, 33 urban and rural municipalities and 67 rural municipalities. The region is divided into three subregions (NTS 3) of Elbląg (31% of the region's territory and 37% of the region's population), Olsztyn (43% of the region's territory and 43% of the region's population) and Ełk (26% of the region's territory and 20% of the region's population). In Poland and Europe, Warmia and Mazury are renowned for their rich nature and diverse natural features such as varying land relief, lakes, forests and clean air. Nature conservation areas, including areas that are part of pan-European programs (Natura 2000, CORINE), account for half of the region's territory. Warmia and Mazury have an extensive network of water bodies, including numerous lakes, ponds, rivers, canals and a section of the Vistula Lagoon (5.7% of the region's territory) that are popular tourist destinations.

Data Collection and Methods

The study analyzed losses sustained by rural areas in the Region of Warmia and Mazury in 2012 in consequence of extreme weather events. Losses totalling nearly PLN 40 million (EUR 1 = PLN 4.18) were recorded in 37 municipalities. The study was performed in two stages. In the first stage, the occurrence of natural disasters in the evaluated region was determined in a quantitative spatial analysis, and in the second stage, the main focus was on the homogeneity of object groups affected by extreme weather (extracting similar groups). Data were supplied by the Regional Agricultural Advisory Center in Olsztyn, the Central Statistical Office and the Geographical Information System. The assessed parameters were selected based on a review of literature, data availability and the researcher's arbitrary decisions. The selected attributes had to represent land use structure in the analyzed territory, local geographic, climatic and environmental parameters, and losses sustained in agriculture due to extreme weather. Data were subjected to quantitative analysis (first stage) and hierarchical cluster analysis (CA) involving Ward's method to identify groups of objects that were homogeneous in terms of the analyzed attributes (second stage). In the second stage of the study, primary data were normalized. A dendrogram was generated by stepwise clustering of operational taxonomic units. Ward's method for estimating distance between clusters relies on the analysis of variance and minimizes the sum of squares of any two clusters. The adopted method was used to identify subpopulations, which were then subjected to factor analysis.

Results and Discussion

Figure 1 presents the level of damages within the studied area in 2012. The largest damages (50% losses) in rural areas (losses in the urbanised and built up areas were not included) are caused by the negative consequences of wintering (about 17 mln PLN). Sudden ground frosts during the late autumn, winter without snow, strong solar radiation during the day and low temperature at night as well as high air humidity coming from water reservoirs are the main causes for such high losses. Hail is the second most arduous extreme phenomenon (36% losses) that in most cases damages crops by hitting them strongly (about 13 mln PLN). Plants in mature stages of growth are unlikely to recover from physical damage inflicted by hail, their development is arrested before full ripening, and much of the crop goes to waste. The latest extreme events bring losses by torrential rain (6% - about 2 mln PLN), lightning (5% - 1.7 mln PLN), hurricane (0.5 mln PLN) and flood (0.3 mln PLN) (where 1 PLN = 4.3 EURO). Extreme weather events contributed to greatest losses in the central-eastern part of the evaluated region in the municipalities of Budry, Reszel, Węgorzewo, Kętrzyn, Sępopol, Biskupiec Pomorski, Mrągowo, Braniewo, Bartoszyce, Korsze and Bisztynek (see Fig. 3). In the pie chart, 50% are not defined on the right side of the chart.

In the second stage of the study, cluster analysis was performed to identify four groups of objects (subpopulations) with similar spatial (geoinformation) features. The analyzed parameters were: value of losses in 2012 (X1), affected area (X2), value of agricultural losses caused by ground

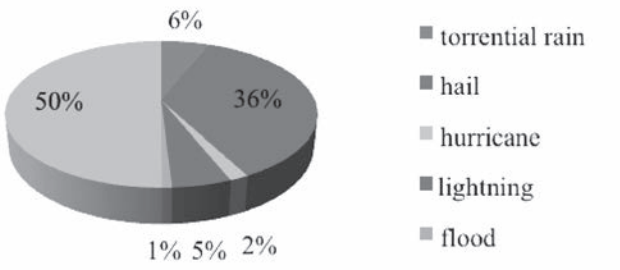

Figure 1. Losses on Warmia and Mazury region in 2012.

Source: own study.

frost (X3), torrential rain (X4), hail (X5), hurricane (X6), lightening strike (X7), flood and submergence (X8), surface area of flowing and stagnant waters in the analyzed municipality (X9), area of swamps and waterlogged soils (X10), area of the analyzed administrative unit (X11), area of farmland (X12), area of grasslands in the municipality (X13), area of land covered by trees, shrubs and forests (X14), soil class and soil complex (X15) established for the purpose of adjustment of agricultural production area in Poland (Witek et al., 1981) [scale 100-points], local climate and crop requirements (X16) established for the purpose of adjustment of agricultural production area in Poland (Witek et al., 1981) [scale 16-points], terrain parameters based on diversity of land relief and farming conditions (X17) established for the purpose of adjustment of agricultural production area in Poland (Witek et al., 1981) [scale 5-points], water availability based on soil moisture levels (X18) [scale 5-points]. Data were standardized. Homogeneous object groups were presented in the form of a dendrogram in Figure 2. Four homogeneous subpopulations were created at cut-off value of 11.2.

The first subpopulation covers observations C_1, C_5 and C_10, the second subpopulation – observations C_2, C_11, C_4, C_7, C_17, C_14, C_16, C_15, C_20, C_21, C_22, C_9, the third subpopulation – observations C_28, C_26, C_30, C_29, C_27, C_25, C_13, C_32, C_12, C_8, C_18, C_6, and the fourth subpopulation – observations C_33, C_34, C_35, C_24, C_37, C_36, C_23, C_19, C_31, C_3. The number, name and average value of diagnostic attribute in a group are given in Table 2.

The greatest losses (722 020 PLN) were noted in subpopulation 4 comprising the municipalities of Bartoszyce, Barciany, Prostki, Węgorzewo, Zalewo, Lidzbark Warmiński, Dobre Miasto, Ostróda, Kalinowo, Mrągowo, Pasłęk and Kętrzyn. Subpopulation 4 was also characterized by the highest values of the following parameters: area of

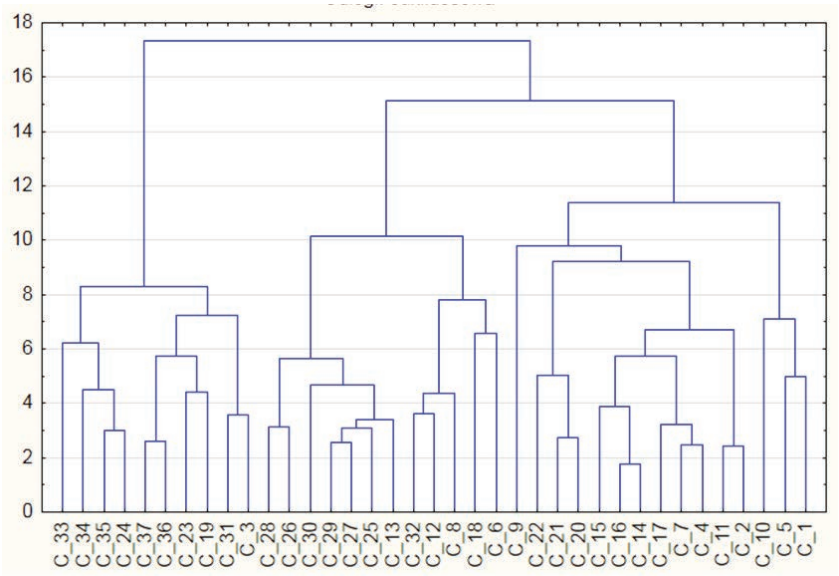

Figure 2. Dendrogram of groups (subpopulations) homogeneous in terms of spatial attributes and losses sustained by rural areas in 2012.

Source: own elaboration in *Statistica* application.

Table 2

Average values of diagnostic attributes in a given subpopulation

Name of the commune	Group characteristics																	
	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14	X15	X16	X17	X18
SUBPOPULATION 1 BRANIEWO (C_1) KORSZE(C_5) BISZTYNEK(C_10)	490.620	2.93	1.0	0.33	0.66	0.33	0.7	0	218.0	606.0	25.325	17.770	3.734	4.543	64.66	9.0	4.16	4.23
SUBPOPULATION 2 RYCHLIKI (C_2) SĘPOPOL (C_11) SROKOWO (C_4) BUDRY (C_7) MARKUSY (C_17) RESZEL (C_14) BISKUPIEC (C_16) KISIELICE (C_15) WYDMINY (C_20) KOLNO (C_21) WILCZĘTA (C_22) GODKOWO (C_9)	693.200	0.55	0.75	0.25	0.33	0.08	0.1	0.1	495.0	827.0	18.099	12.055	3.505	4.032	58.66	8.33	3.59	3.86
SUBPOPULATION 3 BANIE MAZURSKIE (C_3) JANOWIEC KOSC. (C_19) SORKWITY (C_23) GRODZICZNO (C_24) OLECKO (C_31) PIECKI (C_33) ŚWIĘTAJNO (C_34) JONKOWO (C_35) KURZĘTNIK (C_36) DŹWIERZUTY (C_37)	139.200	0.05	0.1	0.2	0.2	0.5	0.4	0	1.005	966.0	19.259	1.1431	3.403	5.230	44.83	7.62	3.15	2.84
SUBPOPULATION 4 BARTOSZYCE (C_6) BARCIANY (C_8) PROSTKI (C_12) WĘGORZEWO (C_18) ZALEWO (C_25) L.WARMIŃSKI (C_26) DOBRE MIASTO (C_27) OSTRÓDA (C_28) KALINOWO (_29) MRĄGOWO (C_30) PASŁĘK (C_32) KĘTRZYN (C_13)	722.020	0.53	0.41	0.08	0.58	0.25	0.1	0	1.734	1.343	30.453	19.631	6.104	6.850	58.11	8.18	3.23	3.82

Source: own elaboration.

municipality (30,453.0 ha), flowing and standing waters (1,743.0 ha), swamps (1,343.0 ha). farmland (19,631.0 ha), meadows and pastures (6,104.0 ha) and land covered by trees, shrubs and forests (6,850.0 ha). The remaining attributes were characterized by average values.

The highest number of extreme weather events (torrential rain, ground frost, hail and lightening) was reported in subpopulation 1 covering the municipalities of Braniewo, Korsze and Bisztynek. Subpopulation 1 was characterized by the highest soil quality index of 64.44 (which represents class III and IV arable land and grassland), the highest climate index of 9 (on a 16-point scale for Poland), the highest terrain index of 4.16 (on a scale where flat land with no obstacles for mechanical cultivation scores 10 points. and land with the most diverse relief – 0 points) and the highest water availability index of 4.23 in the studied population (on a scale where soils with optimal moisture levels score 1 point and permanently dry soils – 5 points). The remaining two subpopulations were not characterized by outstanding land features or excessive losses resulting from natural disasters in rural areas. The spatial distribution of municipalities that suffered losses in consequence of adverse weather effects in 2012 is shown in Figure 3.

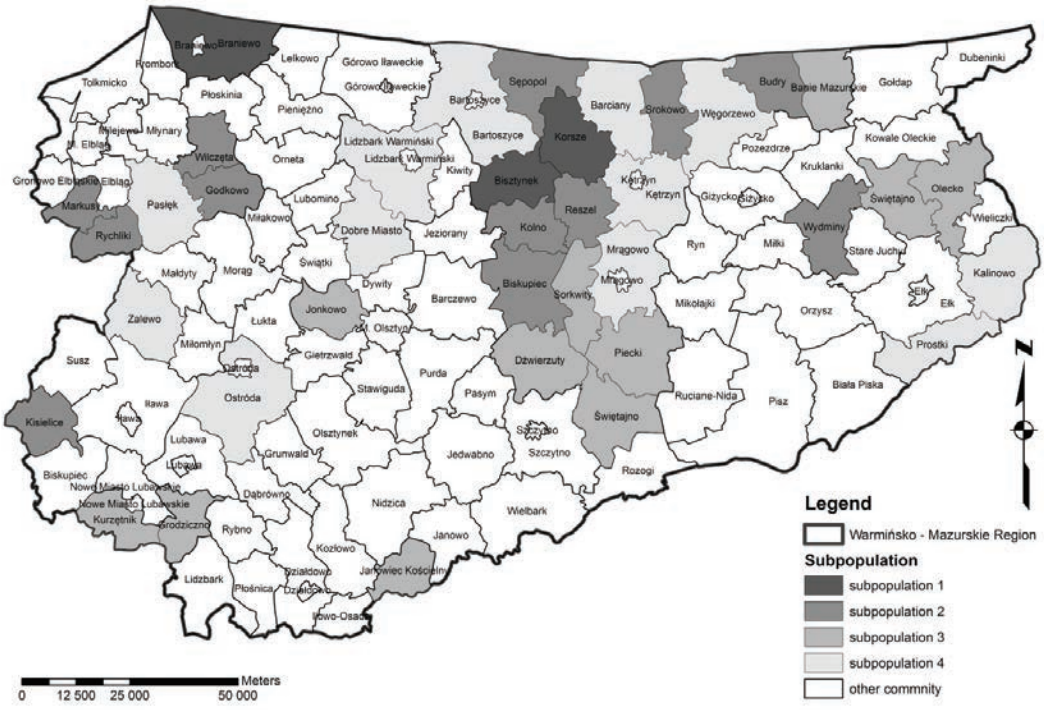

Figure 3. Spatial distribution of homogeneous subpopulations.

Source: own elaboration.

Conclusions

Natural processes and human activities are responsible for climate change and extreme weather events that generate vast financial and human losses (O'Brien et al., 2007). Natural disasters are increasingly often observed in Europe. They are caused mainly by greenhouse gas emissions, ozone depletion, deforestation, desertification, urbanization, land use and various local factors. The main purpose of research was to identify local factors that reinforce the negative effects of extreme events. In this study historic data were used to identify homogeneous objects that had been affected by extreme weather events. The study was performed in 37 rural municipalities in the Region of Warmia and Mazury, which suffered financial losses in 2012 in consequence of natural disasters. A total of 18 diagnostic parameters associated with adverse weather events were described. Four homogeneous subpopulations were identified in cluster analyses. Two groups clearly stood out in the analyzed population. The first subpopulation was characterized by the highest losses and the highest values of the following parameters: area of municipality, area of flowing and standing waters, area of swamps, area of farmland, area of meadows and pastures, area of land covered by trees, shrubs and forests. The remaining attributes had average values. This subpopulation covered the municipalities of Bartoszyce, Barciany, Prostki, Węgorzewo, Zalewo, Lidzbark Warmiński, Dobre Miasto, Ostróda. Kalinowo, Mrągowo, Pasłęk and Kętrzyn. The second outstanding population was characterized by optimal conditions for crop cultivation, including soil quality, climate, land relief and soil moisture and it comprised the municipalities of Braniewo, Korsze and Bisztynek. The identification of local conditions contributes to the reliability of extreme weather forecasts and supports the implementation of effective measures for preventing and alleviating the adverse consequences of natural disasters. Spatial planning systems should be developed for managing high-risk rural areas in a way that minimizes the resulting losses.

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HARMONY IN INDOOR/OUTDOOR CONTEXT OF ARCHITECTURE

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Abstract
Intensity of indoor/outdoor dialogue in the context of architectural history has rapidly evolved due to technological progress while the quality of environment expressed as harmony has proved to be of coincidental character. Reality reveals a pressing need to restore integrity, wholeness and harmony of environment, at the same time searching for a balance in the dialogue between interior and exterior space and a way to harmony in this complicated system. By analysing previous successful and unsuccessful examples in the architecture of publicly most sensitive objects in the re-established Latvia, one obtained a pyramid of subordinated guidelines to be used in architectural theory and practice in support of creating a harmonious architectural environment. The subject is of current importance not only among experts but in all levels of society: we all are users of this environment. In order to create a harmonious high-quality environment in the future, at first, one has to invest substantial resources in all levels of education; it will become a reflection of inner conflicts of a new concept of space, continue historical process of architectural development and introduce diverse approaches to harmonise indoor/outdoor space in a scientifically grounded way.
Key words: indoor/outdoor harmony, indoors/outdoors, interior/exterior space, architecture, glass panels, glass systems.

Introduction

The modern tendency to merge indoor and outdoor space shows a pressing need to restore integrity, wholeness and harmony of environment. Up to now it has been a spontaneous coincidental process, and a need has arisen for a scientifically grounded mechanism to regulate it, along with artistic tasks in planning harmonious spatial structures. Development of new well-grounded principles for a harmonious linking of indoor and outdoor space, taking into account recent trends in this area, has become a central task and an issue for research (Большакова, 2013). 'High-standard living environment in attractive surroundings is one of the main conditions for city development and labour force attraction. Municipalities want to offer attractive residential territories thus securing their main municipal budget resource, i.e. income tax (Treija, 2006)'. Tourism, with its commercial infrastructure and profit coming from high environmental quality, is an important factor.

A harmonious environment consists of interaction between harmony and disharmony (Гликин, 1979); its wide spectre encompasses parallels of material and spiritual aspects (Jencs, 2003, 2010, 2013). The issues addressed by the present study comprise a limitless amount of subjective and objective factors. Previous level of study on architectural conditions, criteria and aspects of indoor/outdoor harmony does not provide sufficient knowledge of the subject; this problem has previously not been a primary focus. Communication of building architecture, interiors and landscape architecture, as a harmony of indoor/outdoor dialogue, has been studied in a fragmentary way, and the studies lack a unifying core in the meaning and scope of real situation in life and architecture where intensive application of glass systems may be observed. The model unifying the form and function in architectural systems created by J. Briņķis and O. Buka (Briņķis and Buka, 2006) today could be supplemented by another essential element, i.e. interior space, or indoors. Theoretical basis of certain aspects in environmental design consists of an extremely long array of studies such as:

- Systems of universal proportions – inspiration of natural harmony in bionics and possibilities it offers for creating harmonious spaces (Kimberly, 2001; Kundziņš, 2008; Neufert, 1970; Гликин, 1979);
- Application of colour harmony and principles in environmental design (Godjevac, 2010; Kundziņš, 2008);
- Harmony in synthesis of architecture and art (Alle, 2013; Spārītis, 2013; Strautmanis, 1982; Гликин, 1979; Швидковский, 1984);
- Harmony in space (Neufert, 1970; Strautmanis, 1977);
- Techniques of harmony in architecture (Jencs, 2003, 2010, 2013; Neuferts, 1970; Zigmunde, 2010a; Гликин, 1979);
- Aspects of harmony in urban landscape (Briņķis and Buka, 2006; Briņķis, 2007; Briņķis and Buka, 2008; Īle, 2010; Ziemeļniece, 2012; Zigmunde, 2010b; Гликин, 1979).

Indoor/outdoor harmony and its future development possibilities anticipate an analysis of endless matrixes made of multiple components to obtain results with maximum precision, taking into account the factor of infinity and specifics of outdoors, nowadays accumulated by indoors - both spaces freely interflowing into each other. In order to adjust the key of harmony to indoor/outdoor dialogue in a possibly

best way – which is the main objective of the present study – the following tasks were put forward:

1. Research of how the dialogue of spiritual and material indoor/outdoor dialogue has evolved in architectural history; chronological summary of main facts with conclusions in regard to present situation and global trends;
2. Summary of conclusions in regard to indoor/outdoor harmony obtained after inspection of objects in nature;
3. Development of conclusions on conditions of indoor/outdoor harmony and its development possibilities.

In order to carry out a full-fledged study one must analyse both objective (material and spiritual) and subjective (emotional and psychological) factors opening way to an endless diversity of opinions from experts and users. A justified question arises: is it possible at all to do such work, taking into account multifariousness, inconstancy and instability against time spans as well as dynamic importance of public space (Geldofs, 2009)? In order to fulfill this task, more realistic limits in time and space have been set, i.e. the last 25 years in the territory of present-day Latvia. From 2013 to 2014 the most sensitive environmental objects and functionally different object groups in the territory of Latvia were publicly inspected and results published in reviewed scientific publications (Balode and Grietēna, 2014; Grietēna, 2013; Grietēna, 2014a; Grietēna, 2014b). After summarising conclusions from theoretical and empirical research, the aim of the study was to come as close as possible to the conditions of harmonious indoors/outdoors serving as a basis for take-off in developing scientifically grounded methods for the advancement of indoor/outdoor harmony in the future.

