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RESEARCH FOR RURAL DEVELOPMENT 2013

Volume 1

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FOREWORD

The four independent reviewers estimated each paper and recommended 68 articles for publishing at the proceedings consisted of 2 volumes, which started life as presentations at the Annual 19th International Scientific Conference "Research for Rural Development 2013" held at the Latvia University of Agriculture, in Jelgava, on 15 to 17 May 2013.

In the retrospect of four months later, we can count the Conference as a great success. The theme – Research for Rural Development - attracted participation more than 150 researchers with very different backgrounds. There were 114 presentations from different universities of Lithuania, Netherland, Poland, Thailand, Kazakhstan, Iran, Nepal 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 19th International Scientific Conference "Research for Rural Development 2013" (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 proceedings will be useful for researchers in educational sciences, too. 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 19th International Scientific Conference
"Research for Rural Development 2013"

Ausma Markevica
Latvia University of Agriculture

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EVALUATION OF EUROPEAN PEAR RUST SEVERITY DEPENDING ON AGRO-ECOLOGICAL FACTORS

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Abstract
Pear (*Pyrus communis* L.) fruits in Latvia are very popular, although orchard areas are not large. In the commercial orchards the control of plant pathogens mainly is performed using a plant protection plan, based on long-term observations. European pear rust caused by *Gymnosporangium sabinae* (Dicks.) G. Winter has become during recent years one of the most important diseases in Latvian pear orchards. Pathogen *G. sabinae* has a complex development cycle, with four types of spores on two different plants: pear and junipers. Favourable development of each stage depends on the specific environmental conditions. The aim of the study was field evaluation of the disease severity depending on agro-ecological factors.
The study was performed at the Latvia State Institute of Fruit-Growing from 2008 to 2012. The severity of European pear rust infection on leaves of cultivars was evaluated in points 0–5, where: 0 – a tree has no infected leaves; 5 – 81% to 100% infected leaves. The disease severity during these years, impact of tree planting year, rootstock, cultivar and tree location in the plot were analyzed.
Results gave the opportunity to determine which factors have positive influence on the development of pathogen and severity of disease. Severity of disease was not directly dependent on cultivar, their country of origin, rootstock and planting year. Severity of disease was influenced by tree location in the orchard; higher severity was observed on larger and more vigorous trees, located in outer rows, exposed to the prevailing wind carrying pathogen spores.
Key words: *Gymnosporangium sabinae*, weather conditions, cultivars, rootstocks.

Introduction

Rusts are important plant diseases, which agents belong to the order *Uredinales* of phylum Basidiomycota. According to the morphology of spore *Gymnosporangium* genus belongs to the *Pucciniaceae* family (Aime, 2006). Causal agent of European pear rust *Gymnosporangium sabinae* (Dicks.) G. Winter is distributed in Canada, North Africa, Asia and also in Europe (Farr et al., 1995). This disease is becoming an important problem also in Latvia – in 2007 symptoms of European pear rust were found in more than half of 33 assessed pear orchards (Rancane et al., 2012). *Gymnosporangium sabinae* has an incomplete development cycle requiring both pear (*Pyrus communis* L.) and juniper (*Juniperus* L.) (Jones and Aldwinckle, 1997). Development of disease starts in early spring on the junipers (Hilber et al., 1990). Teliospores germinate to form basidiospores which infect pears. Critical period for pear orchards is the time when the average air temperature is rapidly increasing and long-term rainfalls occur (Митрофанова, 1970; Hilber et al., 1990). Late and dry spring is unfavourable for development and release of basidiospores. The infection period may continue from April to the end of May (Митрофанова, 1970).

Viability of basidiospores is low, and they are unable to distribute for long distances by the wind (Agrios, 1997). For example, basidiospores of related species - apple rust pathogen *Gymnosporangium juniperi-virginianae* are able to distribute in distances over 3 - 5 km (Agrios, 1997). Massive infection occurs when the pear trees and juniper grow no more than 300 - 500 m away from each other (Митрофанова, 1970). Research has been done in British Columbia to compare the infection rate of pears depending on the location of juniper. It was stated that in distance of 30 m from the juniper 100% of pear leaves were infected, whereas in distance of 150 m - 50% of pear leaves, but in distance of 300 m - signs of rust infection were not found on the leaves (Ormrod et al., 1984).

After some time the first symptoms of the disease – spots on pear leaves appear. Under leaves fruiting bodies - aecia form and after some time aeciospores produce (Митрофанова, 1970; Hilber et al., 1990). Aeciaspores cannot infect the plant on which they were developed; therefore, the spores are spreading back to the junipers and infect those, where pathogen is overwintering in the infected branches (Cummins and Hiratsuka, 2003).

Knowledge about the development cycle of *Gymnosporangium sabinae* and its dependency on agro-ecological factors is quite limited, since there are no long-term evaluations of environmental influence, previous studies mostly are performed *in vitro* conditions. Systematic, long-term studies on the development of pathogen as well as on disease severity in the field conditions have not been performed. Therefore, the aim of this investigation was to perform field evaluation of the European pear rust severity depending on agro-ecological factors: weather conditions, tree planting year, rootstock, cultivars and their country of origin, and tree location in the plot.

Materials and Methods

The study was performed at the Latvia State Institute of Fruit-Growing (LSIFG) (56°36'39.37" N 23°17'48.86" E). The European pear rust severity on twenty five cultivars of different origin was evaluated for five years (2008 – 2012). In the trial, pear cultivars included their origin, planting years, cultivar-rootstock combinations as well as the number of pear trees per planting year. All these parameters are described in Table 1.

Table 1

Pear cultivars, rootstocks and tree planting years used in the evaluation of European pear rust severity

Cultivars	Country of origin	Rootstocks	Planting years					
			2001	2002	2003	2004	2005	2007
AMD-42-5-28	Latvia	Pyrodwarf	×	×	13	×	×	×
Belorusskaya Pozdnyaya	Belorussia	Pyrodwarf	×	9	10	×	×	×
		Kazraušu seedling	×	10	×	×	×	×
		BP-30	×	5	×	×	×	×
Bere Kievskaya	Ukraine	BA-29	×	×	×	×	3	×
BP-8965	Sweden	BA-29	×	×	×	×	5	×
Cheremshina	Ukraine	Pyrodwarf	×	×	10	×	×	×
Concorde	United Kingdom	BA-29	×	×	×	12	2	×
Condo	Netherlands	Pyrodwarf	×	×	×	10	×	×
Conference	United Kingdom	BA-29	×	×	×	×	2	×
Duhmyanaya	Belorussia	BA-29	×	×	×	×	×	5
Fritjof	Sweden	Pyrodwarf	×	×	×	12	×	×
Harrow Delight	Canada	BA-29	×	×	×	×	1	×
Mlievskaya Rannyaya	Ukraine	Pyrodwarf	×	4	8	×	×	×
		Kazraušu seedling	×	4	×	×	×	×
Mramornaya	Russia	Pyrodwarf	×	9	9	×	×	×
		Kazraušu seedling	×	9	×	×	×	×
		BA-29	×	×	×	×	2	×
Orcas	Canada	Pyrodwarf	×	×	×	10	×	×
Orlas-3-8-17	Russia	BA-29	×	×	×	×	×	2
		Plauža kompaktā	×	×	×	×	×	13
Paulina	Latvia	BA-29	×	×	×	×	5	×
Platonovskaya	Russia	BA-29	×	×	×	×	×	7
Rescue	Canada	Pyrodwarf	×	×	×	11	×	×
Striyskaya	Ukraine	BA-29	×	×	×	11	×	×
Suvenirs	Latvia	Kirchensaller Mostbirne	9	×	×	×	×	×
		OH × F 333	9	×	×	×	×	×
		Pyrodwarf	10	8	×	10	×	×
		BA–29	×	×	×	11	×	×
		Kazraušu seedling	×	8	×	9	×	×
		OH × F 87	×	×	×	15	×	×
		Circeņa cidonija	×	×	×	×	×	2
		K-TE-E	×	×	×	×	×	3
		Plauža kompaktā	×	×	×	×	×	22
		PU–20495	×	×	×	×	×	3
		Pyrus ussuriensis	×	×	×	×	×	5
Tavricheskaya	Ukraine	BA-29	×	×	×	×	4	×
Vasarine Sviestine	Lithuania	Pyrodwarf	×	4	×	×	×	×
		Kazraušu seedling	×	4	×	×	×	×
Vizhnitsa	Ukraine	Pyrodwarf	×	×	8	×	×	9
		BA-29	×	×	×	×	×	12
		Plauža kompaktā	×	×	×	×	×	17
Zemgale	Latvia	Pyrodwarf	×	×	9	×	×	×