Materials and Methods

The heavenly dimension of art is embodied in material expressions, especially architecture. One might say that architecture lies between heavens and the earth. Therefore, in order to create a unified typology of architecture, interior and landscape, one must consider links comprising the system in two main directions: historical stages of spiritual experience in architecture and three basic levels of the process: thinking in shape-building terms – ideal level of composition; shape-building in mind; and design stages where an idea evolves into a project (Власов, 2015). In the present study, theoretical and empirical methods were applied employing the comparative-theoretical method as a main one in described stage. The latter methods expressed itself as informative and archive-making, analysing the evolution of material and spiritual indoor/outdoor dialogue in world's architectural history (task 1). In previous stages of the study, the results of which comprise the basis of the present study, theoretical and empirical methods were applied: photoanalysis, inspection of objects in nature in various seasons and various times of the day, and sociological surveys. Material chosen for the study was 15 publicly sensitive buildings and 3 of their groups built in the territory of Latvia from 1991 to 2014; one analysed the dialogue of building design, landscape and interiors (composition, coloristic, proportions – massiveness, level of filigree, glass panel areas, level of emotionality), harmony in mutual interaction of architecture and interiors (task 2).

Application of comparative method in summarising information for the study:

1. Principles of glass system application in architecture:

– Composition of glass panel areas in space and their proportion – massiveness in relation to parts without glass;
– Compositional application of colours and chiaroscuro under insolation impact;
– Research of visual accents created by chiaroscuro play;
– Assessment of semantic correspondence of indoors/outdoors and level of emotionality according to functional programme of respective space.

2. Summary of studies on the impact of mutual compositional build of indoors/outdoors:

– Architectural shape-building, glass systems and landscape around objects as the main criteria in the search for harmony between indoors and outdoors, compliance with a supreme spiritual task following the function of spaces.
– Assessment of indoor/outdoor harmony – summary of opinions by experts and other respondents on correspondence of indoor/outdoor dialogue to the supreme task of an object under consideration.

In analytical generalisation stage, with the help of inductive cognition method, the priority factors for an assessment of visual aesthetic quality of indoors/outdoors were determined by inspecting architectural objects in Latvia and trying to come closer to the general principles of defining the main criteria for the present study on indoor/outdoor harmony (task 3).

Results and Discussion

Evolution of indoor/outdoor dialogue in architectural history

At the start of humanity, seclusion of indoor space, independently of its form, was a characteristic basic feature of homes embodying the initial task of living space or area – to protect and guarantee continuation of human race. Accentuation tended to proceed from outdoor space to indoor area. Sunlight coming from

outdoors threw light into the dark caves. Entrance into homes or place of junction with outdoors, was masked due to safety reasons. During human evolution, house building expanded, yet seclusion was still important although homes became externally more visible. Living houses in Pompeii is a typical example of a secluded living area where living space was separated from outdoors and linked to it by passages leading from vestibule to an exit shaft (Джуха, 2000).

Architecture of Byzantium and European renaissance (castles and dwelling houses) was characterised by a centralised structure. The principle of moving from outdoors to indoors remained but was already expressed in accentuation of entrances, continuity of borders and development towards the centre. An outstanding example of a centralised structure from late renaissance period is Villa Rotonda built by the architect Palladio (1508-1580); classicism ideals in an ideal architecture embodied by the villa interacted with a contrasting adjacent landscape. A perfect architecture in natural surroundings underlined architectural performance in contrast to a seemingly arbitrary landscape and became an example of an ideal architecture in the particular period (Вавилина, 2000).

Baroque introduced new thinking: indoors (divisions of interior space) started reacting to outdoors. Relation of interior and exterior space obtained plastic expressions. Nevertheless, indoor/outdoor dialogue retained continuity of material borders and isolatedness up to 20th century.

Up to the end of the 18th century metal was used in building for producing small decorative elements (bars, dividing elements, fastening details like nails and ties). After inventing coke-melting oven in England metal's quality improved; it became cheaper and opened new possibilities for its use in building. At the same time, glass production technologies were improved and glass and its products became cheaper as well. Englishman J.Paxton made the famous Crystal Palace in London's Hyde Park and deeply moved the world of architects. Already in the second half of the 19th century glass and metal constructions, and glass cupolas as roofs had become very common, especially in England, and became an integral part of Victorian era. 19th century architects and designers tried to use the new opportunities to the maximum: compression members (supports, columns, arches) were made of cast iron, i.e. ferrous alloys and widely employed. Comparing to metal which contained very little carbon, cast iron is more durable in compressive stress. On the other hand, metal works better in cases of tensile and flexural stress thus making a good material for beams and ties. However, the new combination of metal and glass revealed serious drawbacks: metal's high thermal conductivity cooled interior space in winter while in summer it got overheated due to the transparency of glass. The only method to tackle the problem was airing of rooms. It was the time when the first railway station pergolas built in glass and metal appeared winning people's hearts and minds. As a result, such pergolas were built in vast amounts. Exhibition and market pavilions built in glass and metal and easy to mount and demount turned out to be excellent companions of the general trend. This era is well characterised by the controversial Eiffel Tower in Paris built in honour of Exposition Universelle of 1889. It was the time when one of the first suspended facades was made for a German shoe factory designed by W.Gropius. Suspended glass façade panels considered as an early prototype of modern façade panels started a new trend called constructivism. In America Mies van der Rohe led the work of finding new ways for applying glass for administrative needs in skyscraper construction. The optimal skyscraper construction system intended to have carcass structure where, instead of bearing walls, one inserted compression columns supported by a reinforced concrete core penetrating the whole building's height. Bearing brick walls in buildings of such construction lost sense. The spirit of the age found a necessity to rehabilitate itself in the nature, and modern achievements offered an excellent opportunity to satisfy this need. Nature, due to glass panels in walls, became a part of interior, i.e. outdoors entered indoors. This concept was most skilfully embodied in the artwork of L.Mies van der Rohe and F.L.Wright (Новиков, 2000).

20th century brought radical change and new accents in indoor/outdoor communication: a new era in environment design started linking indoors and outdoors into a single whole. According to the opinion of architectural historian Z.Gideon, the essence of the 20th century architecture was rooted in its multifacetedness and endless diversity of inner interrelationships. A new interior design age started, led by the main principle – from indoors to outdoors. Centralised plans in buildings were replaced by open ones. Architectural objects and their interiors gradually turned from frozen organisms closed to outdoors into open, pulsating and kinetic objects related to outdoors. An example of a radical change of the mentioned approach is the creative work of the American architect F.L.Wright. He accentuated and secured an irrefutable link between indoors and outdoors materialising it in his architecture which was free from choking and oppressive boxes and, through its glass panels, widely linked to picturesque natural landscapes. Interior plans of buildings, too, obtained freedom and were pulsating into each other as live organisms. Common interior space divided by zoning (i.e. lightweight interior design forms) contained organs necessary for a live organism, with different

functions subordinated to a single whole. At first Wright broke walls with the help of corner windows, and light from outdoors streamed into indoor spaces where it had never fallen before.

Fireplace, bed and table in room centre, instead of former stationary division walls, organised interior space with their function. Indoors were transparent and linked to outdoors; divisions were employed to the minimum. Creative work of the time by the famous Le Corbusier, too, was characteristic with two accents: structural modifications of interior space striving to reach spatial unity and integrity, and merging of indoors with outdoors. Confirmation of a free room plan came with the architecture of Mies van der Rohe, however, the universal suburban glass living house, i.e. aquarium in the USA already seemed exaggerated and unfit for comfortable living.

Creative work by the Brazilian architect O.Niemeyer, too, is characterised by the replacement of interior division walls with lightweight panels thus merging borders of interior decoration. Idea of maximum openness did not bring the expected results but exposed a number of issues related to it. As a result, architecture, after having run another circle of evolution in the spiral of dialectics, started returning to proven classic values such as fundamental walls and divisions enclosing confined rooms (Курбатов, 2000). At the end of the 20th century one came to a conclusion that in order to create high-standard environment one cannot be led by a principle of moving from indoors towards outdoors only; one should also be guided by the reverse link - from outdoors to indoors. This verity became widely visible in private housing architecture of the 20th century's end. In parallel to indoor/outdoor communication thoroughly shaken in the world's stormy architectural tendencies a serene life has been led by the pragmatic countryside building crafts which have been successfully combining human interior needs with outdoors at all times (Кузнецова, 2000). It is proved, e.g. by traditional Japanese living house architecture which, in case of need, is a shelter from natural elements and, due to its lightweight and mobile divisions can be quickly transformed both from inside and outside thus joining indoors and outdoors. In most cases there are two stationary walls, the rest of them are convertible and made of wood, glass or paper. Garden at a Japanese house is designed as its continuation and after sliding the division, interior space physically and spiritually joins with exterior one (Лунц, 2000).

Another turn of centuries is over, and due to architectural heritage of the last 40 years there has been a new start of a new situation characterised not by graduate change of styles in a logical sequence, not with one particular trend like it was in former historical periods of architecture, but a previously unseen stylistic diversity. Styles are developing in parallel to each other, having mutual impact on each other, fighting, being interpreted and improving. Spatial volumes of architecture develop in geometrical progression always creating new fantastic form mutations, especially underlining multifarious applications of glass systems thanks to new technologies and scientific discoveries in bionics (life+technology). The notion of borders in architecture has transformed and obtained new dimensions – like a new organism it plastically pulsates from outdoors into indoors and the other way round, depending on objective and subjective factors (Лихачева, 2000). Minimalism under the guidance of T.Ando brought the idea of restoring links between artificially and naturally created environment: exterior abstraction, interior representability, clarity, purity, laconism, asceticism, and transparency of concept. Hi-tech, on its part, with its industrial technological focus lays claim to Guinness records in architecture – glass panels in the area of several thousand square metres, fastened in openwork metal structures in all dimensions in a shape-building diversity on a previously unseen scale. One of examples of the creative work by architect Henry Foster, airport terminal in Hong Kong, brilliantly and graphically characterises the new possibilities. Innovation, utopianism, biotechnological modelling, dynamism, transformability and dematerialised walls of warm air separating indoors from outdoors – this is the contribution of this style in the architecture of indoor/outdoor dialogue.

While the world trends develop by leaps, national building craft exists in Japan, China, Bali islands, Maldives, Russia, Latvia and many other parts of the world and with good reason; taking into account climatic specifics of every region it provides for a harmonious environment having been developed and accepted by society in long term in hundreds and thousands of years.

Summary of conclusions on indoor/outdoor harmony in objects inspected in nature

Results obtained in researching education institution architecture confirmed a previous hypothesis that the development of indoor/outdoor harmony in the context of 20th-21st century technologies creates a new architectural scenery. Latvian architecture presents excellent examples of an environment serving for the needs of unprotected members of society such as children, e.g. linking of nature and interior space in recreational areas such as in the new extension of Jelgava Secondary School No. 4 designed by architect A.Ziemeļniece with façade made of glass panels and curved towards outdoors thus integrating nature into interior space in an intensive and propitious way. In the new Latvian Academy of Arts' extension designed by A.Sīlis (SZK&Partners),

an obvious contrast to the historic volume, the linkage with outdoors, or the sky, through proportions of glass areas in windows in relation to parts with no glass, has obtained a value of a finished, framed artwork. A widely popular and favourite technique is an entrance motif designed in voluminous glass panels: it emotionally softens borders, creates gradualness and overcomes interspatial borders. This principle has materialised in many new education institutions, e.g. International School in Piņķi designed by architect D.Zalāne (Grietēna, 2013) as well as in architecture of many shopping and sports centres and administrative buildings. Also, in healthcare institutions the linking of nature and specially designed rehabilitation gardens with interiors has become a key to indoor/outdoor harmony accepted on international level; this niche holds an important potential for development in Latvia (Balode, 2014).

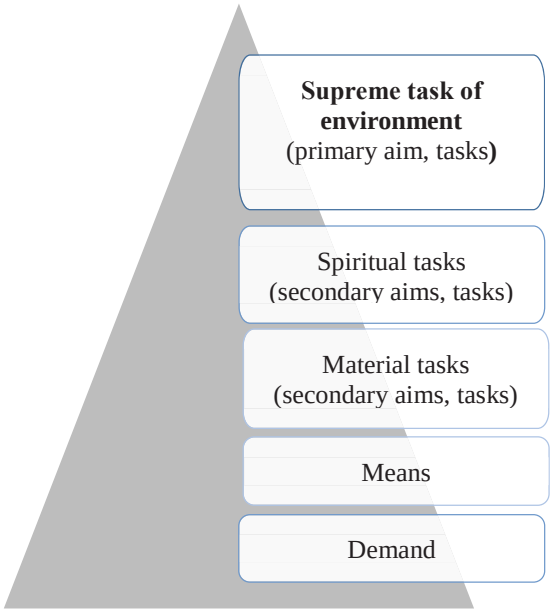
A successful example of a harmonious linking of indoors and outdoors for rehabilitation needs is Brukna Manor complex which, after the 21st century transformation of buildings and landscape, has regained its original aesthetic and architectural quality, and harmoniously states its identity through spiritual dimensions, mental feelings and attitudes. Brukna Manor complex is a harmonious environment for a rehabilitation centre combining values of both material and spiritual harmony. Brukna Manor house, in spite of the changed function, has been renovated according to the values of classicism and may be considered a standard of harmony. Building's new interiors form a unique synthesis of antique heritage and modern art. Environment there exists in a state of continuous transformation like a live organism and is created for living, not for the strict needs of museum. Manor's classic building and interiors are finely supplemented by a garden designed in style of Italian renaissance; buildings of the 21st century render it stylistically diverse. However, the core of the complex, i.e. manor house, interiors and garden, has a strong uniting factor, i.e. central symmetry axes of material and spiritual character that helped preserve harmony in spite of challenges that were posed by functional transformation. The spiritual vertical symmetry axis is expressed through a human one: since the very start of Brukna Manor restoration all works were successfully conducted by dean A.Mediņš. The vertical, as a central symmetry axis for spiritual dimension, is an irreplaceable frame of reference in creating a harmonious environment (Balode and Grietēna, 2014).

Most recent heritage of sacral architecture in Latvia is widely represented in the form of new modern Catholic churches where in a harmonious indoor/outdoor dialogue the main importance should be attached to integration of the idea of transcendence. Compositional application of colours and chiaroscuro under the impact of insolation as well as visual accents created by chiaroscuro play have to underline altarpiece in a space by establishing necessary balance and arranging priorities in the right order. Aesthetic harmony or a picturesque landscape perceived through a glass panel only has a subordinate meaning. For example, in Holy Trinity Roman Catholic Church in Rīga designed by architect U.Šēnbergs, light falls in through asymmetric windows arranged high in walls and accentuated altarpiece which not only organises space opening the view to the sky, but also, in balanced portions, opens interior sacral space for communication with urban outdoors. There are a lot of examples of harmonious indoor/outdoor communication in Catholic church architecture of Latvia: Dobele Roman Catholic Holy Trinity Church, architects J.Kukša and I.Kārkliņš; Saldus Roman Catholic Church, architect A.Andersons; St.Dominique Roman Catholic Church in Liepāja old town and others. Māra Church in Liepāja, architect A.Skujiņa, and St.Meinard's Church and catholic parish centre in Liepāja, architects A.Hupfauf (Germany) (central raised volume) and AKA bureau with A.Kokins, A.Kokina (radial enclosing external part), with their ideological contribution to harmonious indoor/outdoor communication, hold a potential for technical improvements in the future. Analysing a number of sacral buildings within the present study, it was concluded that for maximum result a synthesis of approaches should be employed; a wrong application of light in organising space in sacral buildings sooner or later leads to additional measures and material expenses. Therefore advanced knowledge in creating indoor/outdoor harmony would not only raise the quality of architectural environment, but also help in reaching a higher level of environment design in a more economic and purposeful way (Grietēna, 2014b).

The new building of Latvian National Library (LNB) designed by architect G.Birkerts can be considered as a bright and refined sample of indoor/outdoor harmony in architecture, a successful result of interdisciplinary cooperation of environment makers. The building presents an encounter of noble aims defined twenty five years and respective results of the present day; they have stood the test of time and must be recognised as timeless. Conclusions reached in previous studies on priorities regarding interspatial harmony in other functionally different objects are supported by studies of indoor/outdoor harmony in relation to the new LNB building. In order to come possibly closer to indoor/outdoor harmony in environmental design, upon starting design development for a building one needs long-term thinking and definition of a primary aim, in other words, a supreme task followed by tasks for reaching

it, with subordinated secondary aims and tasks derived from available means and demand. Subordination has the deciding role in reaching harmony (Grietēna, 2014a).

Indoor/outdoor harmony and possibilities for its development today



Source: Graph created by the author (2015).

Figure 1. Subordination of indoor/outdoor aspects as the main condition for creating a harmonious environment.

Summarising conclusions on environmental harmony in functionally different objects and their groups in all successful examples one may notice the same principle: the decisive role of value subordination in reaching harmony in environment design (Figure 1), balance between the spiritual and the material, very much like what is necessary between sciences and humanities. Architecture is a unique area integrating both of these values in itself in balanced amounts. Due to the novelty of the issue, society, people working in public administration and more than often experts as well lack advanced knowledge on conditions of indoor/outdoor harmony and do not pay sufficient attention to it. In order to ensure a harmonious high-quality environment in the future, one must first invest substantial resources in all levels of education; it will become a reflection of inner conflicts of a new space concept, continue historic process of architectural development and introduce diverse approaches to harmonise indoors/outdoors in a scientifically grounded way. With time, a general level of education in this socially sensitive aspect and issues increasing, we may hope for a development of complex practical solutions based in legislation.

Conclusions

Intensity of indoor/outdoor dialogue in the context of architectural history has been rapidly evolving since the 19th century due to technological achievements reaching culmination in the 20th century in living houses with as much glass systems as possible serving as a breaking point of the ruling trend. Since then intensity of the dialogue has diminished, however its trend remains high, looking for balance of a dialogue of interior and exterior space and a way to harmony in this complicated system.

After inspection of education institutions a conclusion was drawn that a close relation of nature and interior space in recreational areas is one of the keys to indoor/outdoor harmony. This communication justifies itself even more in healthcare institutions that have an access to rehabilitation gardens. In a sacral space direct communication with outdoors disturbs its function, therefore in objects like that promotion of indoor/outdoor harmony should be made by indirect, reflected light or a direct communication with the sky. Besides, it is crucially important to organise the light taking into account indoor subordination, i.e. accentuating altars. Library spaces require a solution in-between education and sacral buildings – an indoor presence of nature and urban space has to be tempered. Outstanding results may be achieved by combining light shafts, tunnels, skylights, and reflected lights with traditional windows in facades if the latter are subordinated to the previous in terms of light intensity.

In the summary of conclusions several directions of indoor/outdoor harmony development in modern Latvia appear, both open and closed to communication; however, the leading trend still is towards overtness. By analysing previous successful and unsuccessful examples in the architecture of publicly most sensitive objects of the re-established Latvia, one obtained a pyramid of subordinated guidelines to be employed as a support system for introducing harmonious architectural environments. The subject is of current importance not only in circles of experts but in all levels of society: we all are users of this environment. In order to create a harmonious high-quality environment in the future, at first, one has to invest substantial resources in education; it will become a reflection of inner conflicts of the new space concept, continue historic process of architectural development and introduce diverse approaches to harmonise indoors/outdoors. Due to the novelty of this problem, society, people working in public administration and more than often experts as well lack advanced knowledge on the conditions of indoor/outdoor harmony and do not pay sufficient attention to the issue. With time,

common awareness in this area will increasing, we may hope for a development of complex practical solutions for this publicly sensitive issue based in most recent scientific strategy grounded in research and materialised in a harmonious environment.

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POLLUTION ANALYSIS OF SURFACE (RAIN) WATER FROM PIG-BREEDING ENTERPRISE PRODUCTION TERRITORY

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Abstract
Pollution of surface (rain) wastewater forming in the production territory of a pig-breeding enterprise was investigated in the period 2007–2011. The surface water is collected from the production territory from yards with a hard covering and from roofs, enters the rain sewerage, and is released untreated into the natural environment.
The aim of this work is to find out what influence on the environment is exerted by surface wastewater released into the natural environment from the production territory of pig-breeding enterprises.
Surface wastewater pollution indices and the research frequency were identified according to the requirement to carry out surface water monitoring: surface wastewater samples are collected three times per year.
Because of the uneven rainfall distribution during the year, less surface wastewater runoff occurred during the cold period; however, concentrations of indices were higher compared to the end of the warm period (TSS – 18 %, BOD_7 – 39 %, N_{total} – was unchanging, oil products – 12.5 %). Only P_{total} concentrations were higher in the end of the warm period in 14 %.
The organic pollution in surface wastewater was increased by total suspended solids. The increased concentrations of TSS and biochemical oxygen demand (BOD_7) were related to a higher precipitation level.
The indices' TSS, BOD_7, N_{total}, P_{total} and oil products concentrations in the surface wastewater runoff from the pig-breeding enterprise's production territory complied with the requirements for surface water which is collected from this area and released into the environment.
Key words: environment, production territory, runoff, surface wastewater.

Introduction

Water body pollution is still a crucial environmental problem in both Lithuania and other EU member states. Due to anthropogenic activities, there are many suspended solids, metals, pesticides, polyaromatic hydrocarbons, oil and oil products, miscellaneous bacteria, and biogenic substances in rainwater (Paul and Meyer, 2008; Rentz et al., 2011).