Adapted scale was used similar to scab (*Venturia* Sacc.) spreading evaluation, in points 0–5, where: 0 – a tree has no infected leaves; 5 – 81% to 100% infected leaves from G.C. Percival and colleagues (2009). The response of cultivars to European pear rust was assessed in natural conditions of infection, with fungicide treatment.

The distance between rows was 4 m. Soil management consisted of frequently mowed grass in the alleyways, while 1 m wide strips were treated with herbicides. The soil at the trial site was sod-podzolic sandy loam, the humus content – 3.2%, the soil pH KCl – 6.4, plant available P_2O_5 – 234 mg kg^{-1}, and K_2O – 293 mg kg^{-1} (data of 2010).

Applications of fungicides in 2008, 2009 and 2010 were carried out as for the pear scab (*Venturia pyrina* Aderh.) control. In 2011 and 2012 application scheme in April and May was modified to adapt it for the control of European pear rust, based on basidiospore release observed on junipers (*Juniperus sabinae*) near to the orchard and weather conditions.

Weather information was collected by the meteorological station 'Lufft' at the LSIFG. Weather conditions were recorded every half-hour and analysed by decades. The weather conditions among study years were different. The drier vegetation period was in 2008, but the vegetation period of 2010 had the highest precipitation and temperatures among years of study. During the winter time, low air temperatures were observed in 2010 (the lowest air temperature was in February, -28 °C) and 2011 (the lowest air temperature was in January, -23 °C), whereas in 2008 and 2009 they were the highest ones (up to -17 °C).

Statistical analysis of the data was performed using SPSS v. 15 program modules for descriptive statistics and analysis of variance, correlation analysis and multiple comparison tests. Evaluation of European pear rust severity among years, tree planting year, rootstock and cultivar impact in the plot was performed.

Results and Discussion

Evaluation of European pear rust severity depending on years

Total precipitation (TP), relative humidity (RH) and average air temperature are the most important factors for the development of fungal diseases (Hardwick, 2006), therefore, they were analysed in this study. Meteorological conditions were analysed for the period from 2008 to 2012 (Table 2). Detailed analysis was done for periods which are the most important in the development of the pathogen - 2nd decade of April to the end of May.

Table 2

Characterization of total precipitation, relative humidity and average air temperatures in spring from 2008 to 2012

Year	Month	Decade	Total precipitation, mm m^{-2}	Relative humidity, %	Average air temperature, °C
2008	April	3	0.0	51.8	10.1
	May	1	2.8	70.1	12.2
	May	2	8.6	66.2	10.4
	May	3	0.0	53.7	13.6
2009	April	3	0.0	49.9	11.1
	May	1	0.0	60.4	11.7
	May	2	8.3	69.0	11.3
	May	3	7.1	66.1	14.8
2010	April	3	7.6	67.1	7.4
	May	1	50.0	82.5	8.5
	May	2	10.9	85.0	16.5
	May	3	14.3	73.1	13.4
2011	April	3	0.0	56.8	12.5
	May	1	7.3	63.5	8.7
	May	2	35.2	77.5	13.2
	May	3	11.1	73.4	14.4
2012	April	3	16.0	75.5	11.6
	May	1	11.6	68.9	11.1
	May	2	10.7	74.7	11.7
	May	3	21.8	72.7	14.4

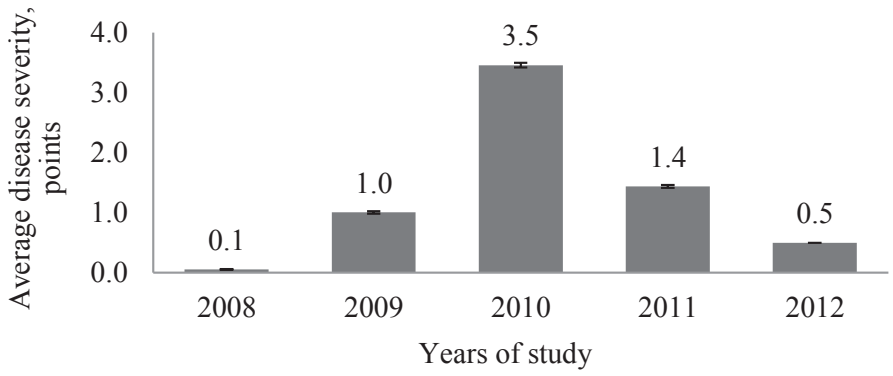

Figure 1. Average disease severity on all cultivars in the years of study (severity scale: 0 - no infected leaves, 5 - 81% to 100% infected leaves).

Overall, the driest periods were in 2008 and 2009 (TP was 11 and 16 mm m^{-2}, respectively). The highest rainfall was observed in 2010 - 82.8 mm m^{-2}. Level of rainfall in the last decade of April and May, 2011 and 2012 was similar (TP was 53.6 and 60.1 mm m^{-2}, respectively). The highest RH during the period analyzed in study years was in 2010, in the second decade of May – 85.0% (Table 2). Statistical analysis showed significant differences between all weather parameters among the study years ($p<0.001$). The highest total precipitation (82.8 mm m^{-2}) and average relative humidity (77%), and the lowest air temperature (11.4 °C) were observed in 2010. Thus, this combination caused the highest severity of disease (3.5 points in average) among the study years.

Analysis of data showed correlation between severity of disease and weather conditions ($p<0.01$). Air temperature had negative correlation to severity of disease ($r = -0.021$, $p = 0.047$). Precipitation and relative humidity had low positive correlation to severity: 0.048 and 0.179, respectively ($p<0.001$).

Average disease severity was statistically different ($p<0.001$) among the years of study and it correlated with the time of the first fungicide application (Fig. 1).

Severity of European pear rust in 2008 was low – only some trees were infected and symptoms were observed only on the leaves. The spring of 2008 could be described as warm and dry; therefore, possibility for development of pathogen was limited. Research in New York about pathogen *Gymnosporangium juniperi-virginianae* Schw., which causes the cedar apple rust on apple (*Malus pumila* Mill.) showed that precipitation is a critical factor that is determining the duration of spore release period (Pearson et al., 1980). Applications of fungicides in 2008 and 2009 were carried out as for pear scab control, and that limited also the severity of European pear rust. Severity of European pear rust in 2009 increased, since environmental conditions in this year was more favourable for pathogen development – during the period of spore release there was heavy rainfall and air temperature was 15 °C. According to U. Hilber and colleagues (1990), such conditions are optimal for infection of pear trees. In 2010, all pear trees were infected by European pear rust, and severity of disease was high – 3.5 points on average. That year symptoms of disease were found not only on fruits but also on branches. Applications of fungicides in 2010 were carried out as for pear scab control. The first application of fungicides was only on May 10, but the first rainfall was on May 3. During the last decade of April and first decade of May the average air temperature was low, – 8.5 °C; therefore, primary infection could occur. In 2011 and 2012, severity of disease decreased. In this period fungicides were applied depending on pathogen live cycle that significantly decreased the disease severity.