According to data of the Environmental Protection Agency of Lithuania, only a small part of all surface wastewater collected in Lithuania is treated. In 2012, 10.6% of all surface wastewater was treated to the set standards. Insufficiently treated wastewater comprised 0.3%, but untreated wastewater - 89.1% (Satkūnas, 2013).

Severe environmental problems in Lithuania are caused by 24 operating pig-breeding complexes with livestock capacities higher than 5000, keeping 573000 pigs, 65 pig-breeding companies and farms with livestock capacities higher than 200, keeping 77000 pigs, 2179 dairy/beef cattle farms with livestock capacities higher than 50, keeping 319400 cattle, and 21 poultry farm with livestock capacities higher than 1000, keeping 9946700 fowls. The key reason is inadequate sewage treatment and poor technologies (Lietuvos..., 2012).

Naturally, environmental pollution increases significantly when a large amount of livestock is kept in one place. However, special attention should be paid to polluted cattle-shed and farm areas, where dung is collected and stored and surface wastewater is formed. Farms usually have inadequate wastewater treatment facilities or do not have them at all. Therefore, untreated wastewater is released into the environment and contaminates it.

The Government of the Republic of Lithuania, in pursuance of implementation of the Framework for the EU Community Action in the field of Marine Environmental Policy and the provisions of the Baltic Sea Action Plan adopted by the Helsinki Commission Ministerial Meeting in Krakow on 15 November 2007, passed the resolution "On Approval of the Baltic Sea Environment Protection Strategy", No. 1264, on 25 August 2010.

The ultimate strategic objective of this resolution is to achieve and maintain the good environmental status of the Baltic Sea up to 2020. The strategy approved by the Government provides that in the period up to by 2015, fewer nutrients (nitrogen: 11750 tonnes, phosphorus: 880 tonnes) should enter the marine environment in comparison to the period from 1997 to 2008 (Dėl Baltijos..., 2012).

For a long time, great attention was paid to the pollution of storm wastewater from urbanized areas, roads, or waste storing places. Storm wastewater from the production territories of stock-breeding farms was less well analysed. Storm wastewater from production territories is the natural atmospheric rainfall precipitated in the production territory of the enterprise during its operation (Hogland et al., 2003).

The aim of this work is to find out what influence on the environment is exerted by surface wastewater released into the natural environment from the production territory of pig-breeding enterprises. The

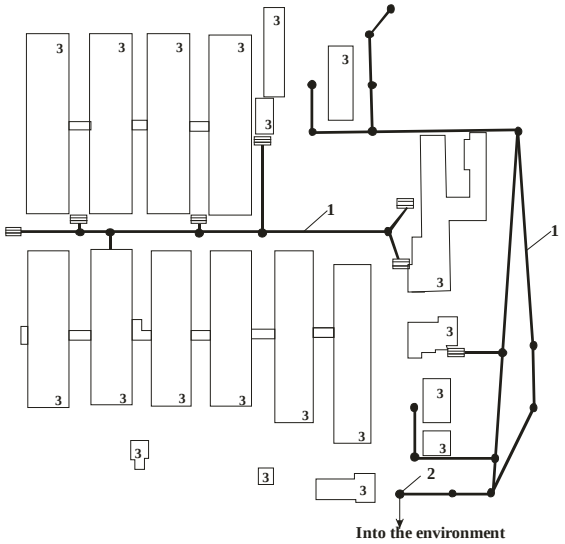

Figure 1. Production territory scheme of the pig-breeding enterprise. 1 – Surface wastewater (rainwater) sewerage, 2 – manhole for release of wastewater into the environment, 3 – roofs of buildings.

tasks are to determine the amount of wastewater buildup in the production enterprise territory and estimate their pollution.

Materials and Methods

The production territory of the pig-breeding enterprise comprises is 11.35 ha. The northern, eastern, and southern sides of the production territory of the enterprise are surrounded by protective ditches, and 5.98 ha of the industrial area without buildings are sown with perennial grasses. The surface water (rainwater) is collected from the production territory from 1.71 ha of yards with hard coverings and from 3.66 ha of cattle-shed roofs, enters the rain sewerage, and is released untreated into the natural environment on the southern side of the production territory (Figure 1).

Surface wastewater pollution indices and the research frequency were identified according to the requirement to carry out surface water monitoring: surface wastewater samples are collected three times per year: in spring, during the snowmelt; in summer, at the first onset of the heavy rainfall period; and in autumn, before freezing. The following indices were identified in the wastewater: total suspended solids (TSS), biochemical oxygen demand during seven days (BOD_7), oil products, total nitrogen (N_{total}), and total phosphorus (P_{total}). The samples were analysed in the Chemical Analysis Laboratory of the Water Resource Engineering Institute of Aleksandras Stulginskis University, which is certified by the Environment Ministry of the Republic of Lithuania.

The following indices were determined: BOD_7 was determined by titrometric method (Vincler), with the difference in oxygen level being calculated after seven days of incubation, and TSS by gravimetric method after filtering the substance through a 0.45 мm diameter filter. Total nitrogen (N_{total}) was determined by the Kjeldahl method, and total phosphorus (P_{total}) was determined by spectrometric method after mineralization with potassium persulphate. Colorimetric analyses were done using an FIA Star 5012 System analyser. Concentrations of oil pollutants were determined with the help of an IKAN-1 infrared spectrophotometric device in the Analytical Department of the Agrochemical Research Laboratory of the Lithuanian Research Centre for Agriculture and Forestry.

The surface water runoff was measured by a volumetric method, falling more rainfall. To evaluate the meteorological conditions, the data from Laukuva meteorological station was used.

In Lithuania, the surface wastewater, which is released to the environment, quality is assessed in accordance to the surface wastewater management regulation. This document states that the concentrations of the suspended solids in the discharged surface wastewater should not be higher than 50 mg L^{-1}, BDS_7 – 57.5 mg O_2 L^{-1}, oil products – 7 mg L^{-1}, total nitrogen (N_{total}) – 30 mg L^{-1}, total phosphorus (P_{total}) – 4 mg L^{-1} (Paviršinių nuotekų..., 2007).

Mathematical and statistical analysis of the data was performed using the computer program Excel and Statistica.

Results and Discussion

As in other countries, precipitation in Lithuania is very uneven. Two rainfall distribution periods may be distinguished: cold (November to March), when the average precipitation per month is from 21 to 81 mm,

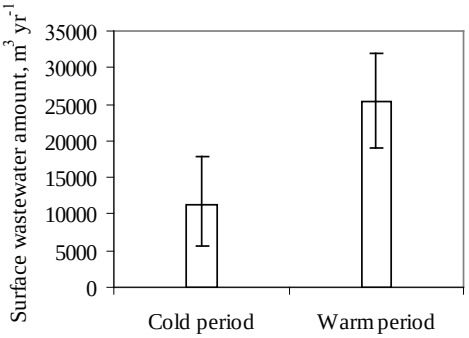

Figure 2. Average surface wastewater runoff the production territory of the enterprise during the cold and warm periods (error bars in figure represent standard deviation).

and warm (April to October), when the precipitation is from 45 to 115 mm.

The most part of the surface wastewater runoff in the production area of the enterprise was find during the warm period of rainfall distribution and accounted for 25402 m^3 on average. During the cold period, the runoff was less by a factor of 2.3, being 11212 m^3 (Figure 2).

The surface wastewater runoff quantity differed considerably between the cold and warm periods ($t_{act.} = 4.61 > t_{theor.95\ \%} = 2.31$), because during the five research years, the precipitation level was 149 mm lower during the cold period on average.

In cold winters in 2009 and 2010 not much surface wastewater formed, 16 and 12% of total runoff amount respectively. In 2007, 2008, and 2010 winter the surface wastewater constituted 39, 28 and 35% of the total runoff amount. During the warm period the largest amount of runoff formed in 2008, 2009, and 2010, (72, 84, and 88% respectively, while in 2007 and 2011 it was 61 and 65% of the total runoff volume). At the beginning of the warm period, the amount of the surface wastewater was greatly increased by precipitation, which, in the form of snow, had accumulated in the production area of the enterprise (Figure 3).

After conducting the research, an extremely strong relationship between precipitation, weather temperature and surface wastewater discharge was determined:

$$z = -192.054 + 37.824\ x + 93.126\ y, \quad (1)$$

where x = precipitation (mm), y = weather temperature (°C), z = surface wastewater discharge (m^3 $month^{-1}$).

If there is a higher level of precipitation and if the average weather temperature is positive, the amount

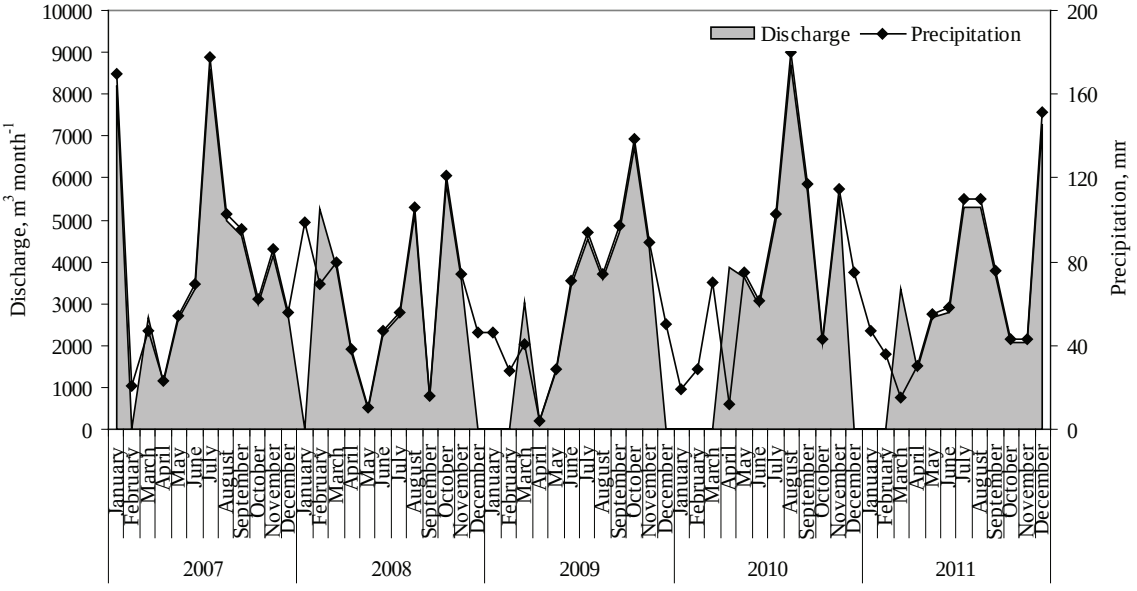

Figure 3. Precipitation and surface wastewater amount distribution.

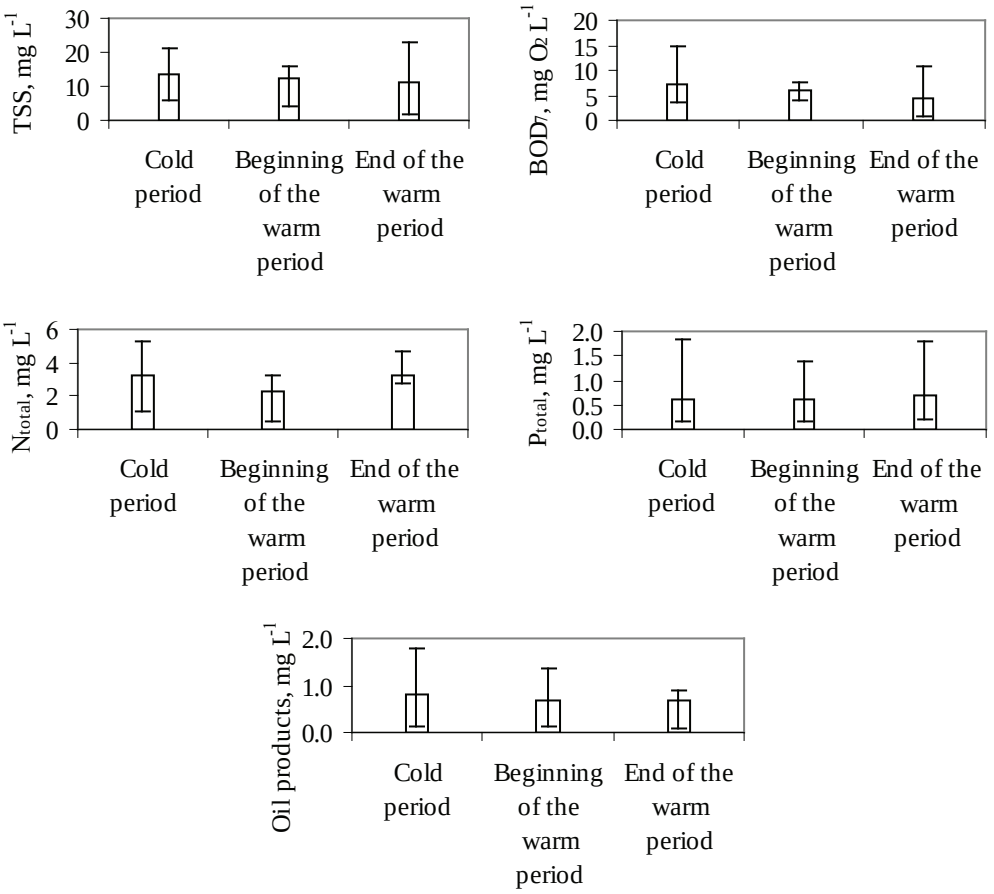

Figure 4. Average concentrations of indices TSS, BOD_7, N_{total}, P_{total} and oil products in the surface wastewater at the beginning and the end of the warm period and the cold periods (error bars in figure represent standard deviation).

of the runoff tends to increase ($r = 0.83$, $n = 60$, $F_{act.} = 61.86 > F_{theor.95\%} = 2.57$). A partial regression analysis has determined that the bigger impact on the formation of surface wastewater has the precipitation ($r = 0.83$) rather than the weather temperature ($r = 0.47$). However, during the cold period, when the weather temperature is usually negative, the surface wastewater does not form.

Due to the sub-zero weather temperature in December, January, and February, precipitation accumulated in the form of snow in the enterprise area and part of it evaporated; therefore, during the spring snow break, the amount of surface wastewater runoff from the enterprise's production territory was lower compared with the warm period. In spring, during the snowmelt, concentrations of indices increase in the surface wastewater, since the cold period, during which these substances gather on the surface area and accumulate in the snow, has the greatest impact on the surface wastewater quality. Higher concentrations of pollutants in the snow are caused by the longer winter period and the lower average temperature (Reinosdotter and Viklander, 2005). When surface runoff starts, pollutants accumulated during the cold period are removed from the area. Higher average concentrations of indices were measured in the cold period compared to the beginning of the warm period: the values of TTS, BOD_7, N_{total}, P_{total}, and oil products in the cold period were as follows: 13.3, 7.4, 3.3, 0.6, and 0.8 mg L^{-1}, respectively (Figure 4).

Brezonik and Stadelmann (2002) have found that the highest concentrations of indices were measured during snowmelt than during rainwater runoff, however Mallin et al. (2009) have determined that TSS, P_{total} and BOD were significantly higher during rain events compared to nonrain periods. This is also confirmed by these research results, as concentrations of the indices in the surface wastewater measured during the warm period were lower. Measured concentrations of TSS, BOD_7, N_{total}, and oil products at the beginning of the warm period were 5%, 20%, 30%, and 12% lower, respectively, than during the snowmelt in the cold period. It is apparent from the analysis of the concentrations of indices at the

end of the warm period that concentrations of TSS, BOD_7, and oil products were 18%, 39%, and 12%, respectively, than during the cold period. However, the concentration of P_{total} increased by a factor of 14% and that of N_{total} showed no change. The increase in concentration of P_{total} at the end of the warm period may be related to asphalted roads and yards in the production territory of the enterprise, as measured concentrations from asphalted roads were found to be higher (Gilbert and Clausen, 2006). Furthermore, concentrations of nitrates and orthophosphates are usually higher in agricultural catchments (Coulter et al., 2004).

It is stated in the literature that at the first part of the wet season concentrations were ranged from 1.2 to 20 times higher than ones near the end of the season (Lee et al., 2004). Such a phenomenon is known as "seasonal first flush" (Stenstrom et al., 2005; Bae, 2013). This statement is confirmed by the research results, which demonstrated that concentrations of TSS and BOD_7 measured at the beginning and end of the warm period were 1.2 and 1.3 times higher, respectively, at the beginning of this period. Concentrations of N_{total} and P_{total} were lower at the beginning of the warm period than at the end of the period, and concentrations of oil products remained unchanged.

Major indices describing the surface wastewater pollution are concentrations of TSS, BOD_7, and oil products. TSS, by forming sediments in surface water bodies, exert the greatest influence on the water quality (Davis and McCuen, 2005; Li et al., 2014).

The quality of surface water runoff from the production territory of the pig-breeding enterprise was very good with regard to TSS, as the maximum permissible concentrations (MPCs) were never exceeded. Within the study period, average measured concentrations of TSS at the beginning of the cold period and the warm period and at the end of the warm period were 13.3, 12.6, and 10.9 mg L^{-1} respectively; that is, 3.8, 4.0 and 4.6 times lower than the MPCs.

Within the study period, the pollution of surface water with organic substances in the study area was very low: average measured concentrations of this index at the beginning of the cold period and the warm period and at the end of the warm period were 7.4, 5.9, and 4.5 mg O_2 L^{-1}, respectively, that is, 7.8, 9.7, and 12.8 times lower than the MPCs, respectively.

The pollution of the surface wastewater with organic compounds is relevant to TSS, since up to 90% of the organic substances comprising BOD_7 are suspended solids, and contamination with other pollutants, that is, oil products and heavy metals, is of random character (Jakubauskas and Račys, 1997). It was established by means of regression analysis that as the amount of TSS in the surface wastewater of the pig-breeding enterprise increased, oil products increased ($r = 0.94$, when $t_{act.} = 5.72 > t_{theor.95\%} = 2.77$; $n = 6$) (Table 1).

Correlation coefficients between TSS concentrations in surface wastewater and researched elements were determined to be strong, average and weak, however statistically insignificant.

It is maintained that oil and oil products are among the most frequent and most dangerous pollutants of the ground and water bodies; their composition changes under the influence of the environment, and natural communities of microorganisms oxidizing the oil decompose them completely only over a long time (Leahy and Colwell, 1990; Čipinytė and Grigiškis, 2000).

Within the study period, the measured amounts of oil products in the surface wastewater released into the natural environment were especially low: average concentrations of oil products at the beginning of

Table 1

Correlation between TSS and BOD_7, N_{total}, P_{total}, oil products in the surface wastewater of the pig-breeding enterprise

Indices	Form of relation	Determination coefficient	Correlation coefficient	n	$t_{theor.95\%}$	t_{actual}
When TSS<10 mg L^{-1}						
BOD_7	$y = -0.23x^2 + 2.54x-2.79$	0.58	0.76	6	2.77	2.31
N_{total}	$y = -0.02x^2 + 0.14x-2.43$	0.007	0.08	6	2.77	0.16
P_{total}	$y = 0.14646x^{0.7773}$	0.20	0.45	6	2.77	1.01
Oil products	$y = 0.0509x^{1.5361}$	0.89	0.94	6	2.77	5.72*
When TSS>10 mg L^{-1}						
BOD_7	$y = 1.12x^2 -3.77x+35.02$	0.48	0.69	8	2.45	2.34
N_{total}	$y = -0.02x^2 + 1.13x-9.44$	0.47	0.69	8	2.45	2.32
P_{total}	$y = 0.009x^2-0.28x+2.65$	0.08	0.29	8	2.45	0.74
Oil products	$y = 0.0001x^{3.0455}$	0.37	0.61	8	2.45	1.89

x – the concentrations of TSS in the surface wastewater, mg L^{-1}; y – the concentrations of indices in the surface wastewater, mg L^{-1}; correlation connection values reliable according to the Student's criterion t ($t_{actual} > t_{theor.95\%}$) are signed with stars.

Table 2

Correlation between the concentrations of indices (TSS BOD_7, N_{total}, P_{total}, oil products), formed in the surface wastewater of the pig-breeding enterprise, and the rainfall

Indices	Form of relation	Determination coefficient	Correlation coefficient	n	$t_{theor.95\%}$	t_{actual}
TSS	y = 0.0393x - 20.479	0.25	0.50	15	2.2	2.41*
BOD_7	y = 0.0217x - 12.153	0.29	0.55	15	2.2	2.80*
N_{total}	y = 0.0054x - 1.4947	0.12	0.35	15	2.2	1.45
P_{total}	y = 0.0016x + 0.7064	0.06	0.24	15	2.2	0.93
Oil products	y = 0.0007x + 0.1359	0.02	0.13	15	2.2	0.48

x – amount of precipitation, mm; y – the concentrations of indices in the surface wastewater, mg L^{-1}; correlation connection values reliable according to the Student's criterion t ($t_{actual} > t_{theor.95\%}$) are signed with stars.