Evaluation of European pear rust severity depending on cultivar

Evaluation of European pear rust severity showed that none of the tested pear cultivars (cvs.) has complete resistance to this pathogen, but have differences in susceptibility level. Similar results were obtained also by M. Fischer and H.J. Weber (2005). Lack of complete resistance was found also in a study of related species *Gymnosporangium juniperi-virginianae* on apples, which showed that each of fifty-eight cvs. and hybrids artificially infected by pathogen showed symptoms of disease (Aldwinckle et al., 1977). The severity of disease did not show significant differences among tested cvs. ($p=0.812$), it ranged from 0.8 and 0.9 points on average (cvs. 'Līva', 'Duhmyanaya' and 'Harrow Delight') to 1.4 points on average (cvs. 'Mlievskaya Ranyaya', 'Fritjof', 'Conference', 'Belorusskaya Pozdnyaya', 'Zemgale', BP-8965, 'Bere Kievskaya', 'Concorde', 'Condo', 'Mramornaya'). The highest variability among years was observed for cvs. 'Harrow Delight', 'Tavricheskaya', 'Platonovskaya', 'Conference', 'Zemgale', 'BP-8965', 'Bere Kievskaya' (Fig. 2). Cultivar 'Suvenirs' had medium symptom severity –

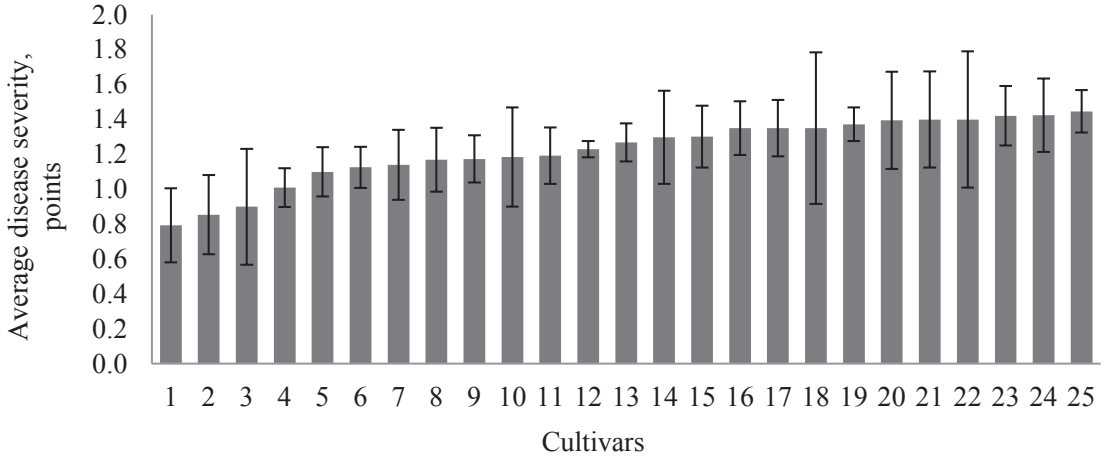

Figure 2. Average disease severity depending on cultivars in all years of study (severity scale: 0 - no infected leaves, 5 – 81% to 100% infected leaves), where: 1 – 'Liva', 2 – 'Duhmyanaya', 3 – 'Harrow Delight', 4 – AMD-42-5-28, 5 – 'Vasarine Sviestine', 6 – 'Striyskaya', 7 – 'Paulina', 8 – 'Cheremshina', 9 – 'Orcas', 10 – 'Tavricheskaya', 11 – Orlas 3-8-17, 12 – 'Suvenirs', 13 – 'Vizhnitsa', 14 – 'Platonovskaya', 15 – 'Rescue', 16 – 'Mlievskaya Ranyaya', 17 – 'Fritjof', 18 – 'Conference', 19 – 'Belorusskaya Pozdnyaya', 20 – 'Zemgale', 21 – BP-8965, 22 – 'Bere Kievskaya', 23 – 'Concorde', 24 – 'Condo', 25 – 'Mramornaya'.

1.2 points on average and the lowest variability among years of evaluation. The favourite cultivar of home gardeners 'Mramornaya' was characterized as highly susceptible to European pear rust. Cultivars used in this trial originated from nine different countries (Table 1), and data analysis did not show significant influence of cultivar origin or their possible genetic background to the disease severity (p = 0.632).