Table 3

Trends of change in concentrations of indices in the surface wastewater of the pig-breeding enterprise within the study period

Indices	Form of relation	Determination coefficient	Correlation coefficient	n	$t_{theor.95\%}$	t_{actual}
TSS	y = -0.7664x + 18.265	0.27	0.51	15	2.2	2.51*
BOD_7	y = -0.4842x + 9.8077	0.39	0.62	15	2.2	3.68*
N_{total}	y = -0.0976x + 3.7674	0.11	0.33	15	2.2	1.33
P_{total}	y = -0.0443x + 0.9918	0.12	0.34	15	2.2	1.39
Oil products	y = -0.0171x + 0.8446	0.03	0.17	15	2.2	0.63

x – study period, yr; y – the concentrations of indices in the surface wastewater, mg L^{-1}; correlation connection values reliable according to the Student's criterion t ($t_{actual} > t_{theor.95\%}$) are signed with stars.

the cold period and the warm period and at the end of the warm period were 0.8, 0.7, and 0.7 mg L^{-1}, respectively, and were thus 8.8 to 10 times lower in comparison with the MPCs.

Average concentrations of N_{total} and P_{total} in the surface wastewater runoff from the production territory of the enterprise at the beginning and end of the warm period and in the cold period were 9.1, 13.0, and 9.1 times and 6.7, 6.7, and 5.7 times lower than the MPCs, respectively. Since measured concentrations of these chemical elements in the surface wastewater were very low, no correlation with rainfall, the number CL of the enterprise, or the TSS in the wastewater was established.

It was established by means of correlation analysis that the increase in concentrations of TSS and BOD_7 in the wastewater is related to higher rainfall (Table 2).

A higher precipitation had no effect on the concentrations of N_{total}, P_{total}, and oil products, because the correlation coefficients were very low and not statistically significant.

Indices in the surface wastewater of the pig-breeding enterprise fluctuate very little and never exceed the MPCs. A downward trend of concentrations of TSS (r = 0.51), BOD_7 (r = 0.62) was observed in the pig-breeding enterprise within the five-year study period (Table 3). It was influenced by the completed reconstruction of the enterprise.

It can be seen from the equations in Table 1 that N_{total}, P_{total}, and oil products concentrations in the surface wastewater, which is released to the environment, due to reconstruction of the enterprise, which was carried out, have a tendency to fall, however, the correlation coefficients were determined to be extremely low.

Conclusions

1. Due to the uneven distribution of precipitation, 2.3 times more surface wastewater ran off the production territory of the enterprise during the warm period than in cold period.
2. Concentrations of indices in the surface wastewater of the pig-breeding enterprise fluctuated within a very narrow range (for example TSS – from 5.8 to 21 mg L^{-1}) and never exceeded the MPCs; however, they were higher during the spring snow break and at the beginning of the warm period.
3. The higher amount of precipitation has led to an increase in the concentrations of TSS (25%) and BOD_7 (29%) in the wastewater.
4. Due to the reconstruction of the enterprise, which was carried out, contamination of the production territory has decreased significantly. A downward trend in concentrations of TSS (27%), and BOD_7 (39%), was observed in the pig-breeding enterprise within the five-year study period.
5. The water pollution formed in the production territory of the pig-breeding enterprise is not an

environmental hazard, as the concentrations of the indices investigated in the surface rain wastewater were 3.8 – 12.8 times lower than the MPCs. Therefore, this wastewater can be released into open water bodies untreated.

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RESEARCH OF DEFORMATION PROCESSES IN REGULATED STREAM CHANNELS OF LITHUANIA

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Abstract
Deformations occurred in natural and regulated stream change beds lateral and longitudinal profiles. This is particularly evident in regulation furrows, where their initial state is known. Research showed that landslips of the upper slopes in regulated watercourses is the most common deformation (72.8% of studied cases), and the largest deformation occurs in the lower part of the slope (63.4%), where the accumulation of the moved silt and soil is. It was found that the deformation changes the bed plan as well. 59.1% of regulated streams distort furrows and make meanders due to deformation effects. The analysis of river beds widths and depths ratio relationship with discharge of channel running flow observed that ratio B/H increases with increasing flow evenly in regulated beds, while the above-mentioned ratio varies unevenly in the natural watercourse. Cross-sectional shape also varies in regulated and natural beds: heterogeneous form beds dominated in natural beds, while parabolic cross section shape is approaching during the deformation in regulated beds, what is more favourable for the living environment.
Key words: deformation of channels, rate of width and depth, processes of naturalization.

Introduction

Recently, the majority of regulated streams have become abandoned and neglected. Banks of abandoned regulated rivers are overgrown with lush grass and dense shrubs or even trees. There are many factors (run-off of surface water, water flow in the bed, growing vegetation in the bed and on the slopes, etc.) that cause various kinds of deformations in regulated river beds. The analysis of regulated stream beds deformation processes showed that erosion of slopes, leaching beds and silt accumulation are the most common deformations of regulated stream bed. That creates favourable conditions for the overgrowth of riverbeds with grasses, shrubs and forest vegetation. Deformation, which occurred on the impact of various natural factors, determines changes in river beds of cross sectional and longitudinal profiles. Landslide soil of slopes accumulates in the foothills of the bed slopes thus narrowing the stream bed. Parts of the soil create resistance for flow of water and distort stream flow. Bed is silting in the low slope areas, while washed out processes are observable in beds with rather great slope areas. Erosion products are brought together with surface runoff from the surrounding areas and complement accumulating silt layers in beds. Bottom of the regulated stream begins to curve during the deformational processes in bottom and the slopes, - that form natural meanders of streams. Stream slopes with overgrown grassy and woody vegetation, the stream bed formed of deformation processes and stream meanders created by water flow create favourable conditions of naturalization processes for regulated streams. This changes the distribution flow energy of rivers and water aeration conditions.

The objective of the research was to evaluate the deformation processes in Jiesia and Merkys rivers basins.

Materials and Methods

The research was carried out in natural and regulated tributaries of Jiesia and Merkys rivers basins. The Jiesia river basin is located in the central part and the Merkys river basin - in the southern part of Lithuania. Amarnia, Čirvija, Grūda, Spengla are tributaries of the river Merkys. Vyčius and Kumė are tributaries of the river Jiesia. Both natural and regulated streches of all these tributaries were investigated. During the research it was attempted to measure lateral and longitudinal deformations of regulated streams, the current configuration of the bed and the bottom, the overgrowth of the slopes with grassy vegetation, beds deformation and naturalization patterns, accumulated silt layers dependence on the prevailing soil.

The longitudinal profiles of streams were measured to adjust deformation in selected sections and to determine the cross-section chances - transverse sections were evaluated. In order to assess the tendency of the transverse deformations, the research objects cross sections were divided into 3 zones: a bed, slope and upper slope parts. The format and amount of deformation in the longitudinal and transverse profile were taken into account. 100-200 m length natural and regulated rivers in the Jiesia and Merkys river basins were selected. Selected sections were subdivided into every 10 m sections. Flow width, slope coefficients and the thickness of accumulated silt layers were measured. The location of profiles was estimated by the GPS device. Straight cuts, geological surveys were made to determine the dominant soils. Leveling was used to evaluate the slope of the bottom of the selected sections. Grass and woody vegetation growing on slopes were visually assessed. The condition of studied streams was evaluated and information was entered into forms. Information was structured, data was processed by methods of mathematical statistics,

the relationship between the formated of silt layer and the prevalence of soil properties was determined.

Results and Discussion

The prevailing deformation was determined in different cross-sectional parts of streams (bed, slope and upper slope part), regardless the researched object parameters. With reference to aggregated data, it can be stated that soil loss was asserted at the upper slope area (an average from 0.09 to 0.17 m), which set 72.8% of the cases studied. The latter area was affected not only by surface run-off, but also by heavy equipment moving in this area and existing human activities. The slight deformation was determined in the middle zone of slope (from 0.02 to 0.12 m, in some cases up to 0.23 m). The fact that erosion of the stream usually starts at the slope and upper part of slope was determined by Ragauskas (2004) as well. This is explained by the fact that poorer vegetation grows in these places and the turf is more vulnerable, and surface runoff also affects the stability of slopes. The largest deformation occurs in the zone of water flow, where the channel is usually affected by water flow. In this area, in the majority (63.4%) of the studied cases, large alluvial soil and landslip from slopes (from 0.25 to 0.33 m) are accumulated. Soil is eroded from the bottom and the slopes are transported downstream. When eroded soil reaches the strech with lower slope or low water flow rate, then the deposited silt settles. That is a result of concentration of alluvial deposits, which determine the design parameters of the riverbed changes. Only in rare cases the transverse profile of the bed remains constant - these were 6.8% of such cases.

Deformation of regulated streams and their distribution in different zones of the cross-section are presented in Figure 1. Along with the supplied cross-section the areas, in which their inherent deformation occurs, are revealed.

Deformation is not evenly distributed in beds, but the positive deformations are pointed up. This is explained by the fact that there is a tendency of siltation dominated in the studied sites and accumulation of soil slopes in the footslopes. Assuming that the determined deformation had formed during the period of 7-9 years and the beds of streams alluvial layers on average range from 0.25 to 0.33 m, it is estimated that over one year from 0.03 to 0.04 m thick layer of silt are formed. These results were confirmed by Varnelis (2004), because he found that in sandy loam soils accumulate 4 cm of deposits and in loam soils - about 3 cm of deposits during the period of one year.

Having made the mathematical-statistical analysis of deformations measuring results it was determined that the weighted average value of deformations is 0.2 m. This implies that the formation of sediment and soil layer is the most frequent type of deformations. Negative deformations are less frequent. The latter deformations most often occur as the leaching and deposition on middle and upper parts of slopes. The variation scope of values is 2.1 m (from -0.9 to 1.2 m). Standard deviation of data set is s = 0.42 m. The dependence of deformations extent on their position in the channel profile is expressed by a correlation coefficient R=0.639 (determination coefficient $R^2 = 0.409$). This shows a medium relation between the distributions of deformations within the channel profile. Correlation coefficient reliability is determined according to Student criterion $t_{actual} = 6.49$. The calculated value exceeds the theoretical value several times ($t_{theor} = 1.304$ when $\alpha = 0.1$) (Kruopis, 1993). This shows a reliable estimation of cohesion of the relation between the distributions of deformations within the channel profile.

Cross-sectional channel deformations highly influence the position of regulated stream channels in the plan. When cross-sectional deformations occur,

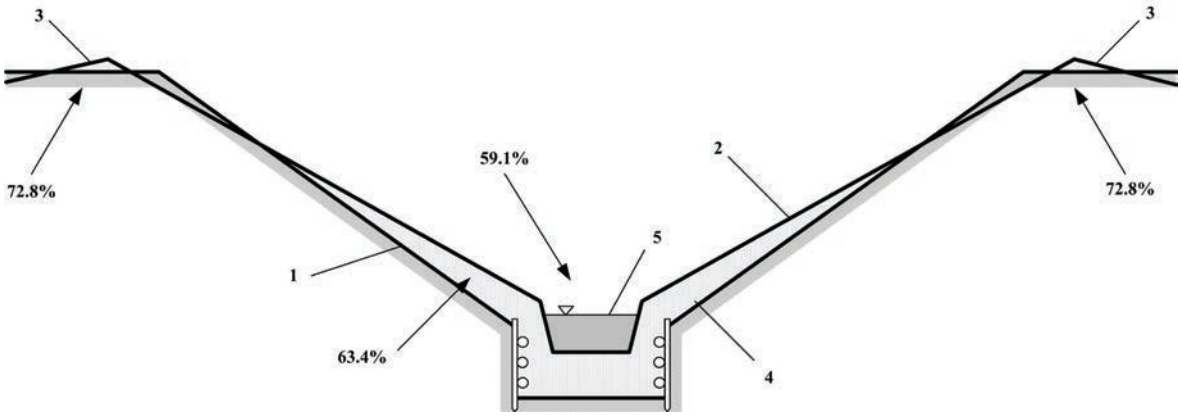

Figure 1. The distribution of deformation in different zones of regulated stream.
1 – designed stream cross section, 2 – present state of regulated stream, 3 – deformation of upper slope part, 4 – soil of landslip slope and accumulated layer of silt, 5 – new stream bed with meanders.

the signs of meandering of channel beds instead of straight channels are observed. Soil slid from slopes on the foot of slopes creates obstacles for water flow, which results in the occurrence of cross-sectional circulation in the channel. Strong water flow starts scouring channel slopes. Due to the flow circulation scoured particles of slope soil are transported into the opposite side of the flow where they form shallows in the course of time. This enhances the processes of side accumulation and side erosion. Having deviated from the designed position, the channel bed creates meanders similar to those of natural streams.

Meandering streambed was observed in 59.1% of all studied stream channels. This is most often found in the stretches of channels where sandy-loam soils are prevailing (69.7%), where sediment is accumulating (42.6%) or where slope deformations are observed (72.8%). Usually in such stretches of channels no maintenance or repair works are carried out.

The balance of water flow velocity and granulometry composition of soil occured in natural streams. In this case, the flow prevents accumulation of silt, but also scour does not exist in stream beds furrow and leaching. However, this process can be influenced by side factors, such as human activities, vegetation and other.

It was found that plants began to grow in the bottom with sediments: sweet calamus (*Acorus calamus L.*), cat's-tails (*Typha L.*). They slow down the flow rate, reduce water permeability and thus capacitate the accumulation of sediments in strem beds. There are suitable conditions for tall grassy vegetation (great nettle (*Urtica dioica L.*), mugwort (*Artemisia*), thistles (*Cirsium*) and etc.) to spread rapidly, which cover all slopes of the stream and displace other plants in neglected streams. Berankienė (1997) confirms the same composition of grassy vegetation species in her studies. Millar (2000) concluded that bank vegetation does exert significant and quantifiable control on alluvial channel patterns. Hession and other researches (2003) also indicated that stream-bank vegetation significantly influences the morphology. Their data indicate that rates of deposition and lateral migration are both higher in nonforested reaches than in forested reaches (Allmendinger et al., 2005). Provided examples show that vegetation growing in river banks and beds make considerable influence on changes of longitudinal and transverse profiles in neglected regulated streams.

Examining the spread of woody vegetation in researched sections showed that the slopes of the streams in 17.2% of the studied cases are overgrown with shrubs and several trees. The density of woody vegetation on the stream slopes is $t = 0.141 \pm 0.011$ pcs./m^2. The genus of willow (*Salix*) plants dominated in target sections: purple willow (*Salix purpurea*), gray willow (*Salix cinerea*), osier (*Salix viminalis*). It was found that the distribution of woody vegetation is unequal in researched objects. The middle zone of slopes is the most densely wooded, where the proper moisture regime formed the most favorable conditions for the development of vegetation. In the source of literature (Survilaite et al., 2006) the data are presented confirming that the middle zone of slope is wooded the most densely. This is because of the lack of moisture in the upper part of slope and because of too much moisture in the lower part of the slope.

The increase of woody vegetation on slopes of the streams is the most often seen in streams discharging near forest or wooded areas. This is confirmed by the literature (Lamsodis, 2002), where results claim that woody vegetation is denser and more common on the slopes of streams located in forests and near them. Moreover, it generates the diversification of plants as well. Lamsodis (2006a) found that herbaceous and woody vegetation growth intensity increases sediment accumulation on the bottom of regulated rivers while the growth of woody vegetation on the slopes reduces it (Lamsodis et al., 2004, 2006a).

The layer of silt accumulation thickness was measured in regulated streams. Correlation was estimated between the thickness of silt and prevailing soil in the area. The summarised research information showed silt layer thickness when light clay loam, medium loam, light loam and sandy loam soils are prevailing. It can be maintained that sandy soils are the most affected, that is why the largest alluvial deposits are evident on beds of streams. Poškus (2008) also indicates that sediment particle size has a realtion with the soil, which dominates in regulated streams. Ragauskas (2004) argues that soil particles washed by surface runoff from surrounding areas also augment the acumulated silt in regulated streams. Studies have shown that the biggest amount of soil is leached in sandy loam ditches, less – in loam (Ragauskas, 2004).

Width of streams. The research showed that the width of stream varied from 5.36 to 12.38 meters in natural and regulated stream sections. The main results of research are presented in Figure 2.

It was found that changes of streams width are related with natural processes there. Another reason for the change of the width of beds is grassy vegetation. It was researched that the changes of width varied to 21% due to the influence of vegetation on slopes. It was found that a greater impact for width of the beds had both grassy vegetation and individual trees. Flow width is reduced, the water flow is tightened and leaching is formed in these places. Self-naturalization processes were started in rivers that are why the width of beds become heterogeneous.

Having measured results of stream widths, it can be said that the flow rate is not the main factor influencing

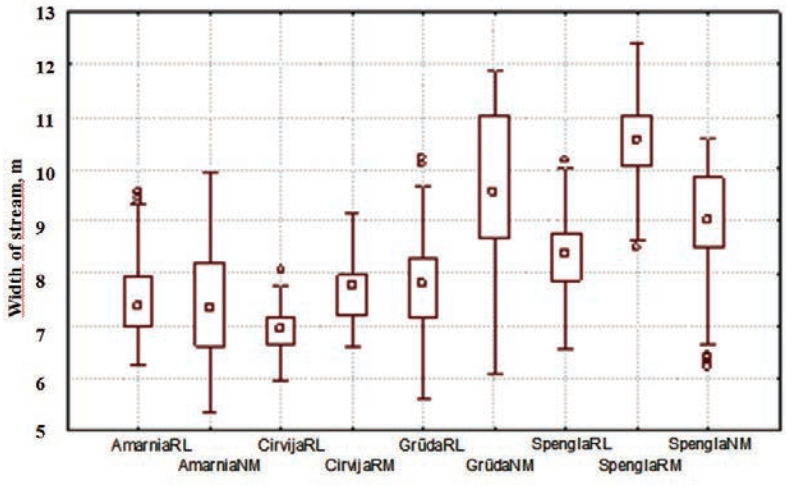

Figure 2. The variation of stream width in researched rivers (RL – regulated stretch located in field, RM – regulated stretch located in forest, NM - natural stretch located in the forest).

the increase of flow widths. Estimating them by the state in terms of naturality, it is clear that the variety of width of natural streams is compared wider to those sections where self-naturalization processes are insignificant. The widths in natural stretch of Amarnia were found 1.4 time higher compared with widths of Amarnia regulate section. Cirvija natural beds widths are 1.2 times higher compared with the regulated bed of Cirvija. The bed width of Grūda natural stretch is 1.3 times higher compared with the bed widths of regulated sections. Spengla natural stretch widths are 1.2 times wider compared with the Spengla regulated river stretch. That shows that the variety is consistent for natural and self-naturalizated rivers. This is a necessary condition ensuring the natural and self-naturalizated processes in stream beds.

The ratio of stream width and depth (B/H). The relation between stream width and depth ratio (B/H) and river flow (Q) was analyzed in natural and regulated rivers. The evaluation of the collected research data is presented in Figure 3.

It is observed that the width and depth ratio are bigger at the flow rate of 50-60 $m^3 s^{-1}$ in natural streams than in regulated ones, where the same B/H ratio value is reached with the flow rate more than 200 $m^3 s^{-1}$. This can be explained by the fact that the natural

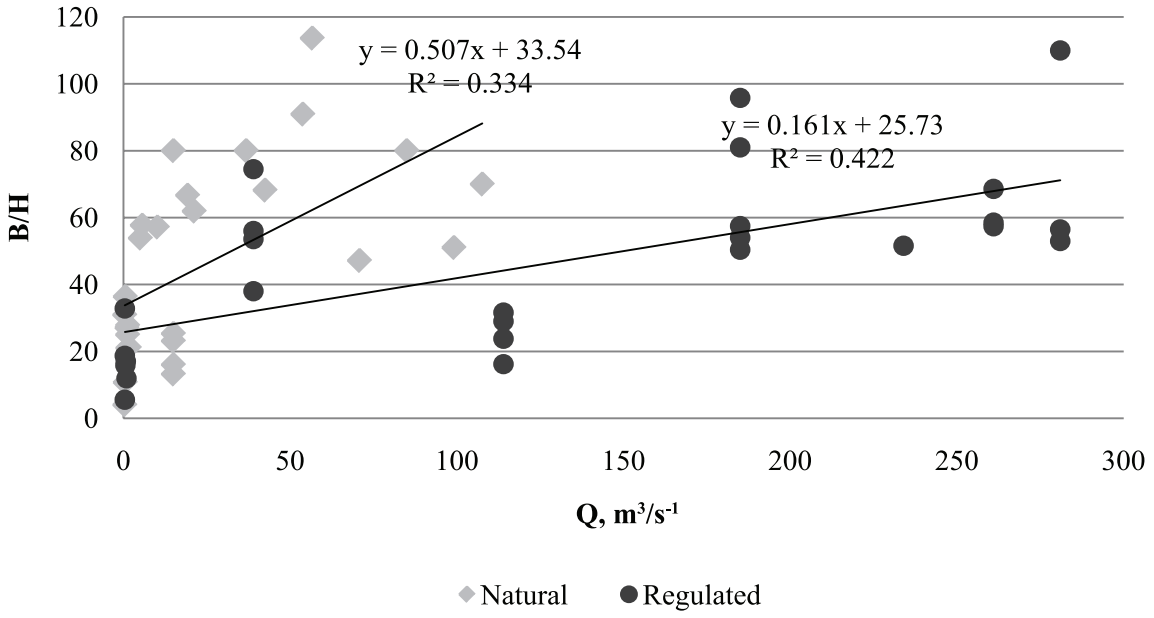

Figure 3. The relation between flow rates (Q), bed width and depth ratio (B/H) in natural and regulated rivers.

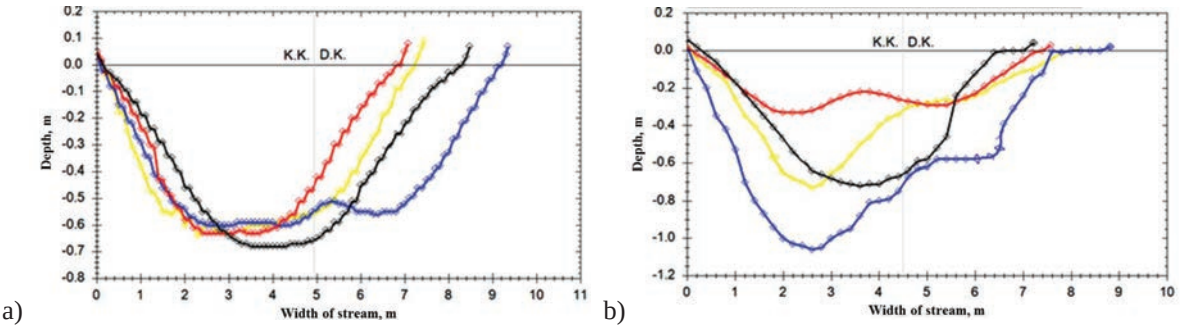

Figure 4. Cross section profiles of regulated and natural river Amarnia.

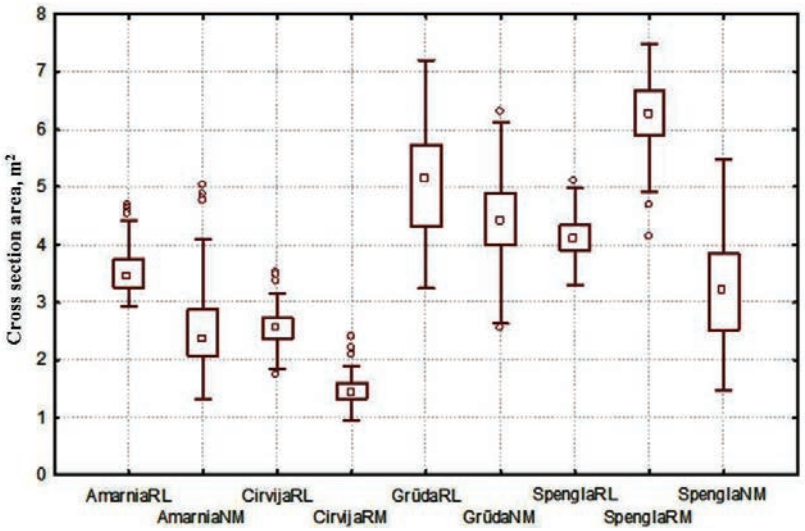

Figure 5. Variations of cross sections in selected streams (RL – regulated stream in field, RM – regulated stream in forest, NM – natural stream in forest).

rivers with higher flow rate have more conveying power than regulated ones. This can be described as a self-regulatory of the bed, which forms contours of the bed. Kleinhans (2010) determined that increasing potential-specific stream power implies more energy to erode banks and indeed correlates to channels with high width–depth ratio. Meanwhile, the width and depth ratio with rivers flow discharge increases gradually in regulated rivers. This shows that the cross section form of the regulated river is artificially formed as trapezoidal, where the increasing flow rate and furrow width and depth ratio increase gradually.

Rivers form and cross section. It was found that the most common form of regulated watercourses is parabolic, regardless the cross section area of the bed. The symmetric parabolas are specific for regulated streams (Figure 4a.). This is mostly due to the fact that natural rivers were regulated by changing them to trapezoidal cross-section profiles. During the deformation, corners of artificial steep profile become rounded and the profile becomes parabolic, i.e. close to natural. Meanwhile, forms of natural beds are more heterogeneous and changing along the stream (Figure 4b.). In Figure 4b, profiles that illustrate the patterns of natural meandering, as well as formation of rifles and pools are presented. In respect of physical sense, big (in this case up to 3.8 times) changes of cross section area and shape of stream determine pulsation of flow rate in furrow. That force aeration and formation of favorable conditions for the aquatic animals and invertebrates.

The research reveled that area of cross sections varied in the range from 1.04 to 7.50 m^2. The most commonly it is founded that broad area range varies in natural streams, while more narrow – in regulated ones. The distribution of cross section area is presented in Figure 5.

Obviously, that variety of shapes and cross section forms dominate in natural streams. That forms different conditions of flow and at the same time creates favourable conditions for aquatic animals. Regulated cross sections remain with similar shape along the river bed, so they do not create favorable conditions for living nature, because bed remains homogeneous. And only during the long recovery

process, it is observed that regulated streams also assume heterogeneous cross-sections, which are more favorable for the living environment.

The analysis shows that the most common deformations of regulated streams are landslip of slopes and accumulation of soil in the footslopes, as well as wash out of the bed or deposition of sediment. One of the most observed types of deformations (63.4%), a landslip from slopes and accumulation at footslopes, mainly depends on soil type, surface and ground water exposure, lamination, inadequate human economic activity and other factors. Maximum deformation affects in water flow area where the water flow keeps an effect of the bed and changes its configuration. Flowing water erodes slopes, thereby enabling the accumulation of silts at the bottom of the stream. Accumulation of silts in flow area helps to develop vegetation. Herbaceous vegetation growing on bed of streams caused the affluent and reduced the flow rate. The deposited silt from upstream is brought with the surface runoff of soil particles (wash products) and form silt deposition. It was found that sediment particles washed from the surface are usually small particles of sludge, which mainly contain organic materials appropriated from the cultivated fields. These particles mostly pollute rivers and streams into which they fall. But it should be mentioned that, depending on conditions, the same factor can cause different deformations of the bed, and the same riverbed deformation can be a consequence of several confounding factors.

Literature (Ragauskas, 2004) also indicates that part of deposites is formed from soil particles with surface runoff from adjacent areas, which riparian strips are not able to protect from. Therefore, sediment retention buffer strips are very important from environmental point of view, because the number of nutrients in fine particles, which were washed out from the fields, is much more higher than in water running from upstream (Račinskas, 1990). Therefore, the erosion products of solid particles not only complement the silt at the bottom of the river, but also they contain chemical elements, which increase the eutrophication of water bodies. These materials also promote the overgrowth of the river bottom and increase resistance to water flow.

One of the means of the protection from surface run-off - streams protection strips, which are very important in those sections, which are near the area of the cultivated land (Ragauskas, 2004). Račinskas (1983) found that 1 meter wide strip covered by grass holds up to 82.4% of deposits of surface runoff. Ragauskas (2004) indicates that inappropriate human activities along the regulated streams cause deformations in upper part of slopes (Ragauskas, 2004). Therefore, the main source of deposition is the basin of regulated streams, because the measured amount of deposits depends not only on the stream flow parameters (bottom widths and slopes) (Poškus et al., 2008; Lamsodis et al., 2006). The results revealed that the eroded material from banks was deposited on the lower bank areas and at the bottom of the ditch where it is potentially transported further during peak discharge events (Stenberg et al., 2013).

In this way, the accumulation of silt and deformed slopes form a new stream bed, so the slope of the stream, flow rate are changing and generate favorable conditions for the development of lateral and longitudinal deformation of regulated stream beds and formation of the stream which is more similar to the natural channel.

Conclusions

1. It was found that the most common deformations of regulated streams are landslips of slopes and siltation in bottom of streams. It was investigated that sufficiently large amounts of deposits and landslips of slopes (approximately 0.25 to 0.33 m) were acumulated in watercourses (63.4% of the cases studied).
2. There are favourable conditions for development of aquatic vegetation in deposited regulated streams which slow down the flow rate, reduce water flow and at the same time increase bed deformations.
3. The research showed that the largest amount of deposits concentrate in regulated streams located in sandy loams. The range of accumulation of deposits on the layer thickness varies from 0 to 1.05 m, while the majority (50%) of the primer layer is formed from 0.1 to 0.73 m thick.
4. In studied streams it was found that their slopes in 17.2% of the cases studied are overgrown with shrubs and isolated trees. The middle zone of slopes is wooded the most densely, where due to the proper moisture regime the most favorable conditions for the development of vegetation are formed.
5. The regulated river beds meandering features occur due to slope deformations. The formation of meanders was identified at 59.1% of the studied streambeds.
6. It was found that the cross section of regulated rivers get parabolic shape due to the ongoing deformation processes. Asymmetry of cross section profiles is characteristic for self-naturalization streams. Meanwhile, forms of natural beds remain heterogeneous with a variety of characteristic forms.

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IMPACT OF HOUSEHOLD ELECTRIC ENERGY USAGE TRENDS ON ELECTRICAL POWER SUPPLY NET POWER FACTOR

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Abstract
The article deals with the research results of the household electric energy usage trends impact on the electric power supply grid power factor. The amount of household appliances has increased tremendously during the last 20 to 30 years, substantially raising the electric energy consumption in private sector. As a serious technological development of household appliances took place, for example, LED lamps, inverters for power supply of TV sets and computers, the introduction of microcontrollers and automated systems, as well as efficiency requirements increase for the devices used in household have led to a reduced specific energy consumption per device and increased overall energy consumption. Analytic and experimental research assured that all these changes have a substantial impact on electric grid quality, particularly on the power factor value. Filed research data proved the theoretical analysis results that there is a trend from minor inductive power factor towards considerably high capacitive power factor, thus reducing power supply grid quality. This trend must be considered seriously by the electric grid operators in order to keep high quality of energy supplied to the end users.
Key words: power factor, electric appliances, household electricity use trends, inductive load, capacitive load.

Introduction

Overall energy consumption in household sector is increasing, and electricity accounts for a significant part of overall household energy consumption not only in Latvia but all over Europe (Raudjärv and Kuskova, 2013; Energy in the Residential Sector 2013 Report, 2013), having a fast growth in the EU 28 countries (Electricity Production, Consumption and Market Overview, 2014). Overall energy consumption in the EU 28 countries reached 1.104 Mt of oil equivalent in 2012, and household accounted for 26.2% (Fetie, 2014). Official sources prove that in Latvia in 2011 household accounted for slightly less than average in the EU countries - just 24.2% of all energy consumed (Latvian Energy in Figures, 2013).

At the same time Latvia showed one of the fastest growth rates in household energy consumption between 2002 and 2012 – average annual growth was 4.00% (Fig. 1.) (Mājokļos izmantotās elektroierīces un elektroierīču vidējais vecums, s.a.).

The main reason for consumption increase could be the increasing number of appliances owned by households – data from Latvia Central Statistics Bureau prove that sales of electronic equipment (e.g. TVs, desktop and laptop computers, mobile phones and kitchen appliances) are stable or increasing, and

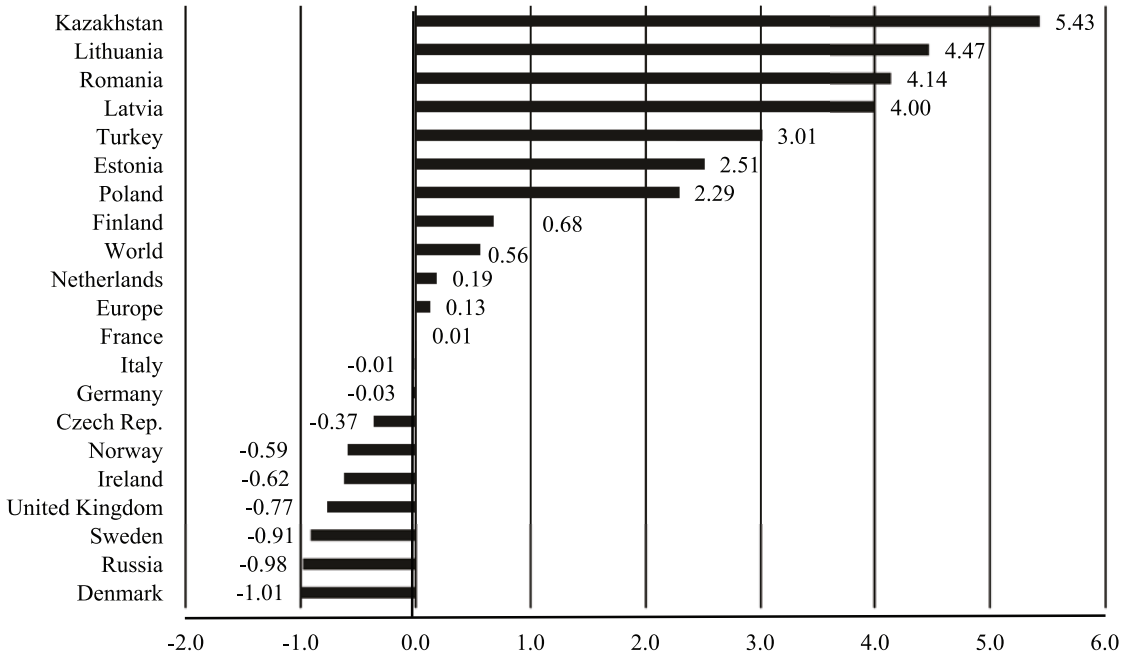

Figure 1. Electricity consumption change per electrified household 2000 - 2012 (% per year).

a large part of households have all basic electric appliances (Mājokļos izmantotās elektroierīces un elektroierīču vidējais vecums, s.a.).

In addition, the rapid technological progress and willingness to live better are the reasons why the appliances are being replaced more frequently than in the past, which also boost electric energy consumption. Increased appliances replacement speed can be caused by shorter newly designed appliances life time and built-in pre-defined product life cycle.

Statistic data show that in Latvia household electric energy consumption increase slowed down during the last three-four years, and showed a drop in consumption (Fig. 2), contrary to Estonia, where the consumption is still growing (Raudjärv and Kuskova, 2013), or the USA, where the overall electricity consumption in household stabilizes although the number of households increases (http://www.eia.gov/consumption/residential).

Although the number of appliances is increasing, due to technology changes energy consumption per appliance has decreased drastically. The sharpest drop took place with the LED technology development – this invention had direct impact on lighting and TV set design, reducing energy consumption on some occasions by more than 5 times (e.g., average electric energy consumption of regular color TV set was 250..300 Wh 30 years ago, but with LED and modern OLED screen technology it dropped below 50 Wh).

Electric energy providers supply energy to end users using electric grid, and current scientific inventions implemented as new devices and products is a challenge for them, because of increasing energy quality requirements and potentially decreasing sales volumes. Technology changes and increased number of household appliances together with changing household lifestyle and daily habits have changed trends in electricity consumption, and also have an impact on electric grid properties.

The power factor is among the main electric grid quality measures. It takes into account the characteristics of the load, and in cases when the load is not only resistance, but also has either inductive or capacitive components, the electric grid is loaded by unnecessary energy, decreasing possibility to deliver enough energy to consumers. The research devoted to the power factor improvement and trends, as well as the control over power factor was provided mainly for industrial grids, as industrial consumers historically were the main electric energy consumers. All Baltic states have established principles of penalties to those industrial consumers, who decrease electric grid power factor - Latvia (MK Noteikumi Nr. 50 Elektroenerģijas Tirdzniecības un Lietošanas Noteikumi, 2014), Lithuania (Цены На Электроэнергию И Тарифные Планы - Для Корпоративных Клиентов - LESTO, s.a.), and Estonia (Типовые Условия Elektrilevi OÜ Сетевого Договора Выше 63 А, s.a.; Прейскурант 0.4 (0.23) кВ - Imatra Elekter, s.a.) have introduced penalties to large industrial users (electric connection current larger than 200 A), who decrease the power factor.

Power suppliers were assuming that power factor in household segment is high ($\cos\varphi > 0.95$), although due to the recent technological changes it is not true anymore. Observations of the latest research trends in electric grid power factor did not reveal any research results evaluating household electric

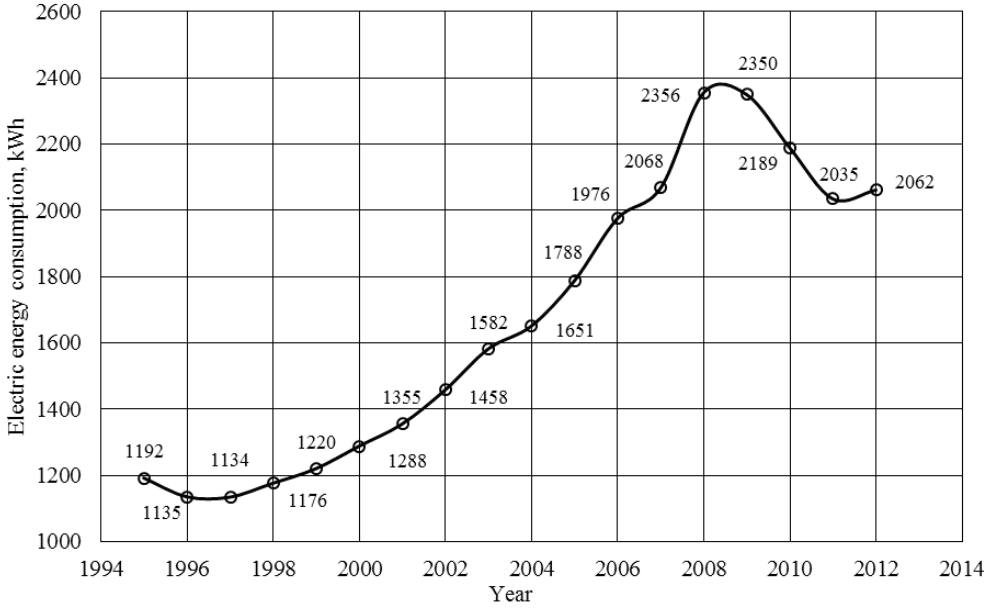

Figure 2. Average annual electric energy consumption in households, Latvia, kWh per household.

energy consumption patterns impact on power factor in the supply grid, although household stake in the electricity consumption is becoming substantial. The necessity to understand the interaction between the household energy consumption patterns, technological changes and electric energy supply grid power factor characteristics has resulted in this research.

Observations prove that households do not care about the power factor and other electricity grid quality issues – they simply consume energy, at the same time requesting more energy and higher quality (stability, availability, etc.). Usually they are not knowledgeable about the electric grid and home appliances interaction. When buying new electrical equipment, they do not care about the grid conditions and the characteristics of the new device load (resistive, inductive or capacitive).

The utility companies - the electricity suppliers, to the contrary, are very cautious about the new reactive energy suppliers into their grid. Their intent always is to establish systems with minimum reactive power.

Industrial electricity users know power factor requirements because of penalties introduced by the electric grid operators. Industrial consumers usually have inductive character of load, because of intensive use of electric motors as mechanical energy suppliers. In order to reduce inductive power factor (to reduce financial penalties), they use capacitive compensation devices – static and dynamic.

The latest trend in the electric grid development is an increased use of insulated cables instead of free air wires because of reduced possibility to break the supply line. Increase in cable lines substantially increase capacitance of electric grid, requesting the utility companies to use additional inductive loads, especially during the off-peak loads and time with low electricity consumption, in order to reduce reactive losses in the grid.

Theoretical research of household electric appliances use in Latvia was done in order to understand the trends in reactive energy changes in household electric energy supply grid. No research results made by other researchers were revealed – currently this is among the first results published in this area.

Materials and Methods

The main research method used in this research was theoretical data analysis about the trends in electric appliances development technologies and consumption trends of households in Latvia. The theoretical analysis results were compared with experimentally acquired long term measurements of the power factor in several household areas of Latvia.

Data about the intensity of electric appliances usage and their power characteristics were collected through on-site observations, technical data from electric appliances producers, and research results from other data sources (state statistics bureaus, etc.), for the time period from 1950 to 2013.

Experimental data collection was provided by using Automated Electric Energy Accounting system (AEEAS), which includes smart energy counters or specialized reactive energy measuring and accounting devices (e.g., PTD 3G, produced by 'Elgama elektronika' (Lithuania)).