Rootstock impact on European pear rust severity

In the trial, pear cultivars were grown on twelve different rootstocks. Statistical analysis of data showed significant differences among rootstocks according to the severity of disease (p = 0.046). The highest severity of disease had cultivars on seedling rootstock Kirchensaller Mostbirne (originated in Germany) and clonal rootstock OH × F 333 (USA) – 1.6 and 1.5 points on average, respectively. Cultivars on these rootstocks were located in the first row of trial block and had larger,vigorously growing crown that possibly increased the severity of disease. Dwarfing rootstock BP 30 (selected at the SLU-Balsgård, Sweden) forms smaller and less vigorous trees. Pear trees on BP 30 were located inside the block behind trees of larger and vigorous size, but severity of disease was high – 1.4 point on average. Trees on BP 30 showed also high variability among years. This phenomenon could

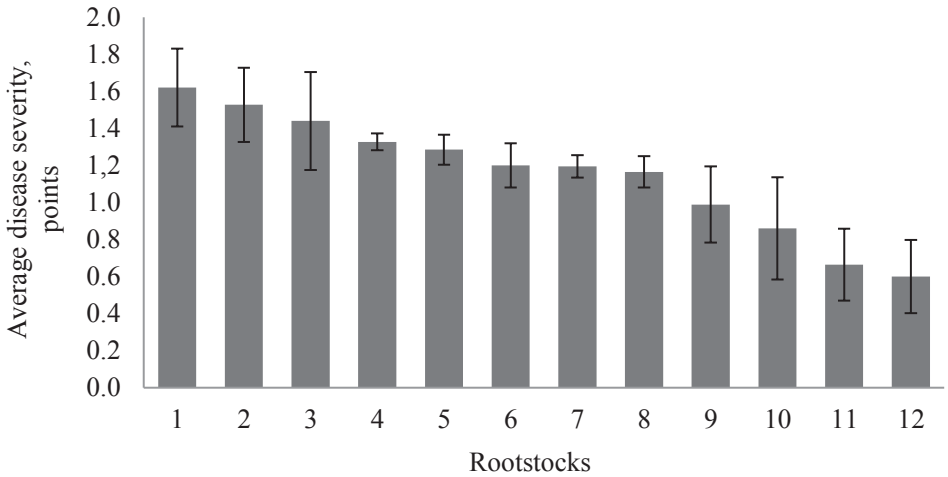

Figure 3. Impact of rootstock to average disease severity in all years of study (severity scale: 0 - no infected leaves, 5 - 81% to 100% infected leaves), where: 1 - Kirchensaller Mostbirne, 2 - OH × F 333, 3 - BP 30, 4 – Pyrodwarf, 5 – Kazraušu seedling, 6 - OH × F 87, 7 – BA-29, 8 – Plauža Kompaktais, 9 – *Pyrus ussuriensis*, 10 – Circeņa cidonija, 11 – K-TE-E, 12 – PU 20495.

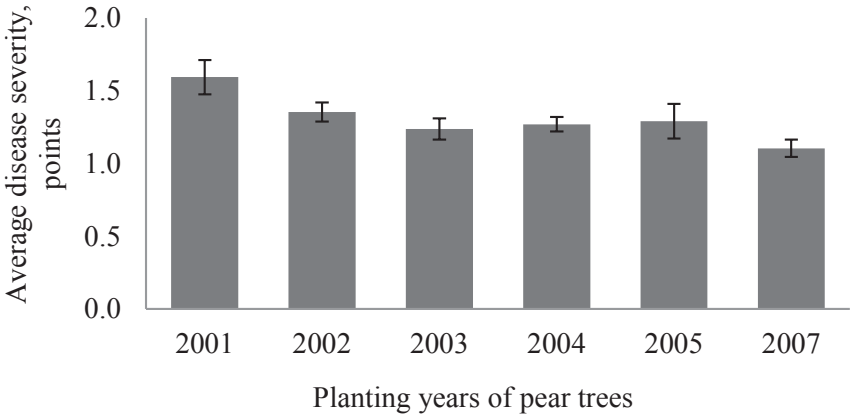

Figure 4. Average disease severity depending on planting year in all years of study (severity scale: 0 - no infected leaves, 5 - 81% to 100% infected leaves).

be explained by only one cultivar grafted on BP 30 – 'Belorusskaya Pozdnyaya', which is very susceptible to disease and had high symptom severity – 1.4 point on average (ranging from 0.0 to 4.0 points). The lowest severity was observed on rootstocks K-TE-E (Czech Republic) and PU 20495 (Latvia), 0.7 and 0.6 point on average, respectively (Figure 3). Pear trees on these rootstocks were located in the middle of the plot, it was the youngest planting and their crowns were smaller.