The power factor calculation formula used for the analytic calculations is the following:

$$cos\varphi = \frac{P}{S} = \frac{P}{U \cdot I}, (1)$$

where
$cos\varphi$ – power factor;
P – active power, W;
S – apparent power, VA;
U – electric grid voltage, V;
I – current in electric grid, A.

Equation (1) can be used to find the instant power factor. As the households use different household appliances together and individually, power factor is changing all the time. So the best way to find critical values of power factor would be to find out precise daily load schedule of all household appliances, and then to observe which appliances with reactive power component are used in parallel, thus reducing the power factor.

This approach is very difficult to implement, because households usually do not think about electric grid loads, and very often even do not know what equipment is consuming electricity and when this happens. Researchers are trying to collect indirect data (Laicāne et al., 2013), to understand the trends in electricity consumption, but the results are just approximate.

In order to find the household power factor indirectly, and to calculate the critical power factor using the information about the household appliances used and available households in Latvia, the equation (2) was developed by modifying the basic power factor equation for more than one appliance with a different power factor and active power consumption:

$$cos\varphi = \frac{\sum(P_i)}{\sum \frac{P_i}{cos\varphi_i}}, (2)$$

where
$cos\varphi_i$ – power factor of the indexed household appliance;
i – household appliance index;
P_i – active power of the indexed household appliance, W.

Table 1

Power factor of some electrical equipment, used in the household development

Electrical equipment	Load type (R/L/C)*	Active power used P, kW	Power Factor
106 cm LED TV	R/C	0.04	0.80
Color TV set (vacuum tube)	R/L	0.30	0.70
Washing machine (Hazra, 2012)	R/L	1.5	0.57
Water pump	R/L	0.25	0.40
Notebook	R/C	0.05	0.58
Laptop	R/C	0.25	0.56
Wi-Fi rooter	R/C	0.005	0.45
Stationary radio (lamp)	R/L	0.20	0.7
Stationary radio	R/L	0.05	0.80
Refrigerator	R/L	0.1	0.80
Microwave owen	R/L	0.15	0.93
Kitchen ventilation	R/L	0.1	0.53
60 W incandescent bulb	R	0.06	1.00
15 W compact fluorescent bulb	R/L	0.015	0.58
3 W LED bulb	R/C	0.003	0.44
Mobile phone charger	R/C	0.01	0.62
Halogen heater	R	1	1.00
Water kettle	R	2.5	1.00

* R - Active power load, L – Reactive (Inductive) power load, C – Reactive (Capacitative) power load.

Data of active power and the power factor for all major home appliances were collected from their technical specifications for the time period from 1950 to 2013. A sample of the major appliances used in the equation (2) is presented in Table 1, which was developed using (Mājokļos izmantotās elektroierīces un elektroierīču vidējais vecums, s.a.).

Results and Discussion

The results of equation (2) and household appliances power factor and active power data application are presented in Fig. 3 by making power factor measurement simulation of that time electric appliances used.

As no comparable research results were found, the research results can be mentioned as unique in this field.

Trend line describing the simulated power factor changes in household shows stability and high values of power factor ($\cos\varphi > 0.9$) up to 1990ies, still showing a substantial part of inductive load growth (RL). Then (starting with 1990ies) the calculated power factor shows the trend of decrease, with a substantial drop in the last decade. This shows a serious impact of the technological trends in household appliances, and especially the recent capacitative (RC) load growth, which is the result of LED technologies and inverters development and introduction into household.

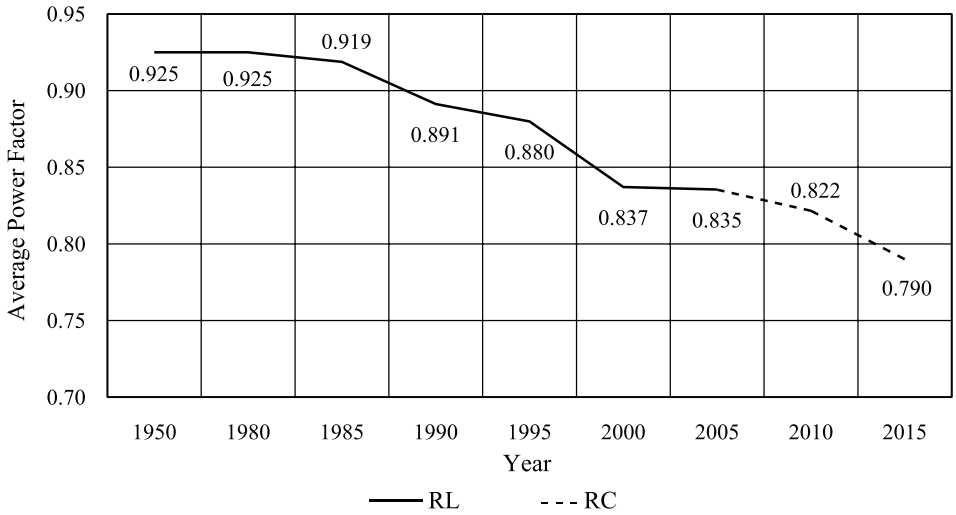

Figure 3. Critical power factor calculation results for household appliances used in Latvia.

As the electricity consumption in Latvia still has a place to grow, this recent trend towards RC reactive load should be taken into account by utility companies, who want to supply high quality electricity to suppliers, and who also want to reduce unnecessary investments in overpowered transformers and electric grid elements, which can only be used for reactive power transfer. Thus, the compensation mechanism of RC load must be developed, or at least considered as a factor to be taken into account in future.

Conclusions

The power factor of household electricity supply grid has changed substantially during the last decade from 2005 to 2015. It not only decreased by number, but also changed the main characteristic – substantial capacitative loads entered the market. Further research is needed to understand the impact of such changes on all electric grid components, including commutation and protection devices, as well as their control principles.

The power factor in households has changed by load type from active-inductive (RL) to active-capacitative (RC) and its value to 0.790 in 2015 (Fig. 3).

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TEMPERATURE PROTECTION METHODS OF INDUCTION MOTOR

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Abstract
In conditions where induction motors are frequently started, overloaded and used in high inertia applications with long starting times, supplied from frequency converter, a temperature protection systems are more reliable to protect induction motor stator winding against thermal overloads. There are different types of temperature protections - thermostat, PTC thermistor, resistance temperature detector (RTD) and thermocouples, so it is important to know the properties of each type to choose an adequate protection system. Analyses of temperature sensor properties and their advantages and disadvantages show, that PTC thermistor is a cost-effective temperature protection solution, but for medium and high voltage induction motor protection RTD are commonly used. A virtual model has been represented to simulate the temperature sensor thermal time constant under different thermal conductivity and thickness of winding magnet wire insulation.
Key words: induction motor, protection, temperature sensor, thermal time constant, heating.

Introduction

Today in industrial and domestic environments moving and rotary mechanisms are mainly driven by electric motors, 90% of which are induction motors (IM). Because of simple construction, maintenance and variable frequency drive (VFD), IM with squirrel cage rotor are widely used. Three-phase induction motors (IM) are used as drive motors in pumps, lifts, cranes, compressors, fans, crushers, mills, cranes, conveyors, etc. Annual IM failure rate is estimated at 3-5% per year, and in extreme cases, up to 12% (Venkataraman et al., 2005), which cause essential direct and technological losses.

The failures of IM may be classified as following:

- electrical faults;
- mechanically related;
- environmental impact;
- other reason.

Statistics show that overheating of IM parts during operation is one of the common reasons of an IM failure (Venkataraman et al., 2005). Stator windings insulation is most sensitive to thermal overheating. Exceeding the thermal limit of the insulation will result in acceleration of the oxidation process that will decrease the insulation life or even cause IM failure. It is well known that the duration of insulation life is decreased by half for each increase of 10 °C temperature above its thermal limit temperature. In the past decades improved materials with better electrical, mechanical and thermal properties allowed manufactures to reduce the materials within motor and production costs of the IM (Glew, 1999). For example, reduced cross section of magnet wire (winding wire) increases the copper losses in IM winding thus increasing winding heating. Therefore, it is important to select an adequate protection system to protect critical IM parts from overheating and prevent failure.

One of effective techniques to detect contamination is direct winding temperature monitoring to archive temperature sensor measurements. Trending the stator winding temperature over time, and if there is a gradual increase in temperature, it may be cooling problems caused by contamination (Culbert, 2008). An operation review (Barbinyagra, 2007) from ammonia factory shows, that majority of IM failures are caused by vibration, overload and hazardous environment. To increase cycled loaded IM reliability, a temperature monitoring system with temperature sensors was installed in the factory. Experimental study on heating of 2.2 kW IM fed by pulse width modulation inverter (Benhaddadi et al., 1997) shows, that at rated load stator winding temperature is 7 °C higher than if IM is fed by sinusoidal voltage. Inverter fed IM stator winding insulation also needs to be resistant to partial discharge (PD). Therefore, magnet wires of IM contain nano sized metal oxides to impart PD resistance (Lynn et al., 1985). This very thin layer of metal oxide material is heat shock and thermal cycling sensitive and when the layer is cracked, the PD resistance property is reduced (Stone and Braswell, 2004). In high inertia application and application, where the rotor can lock or stall, the heating process of IM parts is very rapid and direct temperature sensing is considered to be more reliable than stator or armature current sensing. Studies (Staton et al., 2009; Gedzurs et al., 2014) show, that for low power IM (2.5 kW and 1.1 kW) the temperature rise speed is 6.2 $°C \cdot s^{-1}$ and 5 $°C \cdot s^{-1}$ respectively under locked rotor conditions. These studies show that in the mentioned operation conditions a direct temperature measurement of IM winding is more reliable for an adequate IM protection.

The objective of the study is to analyze temperature sensor properties for temperature protection of induction motors under extreme overloads and hazardous operation conditions.

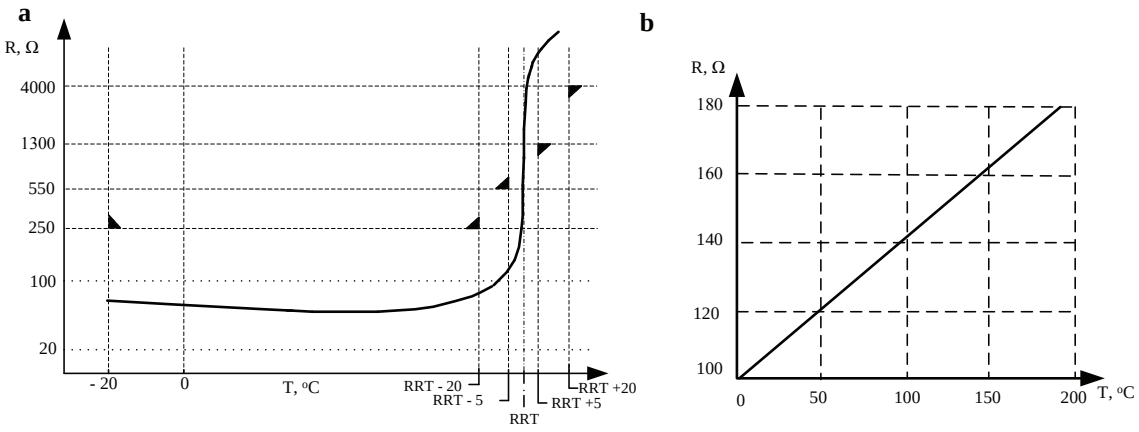

Figure 1. Temperature sensors resistance - temperature characteristics:
a - PTC thermistor; b - RTD PT100 sensor.

Materials and Methods

Induction motor temperature protection monitors the stator winding temperature by directly measuring the temperature using embedded temperature detectors. There are different types of temperature detectors (sensors), that are used for electric motor protection - thermostat, PTC thermistor, resistance temperature detector (RTD), thermocouples.

Winding thermostat is a temperature dependent device that uses bi-metallic strip to change the position of a pair of contacts at the preset rated response temperature. When temperature preset level is exceeded, the contacts switch a control device, relay or contactor. The control voltage can be applied directly to the thermostat, which makes a tripping mechanism unnecessary. The disadvantages of the thermostat are - long thermal delay, tripping temperature can be affected by careless fitting, large size compared to modern sensors.

Thermistor is a small resistance sensor with non-linear resistance - temperature relationship. There are two types of thermistors - negative temperature coefficient (NTC) and positive temperature coefficient (PTC) thermistors. The PTC thermistors are commonly used in IM motor protection systems. The resistance at normal temperature is relatively low and remains nearly constant up to the rated response temperature (RRT). As the RRT is exceeded, the gradient of the resistance increases sharply, giving the PTC thermistor a high sensitivity to small changes of temperature. At the set point, a temperature rise of a few degrees results in a large increase in resistance. The PTC thermistor resistance characteristic is shown in Figure 1(a). The resistance change of the PTC thermistor is monitored by a thermistor protection relay. The advantages of the PTC thermistor are small size, which allows them to be installed in direct contact with the stator winding and a low thermal inertia, which gives rapid and accurate response to the winding temperature changes.

Resistance temperature detectors (RTD) monitors temperature by measuring the change of resistance of an accurately calibrated resistive sensor, usually made of copper, platinum or nickel, but platinum RTD PT-100 is commonly used for stator winding temperature monitoring. RTD sensors can be of the wire wound type, or can be of the metal film type, which are lower cost with faster response but their characteristics can deteriorate over a time. A RTD has a linear resistance and temperature trend (Figure 1(b)), usually 0.4 $\Omega \cdot °C^{-1}$ for PT-100 sensor. A very sensitive instrument, based on Wheatstone bridge, is required to measure the small changes in the resistance of RTD. The instrument passes a small excitation current through the resistive sensor.

Thermocouple is a temperature sensor that consists of two dissimilar metals, joined together at one end and for a junction (a couple). There are different types of thermocouples depending on the combination of metals used to make the sensor. Type J (iron and constantan), K (Chromel and Alumel), T (copper and constantan) and E (Chromel and Alumel) thermocouples are commonly used. The voltage-versus-temperature relationship of most types of the thermocouple is not linear. Thermocouple measurement devices need a cold junction compensation. The size of thermocouple is small so it can be placed between windings in small power IM.

To simulate changes of the thermal time constant of the temperature sensor at different IM stator winding insulation parameters, thermal conductivity and thickness, a virtual model is represented in MATLAB SIMULINK environment. The mathematical model is obtained from the following mathematical operations. Assuming that at start time there is no heat transfer from the sensor to environment, the heat transferred from the winding to the temperature sensor is described by the following equation:

$$\theta_t = h \cdot S \cdot (T_w - T\ ),\ \theta_a = c \cdot m \cdot \frac{dT}{dt},$$

$$h \cdot S \cdot (T_w - T) = c \cdot m \cdot \frac{dT}{dt}, \quad (1)$$

where θ_t – increment heat from windings to sensor, W;

θ_a – sensors accumulated heat flow, W;
h – heat transfer coefficient, $W \cdot (m^2 \cdot °C)^{-1}$;
S – heat transfer surface, m^2;
T_w – winding temperature, °C;
T – sensor temperature, °C;
c – sensor specific heat, $J \cdot (kg \cdot °C)^{-1}$;
m - mass of the sensor, kg.
t - time, s.

According to expressions (1) a differential equation of sensor`s heating in normal form is as follows:

$$\tau_s \cdot \frac{dT}{dt} = T_w - T, \quad (2)$$

where τ_s – sensor thermal time constant, s.

From equations, (1) and (2) it is possible to get thermal time constant τ_s of the temperature sensor:

$$\tau_s = \frac{c \cdot m}{h \cdot S} = \frac{c \cdot m \cdot R}{S}, \quad (3)$$

where $R = 1 \cdot h^{-1}$ - thermal resistance, $(m^2 \cdot °C) \cdot W^{-1}$.

Results and Discussion

Table 1 shows comparison of the temperature sensors. PTC thermistors have high sensitivity to small temperature changes in the rated response temperature range and due to higher resistance of the thermistor, lead wire resistance error has a very small effect, therefore, only two lead wires are required. A PTC thermistor can be used either for alarm or trip function. If both functions are needed, then the one thermistor is used for alarm and the second one for trip. Like RTD, thermistor needs a constant current/ voltage source, but one controller can been used for several thermistors connected in series. This makes a PTC thermistor a cost-effective temperature protection method. RTD among above mentioned temperature sensors has the best accuracy due to linear resistance-temperature relationship and the highest stability. Lead wire resistance and self-heating errors affect the RTD measurements. Three lead wire configurations will been used to decrease the lead wire resistance effect and four lead wire configurations for very precise measurement needs. A Wheatstone bridge based instrument needs to measure small resistance changes of RTD. These facts make RTD a more expensive temperature protection system. Thermocouples are simple, self-powered and have wide temperature measurement range, but for temperature protection of medium and high voltage induction motors the RTD are more conformable, because of their better accuracy and stability.

Thermal time constant of temperature sensor.

As mentioned in (Venkataraman et al., 2005), due to thermal inertia of temperature sensors, the temperature protection system cannot react to fast heating process of the IM stator winding, such as locked rotor or prolonged starting of IM modes. It is important to know the thermal time constant of the temperature sensor to choose properly settings. Heating process model of the temperature sensor, placed on the surface of end winding, shown in figure 2 (Fraden, 2010).

To evaluate the thermal time constant of the temperature sensor, the technical data of a Minco

Table 1

Comparison of temperature sensors

Sensor	PTC Thermistor	Platinum RTD	Thermocouple
Advantages	- sensitivity; - 2 lead wires required; - can connect several thermistors in series to one relay; - cost-effective; - low thermal mass.	- accuracy; - linearity; - stability.	- self-powered; - less expensive than RTD; - small size and thermal mass; - wide temperature range - simple.
Disadvantages	- non-linearity; - each thermistor has pre-set switch point (can be used only for alarm or for trip); - current/voltage excitation.	- lead wire resistance error (3 or 4 lead wire required); - generally high response time for wire-wound RTD; - low vibration resistance; - self-heating; - current/voltage excitation; - requires very sensitive instrument to measure.	- cold-junction compensation; - non - linearity for most types; - least sensitive; - least stable; - low output signal.

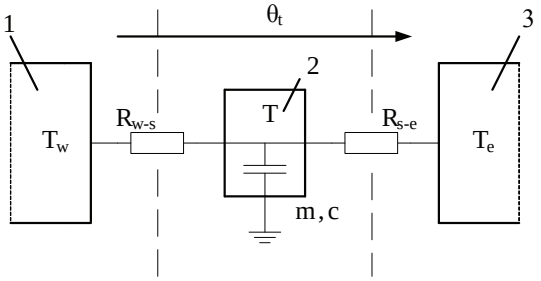

Figure 2. Heating process model of the temperature sensor:
1 - Stator winding of IM; 2 - temperature sensor; 3 - air gap between stator winding and IM case; R_{w-s} - thermal resistance between winding and temperature sensor; R_{s-e} - thermal resistance between temperature sensor and air gap.

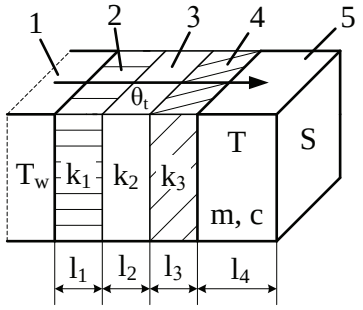

Figure 3. Simplified heating process models of the temperature sensor:
1 - stator winding of IM; 2 - magnet wire insulation; 3 - winding impregnation; 4 - sensor element insulation; 5 - sensor; l_1, l_2, l_3 - thickness of insulation layer; l_4 - sensor thickness; k_1, k_2, k_3 - thermal conductivity of insulation layers.

thin-film RTD stator temperature sensor (model S200050PD) have been chosen as an example. The temperature sensor - PT100 has the following parameters: sensing element dimensions - 2 mm x 2.3 mm, thickness - 2 mm, insulation material of the sensing element (epoxy glass). If the sensor has been placed on the end winding surface and the contact is ideal, then there is a layer of insulation between stator winding and the sensor. The insulation layer consists of a winding magnet wire insulation, winding impregnation and sensor insulation layer. A simplified heating model of the sensor is shown in Figure 3. The thermal resistance of the each insulation layer can be calculated using equation 4.

$$R = \frac{l}{k}, \tag{4}$$

where k – thermal conductivity, W·(m ·°C)$^{-1}$;
l – insulation layer thickness, m;

Thermal conductivity of sensor epoxy glass insulation layer is k_3 = 0.343 W·(m °C)$^{-1}$ (Sarvar et al., 1990). Thermal conductivity of magnet wire and winding impregnation depends on the quality of insulation. According to (Dorrel et al., 2006) thermal conductivity $k_1 = k_2$ = 0.2 W·(m ·°C)$^{-1}$ for normal raisin and $k_1 = k_2$ = 0.2 W·(m °C)$^{-1}$ for high performance insulation material. Thermal conductivity of platinum k_4 = 71.6 W·(m °C)$^{-1}$. According to (Stone and Braswell, 2004), the insulation thickness of magnet wire l_1 = 0.05 - 0.15 mm and thickness of winding impregnation usually is l_2 = 0.025 - 0.05 mm and rarely l_2 = 0.38 - 0.5 mm. Since the thickness of insulation layer of the temperature sensor is not given in specifications, it was assumed to be equal to the average thickness of the magnet wire insulation, i.e., l_3 = 0.1 mm.

Using equation (3) and parameters of insulation materials and the temperature sensor, a virtual model in MATLAB SIMULINK has been developed (Figure 4) to simulate effects of different insulation layer thickness and thermal conductivity on the thermal time constant of the temperature sensor. Thermal time constant τ_o = 4.69 s at the following parameters $l_1 = l_2$ = 0.05 mm, l_3 = 0.1 mm, $k_1 = k_2$ = 0.2 W·(m °C)$^{-1}$.

Figure 5 represents simulation results of the thermal time constant of the temperature sensor depending on the thermal conductivity k1 (0.2 - 1 W (m °C)$^{-1}$) of magnet wire insulation. At k1 = 0.2 W. (m °C)$^{-1}$ thermal time constant is τ_o = 4.69 s, at k1 = 1 W (m °C)$^{-1}$ - τ_s = 3.55 s, so the thermal time constant decreased by $\Delta\tau_s$ = 1.14 s. As Figure 5 shows the trend is not linear and from k_1 0.2 - 0.4 W·(m °C)$^{-1}$ thermal time constant change is the biggest $\Delta\tau_s$ = 0.71 s.

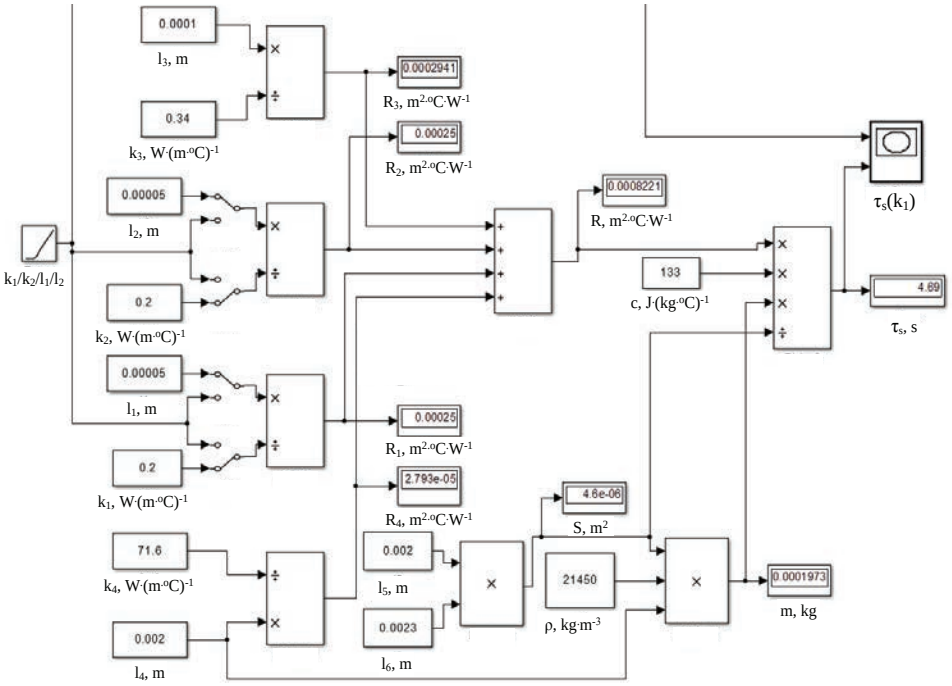

Figure 4. Simulation block diagram of thermal time constant of temperature sensor.

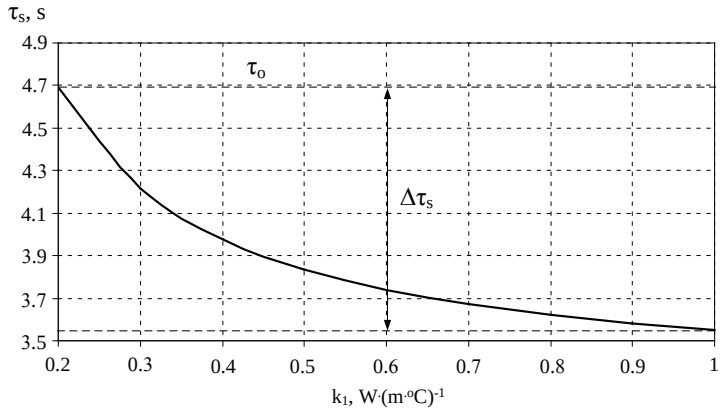

Figure 5. Simulation results of thermal time constant of temperature sensor at different thermal conductivity k_1 values of magnet wire insulation.

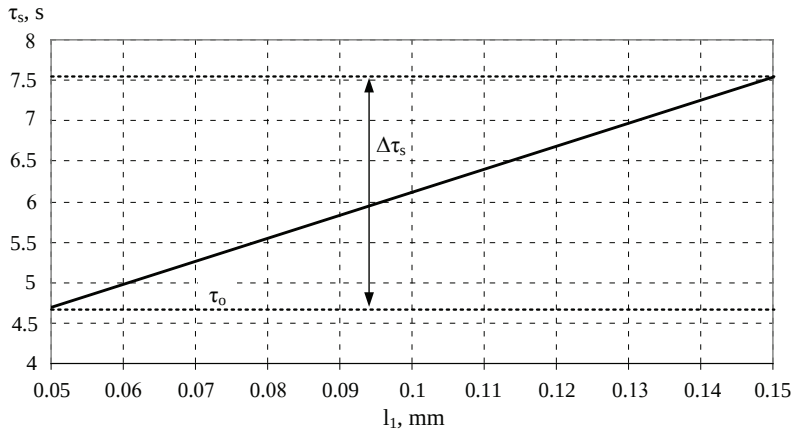

Figure 6. Simulation results of thermal time constant of temperature sensor at different insulation layer l_1 values.

Figure 6 represents simulations results thermal time constant of the temperature sensor τ_s and magnet wire insulation wire thickness l_1 relationship. Thermal time constant increase is linear from τ_o = 4.69 s at l_1 = 0.05 mm τ_s = 7.54 s at l_1 = 0.15 mm. The thermal time constant increased by $\Delta\tau_s$ = 2.85 s or by 64%.

Simulation results show that, the thickness changes of magnet wire insulation have a bigger influence on the thermal time constant than that of thermal conductivity.

Conclusions

1. Analyses of PTC thermistor sensor properties show that it requires mostly two wire leads, is very sensitive to temperature changes at rated response temperature range: several thermistors can be connected to one controller, small size of the sensor and low thermal mass, which makes PTC thermistor a cost-effective solution for induction motor temperature protection.
2. Analyses show that to measure small resistance changes of RTD a sensitive instrument and three or four lead wires are required for precise measurements, but accuracy, linearity and a long-term stability of the RTD makes it a conformable solution for temperature protection of medium and high voltage induction motors.
3. Thermal time constant simulation results of the thin-film RTD PT100 temperature sensor show that at stator winding magnet wire insulation thickness l_1 = 0.05 mm the thermal time constant is τ_o = 4.69 s and at insulation thickness l_1 = 0.15 mm thermal time constant increases to τ_s = 7.54 s. Therefore, insulation material thickness between the stator winding and temperature sensor has an essential impact on the thermal time constant of small size temperature sensors.

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QUALITY ASSESSMENT OF ELECTRONIC LEARNING MATERIALS

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Abstract
Information and communication technology combined with multimedia and networking have enabled development of e-learning. E-learning opportunities expand access to education, without the learner's social, economic and geographical barriers. The main question in this situation has to take into account the quality of e-learning materials. The aim of this research was to explore the definition of the quality and scientific research literature on e-learning quality, and identify the quality influencing factors. This article looks at quality standards and summarizes the existing literature on the quality aspects of electronic materials. During the research was analyzed the literature of the last 13 years. Findings showed that the quality evaluation model covers a wide scale — from one e-course to e-learning system implementation quality aspects. The quality of electronic learning material can be measured by technical, pedagogy and content criteria. The technical quality of the e-material (text, picture, video, sound recording, visual presentation, multimedia, etc.) is influenced by many factors and specifying for each type. The research should be continued to indicate the quality criteria for each type of e-materials including the degree of importance.
Key words: e-learning, quality, e-material.

Introduction

E-learning has taken an important role in the higher education and distance learning industry. E-learning can support students who are employed and need access to study materials at a distance. Accordingly increases the need to update the e-learning quality assurance procedures. Literature review revealed that online training and its effectiveness has been widely studied (e.g. Henderson, 2005; Henderson and Chapman, 2007; Jung, 2010). There is a lot of research that affects the quality issue, but there is not a single point of view on this matter. Studies on the measurement of the quality problems are still valid. Quality assurance is an important task in any sphere, including education and e-learning. The challenge is in the issue of quality, which is expressed in different views and approaches (Stracke, 2006).

The first International Organization for Standardization (ISO) quality standards of training, education and training ISO / IEC 19796-1 (International Organization for Standardization / International Electrotechnical Commission) was developed, approved and published in 2005. E-learning standards is a challenge that affects two important areas — pedagogy and technology. Although in recent years, both the US and Europe in several standardization consortiums have defined an open technology interoperability standards, the emphasis is on the technical and substantive aspects of the didactic concept only slightly affected (Baumgartner et al., 2002). Research on the e-learning quality in higher education has identified several aspects and criteria for quality assessment, but they are different (Ehlers et al., 2005; Ehlers, 2007; Shah, 2013). The problem relates to the fact that e-learning affects many quality indicators in different dimensions — various stakeholders (the student and lecturer), technology, educational content, etc. and each dimension has its own status.

The aim of this article was to explore the literature about e-learning quality in general and to identify the real situation, which might help to improve the quality of e-learning.

Materials and Methods

Monographic method has been used for this article. Information was searched in electronic databases and printed publications, published in Latvia and Europe. The study analyzes the literature of the period from 2000 to 2013, except one source about the definition of quality from 1980. The general term 'quality' characterisation used quality management standard ISO 9001:2008, but the e-learning quality performance — quality standard ISO / IEC 19796-1. Broad spectrum is discussed and analyzed in the scientific research literature on studies of how to identify the factors that influence e-learning quality.

Results and Discussion

Quality

Quality is defined as a set of product or service features and characteristics related to their ability to satisfy certain needs of product or service. According to ISO 9001, quality is the degree to which a set of inherent characteristics fulfills requirements (ISO, 2008). Quality development in its broad sense can be defined as follows - 'Quality is of fundamental importance, this is true over all the borderlines of organizations, branches and political economies' (Stracke, 2006).

The definitions of quality vary and commonly reflect the different perspectives of the individual and of the society. The quality of a product is its ability to satisfy the needs and expectations of the customer.

D. F. Davok defines quality as a set of attributes related to a specific object or process that allow to compare with a set of benchmarks (Davok, 2007 cited from Casanova et al., 2011). This definition alludes to a comparison between the object evaluated and a set of criteria related to quality (Ehlers, 2004). In education the term quality is a client-oriented concept in which quality requirements are defined through a participation process between clients and providers. J. M. Pawlowski defines quality as 'appropriately meeting the stakeholders' objectives and needs, which are the result of a transparent, participatory negotiation process within an organization' (Pawlowski, 2007).

The quality itself is too abstract concept and therefore is defined in accordance with the given situation and context, taking into account the individuals involved. It is important to determine the relevant aspects and requirements to determine appropriate criteria. In order to obtain a common understanding of the quality, it is necessary to reach a consensus between the different views. This, in turn, would allow to avoid the sometimes contradictory meaning of quality and the needs of stakeholders (Donabedian, 1980; Deming, 2000).

Quality can be used to confirm that a specific process or object is made with quality, or it can be used to improve the process or object.

The process of the adoption, implementation and adaptation of quality development can be divided into three steps based on three different levels of quality development concept (Hildebrandt et al., 2006):

- Level of the individual person — the objective is to ensure that every stakeholder knows what quality development means and is standing for,
- Level of the organization — develop a quality vision and a common understanding of the quality objectives and the resulting mission statements, each individual is aware of the necessary base for this,
- Integration of quality development involving all stakeholders — to look for ways to improve organizational vision and quality objectives into the educational and business process to become a part of the daily business, all stakeholders are important for ensuring their motivation and contribution.

M. Jara and H. Mellar (2007) emphasize that quality assurance makes a comparison with a predetermined standard and quality improvement and is related with the relation between the current benchmark and the pathway to achieve this benchmark. For e-learning, and because of its characteristics, quality can be related to all the processes, products and services supported by information and communications technology (ICT) (Ehlers et al., 2005; Pawlowski, 2007). U. D. Ehlers prefers to address the importance of understanding what quality is for learners in e-learning suggesting some preferences for each specific target group of students (Ehlers, 2004). In conclusion, quality in e-learning must involve the different actor's interaction and participation and, at the same time, must introduce two different perspectives of quality: to assure that quality exists and to be used as a tool to its improvement (Casanova et al., 2011).

The quality of e-learning has often been viewed with skepticism and been the target of criticism. This criticism has focused on the lack of physical interaction (Yeung, 2003), technical problems (Zhao, 2003), or a technological and aesthetic focus instead of an educational one (Barbera, 2004). Other research reports show that the course delivery medium is rarely the determining factor for quality, or that online education in itself can be a quality enhancement factor in terms of accessibility, collaboration or community-building, for either teachers or learners (Connolly et al., 2005; Jara and Mellar, 2007; Dondi and Moretti, 2007).

E-learning quality assurance is a faculty evaluation process that 'judges, measures, or assesses the quality of the development and delivery of online courses/ learning environments focused on appropriate design and best practice, and is aimed at self-improvement ensuring quality instruction in a non-threatening way' (Quilter and Weber, 2004). According to Q. Wang, some of the main criteria are:

- Learning outcome assessment,
- Curriculum and instructional development,
- Institutional commitment,
- Student support,
- Faculty support (Wang, 2006).

Quality Standards

In the field of quality standards there are some formal and informal international standardization organizations. The most popular of formal standardization organization is ISO. As an informal standardization organizations can be mentioned the community and professional associations which develop industry specifications, for example, the Institute of Electrical and Electronics Engineers (IEEE), Instructional Management Systems Global Learning Consortium (IMS GLC). The above mentioned organizations standards are generally developed and adapted to the specific situation, while the consortium developed recommendations are often only available to consortium members and are not published. Below will be discussed standards relating to the quality of e-learning.

ISO 9001:2008 Quality Management Standard. ISO 9000 is a family of standards for quality management systems. ISO 9000 is maintained by the International Organization for Standardization and

administered by accreditation and certification bodies. ISO 9001 is one of the standards in the ISO 9000 family. ISO 9001:2008 Quality management systems — Requirements (ISO, 2008).

ISO 9001:2008 should be applied to the process approach of quality management systems development, implementation and efficiency in order to enhance the customer satisfaction with their requirements.

Characteristic is the distinguishing feature (ISO, 2008). It can be:

- Physical (mechanical, electrical, chemical, biological),
- Sensory (related to smell, touch, taste, sight, hearing),
- Behavioral (courtesy, honesty, veracity),
- Temporal (punctuality, reliability, availability),
- Ergonomic (physiologically characteristic, or related to human safety),
- Functional (e.g. maximum speed of an aircraft).

However, quality characteristic inherent characteristic of a product, process or system is related to a requirement. Standard does not describe how to take measurements in the field of education.

ISO/IEC 19796-1:2005 Information technology — Learning, education and training — Quality management, assurance and metrics. This is the first ISO quality standard of training and education, and training. The ISO/IEC 19796-1 standard was developed by the Working Group 5 'Quality Assurance and Descriptive Frameworks' of the standardization committee ISO/IEC JTC1 SC36 (International Organization for Standardization / International Electrotechnical Commission Joint Technical Committee 1 — Information Technology — Subcommittee 36 — Information Technology for Learning, Education, and Training). ISO/IEC 19796 is a formal standard for quality management and quality assurance in education and training, composed of several parts (ISO, 2005). The first part provides a common framework available to the critical properties, characteristics and metrics for quality to understand, describe and specify by existing approaches, concepts, specifications and terms for the education and training to be harmonized. The reference process model is Reference Framework for the Description of Quality Approaches (RFDQ) (Stracke, 2007a). The quality standard contains the reference process model RFDQ to help stakeholders in learning, education, training, and especially in e-learning or blended learning to document and (re-)define their everyday business and processes. It will be shown that the reference process model can serve as a valuable instrument for the implementation and the establishment of quality development in learning, education, and training (Stracke and Hildebrandt, 2007; Stracke, 2010).

This standard is an instrument for developing quality in the field of e-learning. It consists of three parts:

- A description scheme for quality approaches,
- A process model as a reference classification,
- Reference criteria for evaluation (Pawlowski, 2007).

The ISO/IEC 19796-1 standard is a basic model or road map for educational organizations and has to be adapted to each organization's specific context. However, the standard does not contain detailed guidelines of how to use the model.

The reference process model covers the whole e-learning or blended learning cycle and therefore it can be used to describe any offer of learning, education and vocational training scenarios. The reference process model can be characterized by the following aspects (Stracke, 2007b):

- Integration,
- Completeness,
- Openness,
- Adaptability,
- Uniqueness.

It is important to note that the reference process model does not include any regulations about the sequence of the processes or interdependencies between them or any specifications on its implementation. It serves as an open descriptive framework that always needs the adaptation to the organization, the educational context and the given situation.

Quality standards are not able to guarantee high quality and success. Any standard should be seen as a set of recommendations to be adapted to the particular situation. By adapting quality standards correctly, customers benefit from the significant advantages in the long run.

The European Association for Quality Assurance in Higher Education (ENQA) aims to achieve integration across the European Union (EU), but it faces a long journey with many starting points (Grifoll et al., 2010). Points of difference include: whether the function of a quality assurance system is to check compliance with standards or to promote quality enhancement; the extent to which external oversight is required; and the applicability to e-learning compared to face-to-face contexts (Inglis, 2005; Jara and Mellar, 2007). EU policy documents that affect the e-learning are: the European Commissions e-Learning Action Plan, the European Unions e-Learning Programme, the European Commissions Lifelong Learning programme and Digital Strategy Programmes. All these documents are intended to increase the new multimedia technologies for education and update the importance of lifelong learning. E-learning quality in these documents is not accessed. Instead they refer to

the arguments and initiatives to promote e-learning. This appears to be the common pattern in EU initiatives related to e-learning.

E-material and Quality Assessment

D. Dinevski has subdivided the electronic learning material into three types: technical parts, learning units, and learning entities (Dinevski et al., 2010). The technical details consist of:

- Text,
- Picture,
- Animation,
- Video,
- Sound recording,
- Programme supported presentation of the contents (Dinevski et al., 2010).

If the technical units provides with a didactic description to turn into a learning and it is the most useful material. For its part, the e-material quality assessment criteria can also be divided into three groups — pedagogy, content and technical implementation requirements. Successfully defined quality requirements give the course developers unlimited choice of teaching methods, training materials, or use of technical tools (Judrups, 2010).

Teaching units didactic quality is probably the most important point in education, but in this study it is not discussed. Didactic quality assessment focuses on the learning content — connections between learning objectives, content, methods, and the student.

In general, electronic material has to undergo similar procedures as comparable classic material if it is to be certified as a learning aid, textbook, or supplementary material. Practice shows that customer composes the quality of services. M. Badri et al. (2005) offers to use five criteria for the assessment of the service:

- Tangibility: the appearance of physical facilities, equipment, personnel and communication materials,
- Reliability: the ability to perform the promised service dependably and accurately,
- Responsiveness: the willingness to help customers and to provide prompt service,
- Assurance: the knowledge and courtesy of employees and their ability to convey trust and confidence,
- Empathy: the provision of caring, individualized attention to customer. Customer Satisfaction.

E-material may be performed in accordance with the technical implementation and compatibility evaluation. E-material quality of the technical parts (text quality, graphics quality, the quality of visual presentation, use of multimedia) is influenced by many factors: legibility and clarity of the text, grammatical correctness of the text, consistent use of style, organization and clarity of presentation, structuring text, hyperlinks, etc. Others measure the quality of e-materials is important not only for technical quality, but to achieve learning objectives use methods and technologies.

A Model for Quality Assessment of Electronic Learning Material, developed by a group of e-learning experts established by the National Education Institute of the Republic of Slovenia, proposed that e-material could be evaluated according to the following elements: technical implementation and compatibility evaluation (Dinevski et al., 2010). Those elements of e-materials are to be focused on things that specifically determine the quality of production, installation, upgrading, and uninstallation in different systems and environments: availability of learning materials, installation, registration, starting the programme, use of material; end of use (Khazaaleh et al., 2011). To evaluate e-materials it is recommended to pay attention to three things:

- Description of the material with metadata,
- Technical evaluation,
- Content and didactic evaluation.