Planting year impact on European pear rust severity

Statistical analysis of data showed significant influence of tree planting year (corresponds to the age of plant and size of tree canopy) on the disease severity ($p = 0.002$). According to the results shown in Figure 4, average value of severity was higher for trees planted in 2001 (1.6 points).

These differences could be explained by location of trees planted in 2001 as well as by the size of tree. In 2001, the first and second rows of the trial were planted, which are located at the edge of the block, adjacent to the highway, and across the road there is a residential district with ornamental junipers in almost every home yard. These junipers were probably one of the sources of infection due to prevailing winds in the spring, which can transfer spores from junipers to pear trees. Trees in the first lines were larger and vigorous; therefore, they ensured the protection for next rows of pear trees. The trees planted in 2002 and 2003 are located in the middle of the block and were smaller and less vigorous, and thus allowed spores transferred by wind reach more distant rows, where stronger and larger sized trees (planted later, in 2004 and 2005) grow. Severity of disease in both years 2004 and 2005 were 1.2 points. The block planted in 2007 was the youngest one and was bordered on all sides by more vigorous trees, probably therefore severity of disease was the lowest – 1.1 point on average. Low disease severity was found also for orchards bounded by windbreaks. Although statistical analysis of data did not show significant influence on tree location ($p = 0.999$), more infected trees were located at the edges of trial block, whereas in the middle of planting the severity was lower regardless of cultivar.

Cultivar 'Suvenirs' grown in different places of pear trial had different tree planting years (2001, 2002, 2004 and 2007) and combinations with different rootstocks. Statistical analysis of data for this trial showed just the same results as previously named in this study. The data showed significant impact of tree planting year ($p < 0.001$) and rootstock ($p = 0.007$) (both correspond to the size of tree canopy) to the disease severity, but did not show significant influence to tree location ($p = 0.983$).

Conclusions

1. Severity of European pear rust was significantly influenced by weather conditions, showing great variation among years. The highest severity of disease could be observed in years with high total precipitation and average relative humidity as well as moderate air temperature in the period of 3rd decade of April to the end of May.
2. Severity of European pear rust was not directly dependent on pear cultivar or its origin as well as rootstocks and planting year.
3. Severity of disease was influenced by pear tree location in the orchard block, higher severity was observed on larger and more vigorous trees, located in outer rows, which are more exposed to the prevailing wind carrying pathogen spores.

Acknowledgements

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UREA APPLICATION AS A SANITATION PRACTICE TO MANAGE PEAR SCAB

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Abstract
Several fungicide applications are used to control a pear scab (*Venturia pirina* Aderh.) on pear (*Pyrus communis* L.) trees. Minimal chemicals use in fruit-growing constantly has been important condition in the integrated fruit production; therefore, sanitation is recommended to reduce a primary inoculum in orchards. The study was carried out in an integrated pear orchard located in Sigulda district, in the central part of Latvia to estimate if a fall treatment of a pear orchard with urea reduces an amount of pseudothecia and pear scab incidence level the following season. Six treatments trial was arranged in the autumn 2011 on a moderately susceptible pear cultivar 'Belorusskaya Pozdnaya'. An amount of leaf litter, pseudothecia and incidence level of a disease on leaves and fruits were determined. The results showed that urea application reduced both an amount of leaf litter and a number of pseudothecia. An average amount of leaf litter was 127 leaves per 0.25 m^2 in a control and 89 leaves in a treatment with urea application. The number of pseudothecia reached 250 pseudothecia per one leaf disc in a control and 160 pseudothecia in a treatment with the urea application, the difference was not significant ($p>0.05$). The reduction of disease incidence level on leaves was significant ($p<0.05$) only in the first assessment of five in total.
Key words: *Venturia pirina*, pear disease, fungicide applications, pseudothecia.