Swedish National Agency for Higher Education (E-learning..., 2008) has defined a model for quality assessment of e-learning. This model is made up of ten quality aspects which we consider crucial when assessing quality in e-learning:

- Material/content,
- Structure/virtual environment,
- Communication, cooperation and interactivity,
- Student assessment,
- Flexibility and adaptability,
- Support (student and staff),
- Staff qualifications and experience,
- Vision and institutional leadership,
- Resource allocation,
- The holistic and process aspect.

The quality aspects are thematic areas, each with a set of specific e-learning problems and issues. For each quality aspect, 3–4 quality criteria have been developed. These criteria are recommendations for concrete measures for dealing with the problems and issues identified at an institutional level.

Educators believe that a combination of all these aspects is needed — and not only as the sum of the different parts, but aligned in a functional manner that adopts a systemic view. It is important for all elements to fit together in a coherent manner on the basis of a pedagogical philosophy.

B. F. Chapman and R. G. Henderson (2010) emphasize 18 quality criteria. Their study showed that the most important quality criterion is ‘rich content’. More importantly, it is referred to as ‘user friendly’, ‘interaction’, ‘reliability’, ‘flexibility’, ‘technical support’ and ‘informative’.

A. Usoro, A. Abid and G. Majewski (Usoro and Abid, 2008; Usoro and Majewski, 2009) defined the nine factors, such as e-learning in higher education quality ingredients.

Since e-learning is related to different target groups (the material interests of creators and users interests) and e-learning material users have different needs and desires, the quality aspects are to be seen in the context of many influencing factors.

Conclusions

In recent years e-learning quality aspects have been widely studied. Several e-learning quality models of technical realization, platform choice, and improvement of accessibility are developed. The main role of quality standard is to support the educational process implementation and management. The quality criteria affecting electronic learning materials can be divided into three groups — technical, pedagogical and content criteria. Technical quality of electronic teaching materials, which are different for each technical unit, and quality criteria are still investigated. The study should continue with specifying the quality criteria for each type of e-materials and indicating the degree of importance of the criteria.

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INFORMATION LITERACY IN COMMUNITY DEVELOPMENT

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Abstract
The purpose of this paper is to describe the information literacy of people as essential competencies required for the generation of social capital and use of social capital in sharing and obtaining information, which in its turn, is considered to be an important resource in the community's development.
The paper analyses the terms: social capital, information literacy and their mutual influence, as well as describes the research where informational literacy of the population of Latvia, its levels and the determined groups of knowledge and skills, which need to be improved in order to contribute to the generation and use of the social capital, through various methods (population surveys, focus group discussions, information literacy knowledge assessment questionnaires and performing practical tasks) were assessed. The research is based on the UNESCO Media and Information Literacy (MIL) Competency Matrix and UNESCO MIL Assessment Framework. The main conclusions of research are the following: information literacy is an important competence for developing social capital; but based on the completed research there are skills of information literacy – processing of information, critical assessment of information resources, legally correct use of information sources, as well as effective use of information technologies – which should be improved.
The research was conducted within the framework of the European Social Fund project 'Development of Innovative Diagnostic Instruments for Regional Growth' (No.2013/0057/1DP/1.1.1.2.0/13/APIA/VIAA/065).
Key words: social capital, information literacy, competencies of information literacy, assessment of information literacy, levels of information literacy.

Introduction

Community development depends on resources accessible to it and optimally used by it. Among several resources, like economic capital, starting from 1990 both in theoretical literature and empirical research social capital is recognized as important resource in community development (Daugavietis, 2014). Social capital is given an important role in the functioning of the society as a whole. So, for example, based on (Grootaert, 1998) the idea that 'social capital is the glue that holds societies together and without which there can be no economic growth or human well-being', the UK performs the social capital assessment in the country, measuring different kinds of connections between people (for example, by such indicators as numbers and ties of actors in personal relationship networks; the frequency of contacts with others; feeling lonely; receiving social support from other people etc.) (Siegler, 2015).

Based on the ideas of different social capital theories, Huvila I. et al. asserts that 'social capital is in the structures of relationships between people' (Huvila et al., 2010). To define shortly what social capital is, Siegler Veronique writes that 'social capital represents social connections and all the benefits they generate' (Siegler, 2015).

To map the benefits and outcomes of social capital both for the individual and for local community and society, it is divided in three dimensions: 1) structural dimension; 2) relational dimension; 3) content dimension (Huvila et al., 2010). The *structural dimension* is defined by network structures and the nature of network ties which provide information flows and can give benefits for an individual. The *relational dimension* includes trust, identity and roles of actors (individuals) connected in social networks. Trust is an important element to share and exchange information with somebody else. *Content dimension* includes shared meanings and collective knowledge. One of the main benefits of social capital is information exchange and possibility to get the necessary knowledge which is closely related to the concept of information literacy.

Information literacy as a concept was first presented by Paul G. Zurkowski in 1974 (Zurkowski, 1974) and still is at the stage of development. In 2013 UNESCO defined 'information literacy' as 'a set of competencies that empowers citizens to access, retrieve, understand, evaluate and use, to create as well as share information and media content in all formats, using various tools, in a critical, ethical and effective way, in order to participate and engage in personal, professional and societal activities' (UNESCO, 2013). It is accepted that 'information literacy competencies can both foster lifelong learning practices and generate social capital' (Stevens et al., 2006).

Interaction between social capital and information literacy could be bilateral: 1) social capital could be used as a source for information access; 2) information literacy could be used to affect generation of social capital. If we use social capital for information access, then knowledge about information sources (especially other people as information sources), search tools, quality criteria of information sources, as well as

skills of how to create new information and share it depends on individual information literacy level. If we are information literate enough about the possibilities to create social networks in digital environment, we can participate more successfully in the generation and use of social capital.

The purpose of this paper is to describe the information literacy of people as essential competencies required for the generation of social capital and use of social capital in sharing and obtaining information which, in its turn, is considered to be an important resource in the community's development. Therefore the main tasks are the following: 1) how to measure levels of information literacy; 2) to understand what is the level of information literacy in the local community (for economically active adult population); 3) what knowledge and skills are necessary to be improved.

The study described in this paper was based on the implementation methodology of the UNESCO Media and Information Literacy (MIL) Competency Matrix in assessment of adult information literacy and UNESCO MIL Assessment Framework (UNESCO, 2013). The study was carried out within the framework of the European Social Fund project 'Development of Innovative Diagnostic Instruments for Regional Growth' (No.2013/0057/1DP/1.1.1.2.0/13/APIA/VIAA/065).

The MIL Competency Matrix consists of three components. Component 1 is *Access*. It 'includes the ability to recognize the need for information, media content and knowledge, and to be able to identify useful information and media content from all sources and formats'. Component 2 is *Evaluation.* It 'is defined as ability to understand, critically analyse and evaluate information, media content, the work and functions of media and information institutions'. Component 3 is *Creation.* It 'defines the ability to master the production know-how of information, media content and new knowledge, and effectively communicate with others' (UNESCO, 2013).

The performance criteria for assessment of information literacy competence levels is based on the UNESCO MIL Assessment Framework (UNESCO, 2013): the 1st information literacy level (basic) indicates that a respondent has basic skills and knowledge, 'but significant improvements are needed for effective application'. The 2nd level (intermediate) indicates that 'a respondent has a good level of knowledge and skills, but there are gaps in certain areas'. The 3rd level (advanced) indicates that 'a respondent has a very good level of knowledge and skills'. Additionally, in this study, a zero level was introduced, projecting that there are people who do not possess the knowledge and skills needed for the basic level. The zero level indicates that an individual's information literacy is so low that it can become a serious constraint for obtaining and sharing information, and it can be a barrier for both use and generation of social capital.

Materials and Methods

There were several data collection methods chosen in order to perform as thorough study of the population's levels of information literacy as possible. In the beginning of the research from August 19, 2014 to October 2, 2014 a survey of the resident population of Latvia (between the ages of 18 and 74, size of sample achieved – 1004 respondents) (survey conductor - the Research Centre SKDS) consisting of seven questions related to self-assessment of the skills of information search, assessment and use, was conducted. It was possible to respond to every question, assessing their skills using a 4-point grading scale from "I know it very well" to "I don't understand it at all".

In order to determine the levels of information literacy and study needs, there was a pilot study conducted in two residential areas in Kekava County – Kekava Parish and the town Balozi, in April 2014. There were three data collection methods used in every field study – focus group interviews, questionnaires and performing a practical task, applying the think aloud method (Holma et al., 2014).

Focus group interviews were organised in the local public libraries and among the questions discussed were: the daily information needs of people, problems encountered during the process of information search and use, and their education needs. The total number of people, who took part in both discussions, representing various groups of occupation, was 23 persons (average age – 43 years). The data of focus group discussions were encoded and analysed using the software programme NVivo.

Before the discussions commenced, their participants answered to the questionnaire questions, where they provided information about themselves and assessed their computer literacy. According to the questionnaires there were four participants selected to perform a practical task.

After the discussions all participants filled in some knowledge questionnaires, consisting of 23 different closed-ended questions on various daily situations related to the aspects of information search, assessment and use. The questions were grouped into three sections according to the MIL matrix (Access, Evaluation, Creation). Depending on the selected answers, there was a corresponding level of information literacy given (from zero to three). This questionnaire was disseminated in local libraries and kindergartens. A total of 98 respondents took part in the survey.

In order to study how the information related to dealing with a particular daily problem situation is

searched, assessed and processed, there were four participants selected from each group after filling in their knowledge questionnaires (considering their level of education, age, occupation and self-assessment of computer literacy), who took part in completing a practical task. The think aloud method was applied, permitting to record both the process and respondent's thoughts. The screenshot recording software BB Flash Back Express was used in order to record the course of completing the practical tasks given (screenshots) and to record the 'thinking loud' – comments.

The practical tasks given were related to dealing with various daily life situations, such as, searching for a job, dealing with health related issues, making online purchases, preparing a culture programme for the relatives visiting from Australia or planning a business trip. All records were transcribed and encoded according to the MIL information literacy matrix, assessing and assigning a level of information literacy at each stage of three.

Results and Discussion

The results of population surveys will be analysed in more detail in this paper. The detailed results of focus group discussions, knowledge questionnaires and think aloud tasks are described in the publication in Communications in Computer and Information Science (Holma et al., 2014).

Having analysed the data obtained through the use of all four methods, it was possible to conclude that the questionnaire data showed, as it could be expected, that the respondents had assessed their information skills in quite a positive way.

According to the questionnaire data of the population of Latvia, the skill of using various information search tools and selecting the most suitable sources (see Fig 1) was assessed in the most optimistic way (72.4% and 78.4%, respectively, assessed their skills as very good or rather good). Comparing the answers to these questions in various groups of people, people at the age of 25 to 34 assessed their skills higher (93% indicated that they can use various tools of searching for information very well or rather can do it). At the age group between 55 and 74, only 38% consider their skills as very good or rather good.

Much more critically people have assessed their skills of evaluating the sources of information (65.3% answered that they can do it very well or rather can do it). The lowest assessment of these skills is in the age group from 55 to 74 years – 39% of population stated that they cannot assess the sources of information at all, but the lowest assessment was for the skills of using sources of information without breaching copyright (only slightly more than a half of all respondents indicated that they can do it very well or rather can do it).

Comparing the way people with different levels of education assess their information usage skills, it can be concluded that the respondents with university (higher) education rate their skills much higher, for example, 48% people with basic education, 67% respondents with secondary education, but 89% with higher university education rated their skills of using various information tools as very good or good. A similar picture can be drawn with the assessment skills of the obtained information. 36% respondents with basic education, 56% with secondary education, but 82% with higher university education rated their knowledge as good or very good.

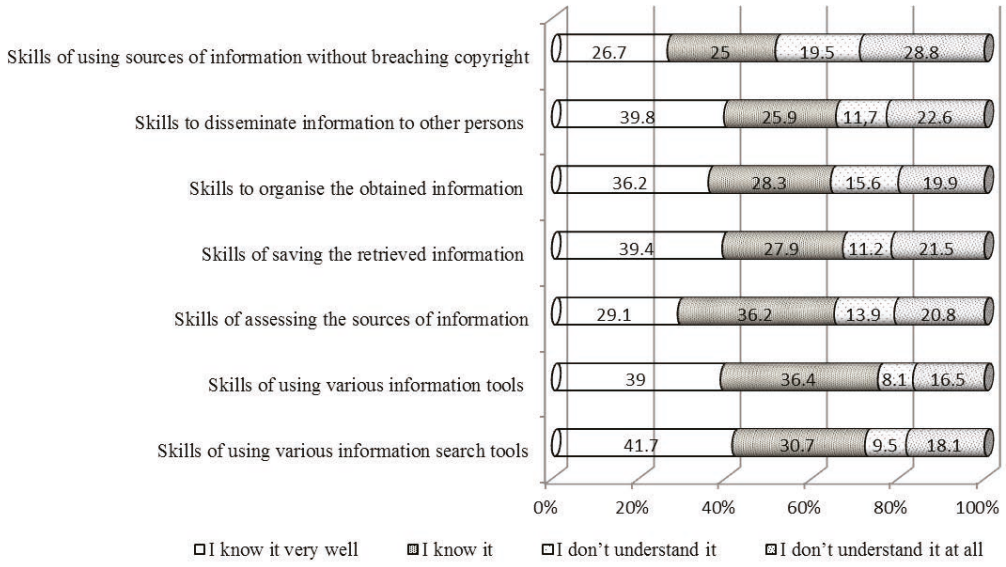

Figure 1. Opinions about dealing with information skills. N=1004.

In terms of the skills to organise and save information, the highest assessment was among the people with university education, where 85% rated it as very good or good.

The last two questions refer to the third information literacy component – summarising information, creation of new information and sharing it with other people. Responding to these questions skills were also rated relatively high, for example, 65% respondents rated their skills to disseminate information to other persons as very good or good. The highest self-assessment was also by people with higher education.

Comparing the obtained results by profession, managers and civil servants rated their skills highest, but seniors gave themselves the lowest rating (only 30% admitted that their skills of assessing the information found is very good or good) and unemployed persons (41% rated them as very good or good).

Comparing by regions, the inhabitants of Riga had a slightly higher assessment of skills, the inhabitants of Latgale has the lowest. For example, 74% inhabitants of Riga, 65% of those residing in Greater Riga, 57% inhabitants of Vidzeme, 65% inhabitants of Kurzeme, 65% inhabitants of Zemgale and 54% inhabitants of Latgale rated their skills of saving the retrieved information as good or very good.

The focus group discussion data were analysed, using six categories of the content analysis:

1. Information required;
2. Information sources;
3. Information channels;
4. Problems encountered during the process of retrieving information;
5. Lack of knowledge and skills;
6. Information literacy education needs.

In the 1st category participants of discussions had named 53 different themes of daily information needed, which were later arranged in 23 subject categories relating to the district or parish life, medicine, entertainment, housekeeping, gardening, public transport.

As the most commonly used sources of information the respondents most frequently indicated websites and Internet search engines (e.g. Google). Other people (e.g. friends, colleagues, neighbours, a postman) were mentioned as important sources for obtaining information. They were followed by databases and printed sources of information according to the frequency of mentioning. Internet was indicated as the most important information obtaining channel.

In the category 'problems encountered during the process of information retrieval', the most frequently used problems were the large volume of available information on the Internet and the usability problems of information sources. Insufficient knowledge of foreign languages and a lack of knowledge of computer software were also mentioned.

The information literacy education needs also resulted from the first five categories. They were related to the information search process, assessment of the retrieved information and summarisation, and presenting of the information found. Problems with the knowledge of foreign languages were mentioned in this category, too.

The data obtained through the qualitative method - *think aloud* - indicate that the highest results were

Table 1

Results of Think Aloud Practical Tasks (Information Literacy Level)

No.	Respondents	1. Access *Level*	2. Evaluation *Level*	3. Creation *Level*	Total
1.	Female (61, higher education, master's degree)	3	3	3	3
		3	3	1	2
2.	Female (34, higher education, master's degree)	3	2	2	2
		3	2	2	2
3.	Female (51, higher education)	2	2	2	2
		3	3	2	3
4.	Female (29, secondary education)	3	2	1	2
		3	3	2	3
5.	Man (41, secondary education)	1	0	0	0
6.	Man (33, secondary vocational education)	1	0	0	0
7.	Female (59, secondary education)	3	2	2	2
8.	Man (47, higher education)	1	1	1	1
		3	3	1	2
On average		2	2	1	2
Moda		3	2.5	2	2

Table 2

Lack of Knowledge and Skills – Education Needs

MIL Component	Problems Encountered
1 Access	To phrase the information need To select and use the search tools
2 Evaluation	To assess and compare the retrieved information sources (as often as not only the first source is chosen and viewed)
3 Creation	To summarise the selected information To compare and arrange information To lay out a text To provide references to the sources – specifying the Internet address To save information To observe the e-mail etiquette

obtained in the MIL Component 1 *Access* (average information literacy level – 2, moda – 3) (see Table 1). Majority of respondents used Google to search for information. Usually there also were no problems with the selection of keywords, as often as not there were words and expressions from the text of a task used as keywords.

It was a bit more difficult with the MIL Component 2 *Evaluation* (average information literacy level – 2, moda – 2.5) (see Table 1). Many respondents chose and viewed only the first or first three results found from the list of retrieved information.

It was most difficult to do the MIL Component 3 *Creation* (average information literacy level – 1, moda – 2) (see Table 1). In all tasks it was asked to summarise and compare the information coming from several sources. As often as not it was done by means of copying, and it was very rare when a text was created. There were no references to the retrieved information made – no addresses were recorded as to where this information was found. This is the stage where the computer literacy problems appeared most of all, e.g. copying a text, saving a file, creating a new folder. Two respondents also experienced problems with using their e-mail account, because in the rules of the task they were asked to send the information found by e-mail. In several cases there was a failure to follow the e-mail etiquette observed – messages were sent without any text, unsigned, or only with a subject.

Comparing the results obtained according to the qualitative method, it is possible to see a tendency that the older respondents and with higher education level scored the highest rating. It took them longer to complete the tasks given, nevertheless they assessed the retrieved information much more carefully and summarised it more skilfully.

The results of knowledge questionnaires showed a similar picture. Answering to the Block A questions (about the selection of information sources; *Access*), the average information literacy level was 2. In the second block (*Evaluation*) of questions there were some questions about the assessment of various information sources included. Similarly to the data obtained through the use of other tools, the questionnaire results indicated that the biggest difficulties were encountered during the third MIL stage (*Creation*) – creation and communication of new information (Holma et al., 2014).

Comparing the data obtained through the use of all four methods it can be concluded that the people are aware of a partial lack of knowledge and skills in all MIL stages. Younger people were more optimistic in their self-assessment. Older people assessed their searching for information and assessment, as well as new information creation skills in a more critical way.

The think aloud method demonstrated vividly what the problems are people encounter when searching for the daily information needed. Although answering to the question, how they assess their skills of searching for the information of daily use, 75.5% respondents had answered that they can do it very well, the results of practical tasks showed many shortages in searching and assessing information, but especially in summarising it, creating new information and saving or sending it. Respondents mentioned some of the problems they had encountered during the practical tasks in the focus group discussions, e.g. comparing and assessing the information sources, using their e-mail accounts, mastering computer software, comparing product and service prices, but the think aloud method helped to reveal several other problems (see Table 2).

Conclusions

The following conclusions can be drawn based on the description of importance of social capital and information literacy in community development, and the results of the conducted research.

1. Social connections represent the social capital and all the benefits they generate. The social capital is considered to be an important public resource for the community functioning. It has effect both

on the economic development and well-being of the society. One of the benefits of social capital is information exchange and sharing. Effective information exchange depends on information literacy of an individual.

2. To evaluate information literacy for adults in the local community several methods (both subjective (self-evaluation) and more objective (practical tasks; knowledge questionnaires)) should be used and obtained results should be compared. The carried out research results showed that the middle level of information literacy dominates among adults.
3. The results showed the following situation: people often use other persons as the source of information (e.g. friends, colleagues, neighbours, postmen, etc.), which shows that the social capital has an important role in obtaining information. Learning the information literacy competency level of people through application of several methods, it is apparent that it is the exchange and sharing of information (MIL Component 3 *Creation*) that is in the lowest level of skills: there is a lack of knowledge on summarising the retrieved information, critical assessment, use of legally correct sources of information, as well as efficient exchange of information, using the possibilities of modern technologies.
4. In terms of generation and use of social capital, there should be a special study conducted on the social media literacy of population, because the social media promote the development of social networks and exchange/ sharing of information.

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