Introduction

Venturia pirina Aderh. (anamorph *Fusicladium pyrorum* (Lib.) Fuckel is the causal agent of scab on pear (*Pyrus communis* L.) trees. The disease is common in all pear-growing areas of the world and causes heavy crop losses by producing lesions on leaves, young shoots and fruits (Shabi, 1990). In pear orchards, crop losses due to pear scab would be about 50-100% if appropriate control measures are not applied; therefore, several fungicide applications are used to suppress disease development. In pear orchards in Latvia, a number of applications could reach seven times, depending on weather conditions, the amount of inoculum and cultivar susceptibility to the pathogen.

The pear scab pathogen overwinters primarily as pseudothecia in pear leaf litter which is the main inoculum source in spring (Shabi, 1990). In addition, the fungus can overwinter as mycelium in twig lesions (Stensvand et al., 1996); this phenomenon has never been investigated in Latvia. During spring, ascospores are released from the pseutothecia and cause primary infection. Fungicide sprays against pear scab are most effective when they coincide with discharge of ascospores; therefore, primary infection period is the most critical time for controlling pathogen. Under Latvian conditions ascospores are released over a 1.5 to 2 month period, from the middle of April until the middle of June when leaves are decomposed.

An important practice for managing apple scab (*Venturia inaequalis* (Cooke) Wint.) and pear scab (*Venturia pirina* Aderh.) in an integrated plant protection program is the reduction of primary inoculum in order to lessen fungicide use during the growing season (MacHardy, 1994). Several nonchemical sanitation procedures against apple scab have been evaluated, such as burning or ploughing (Curtis, 1924), the shredding of leaf litter (Sutton et al., 2000; Vicent et al., 2004), covering the orchard floor with plastic (Holb, 2006) and the use of fungal antagonists to suppress the ascigerous stage of *V. inaequalis* (Vincent et al., 2004). Several studies revealed that mulch cover increased the biological activity of soils, which enhanced leaf degradation (Haynes, 1981). An autumn application of dolomitic lime reduces the percentage of apple and pear leaves with pseudothecia, the number of pseudothecia per leaf, and number of asci per pseudothecium (Spotts et al., 1997). Leaf collection has a direct reduction effect on fallen leaves and primary inoculum (Heitefuss, 1989). Applying urea to leaf litter and in a tree canopy is one of the mostly used sanitation practices to reduce an apple scab inoculum.

Urea applications between all those sanitation procedures have showed the highest efficacy. Urea applied to trees in autumn before leaf fall or to the leaf litter in autumn or spring before bud break reduced the ascosporic inoculum 50 to 90% (Sutton et al., 2000). A urea solution in water should be applied to apple (*Malus x domestica* Borkh.) trees as leaves begin fall in the autumn. This should been done as late as possible to prevent the urea from being translocated into the tree. Trees sprayed with urea may defoliate more quickly than unsprayed trees. Urea inhibits the development of scab fruiting bodies on the fallen leaves. High nitrogen content also helps the leaves to decompose much faster than normal (Jesperson, 1995). Urea increases the softening rate of leaf litter and their palatability to earthworms (Burchill et al., 1971). Urea has been widely tested in apple orchards,

but there is a lack of information how effective it is in pear orchards.

The purpose of this study was to determine the effect of an autumn application of urea to manage pear scab. In this study the potential of urea application to reduce the amount of pseudothecia and scab lesions on leaves and fruits was investigated.

Materials and Methods

The study was carried out in an orchard at Sigulda district, in the central part of Latvia, in 2011 and 2012. In this orchard integrated fruit production practice was used.

The planting distance of pear cultivar 'Belorusskaya Pozdnaya' on seedling rootstock: 5 × 3 m (tree density – 666 pear trees per 1 ha. The pear orchard was planted in 2002.

Soil: sandy loam with the following characteristics: pH KCL – 6.3, content of organic substance 26 g kg^{-1}, content of plant-available K – 183 mg kg^{-1}, P – 302 mg kg^{-1}. In the orchard an apple scab warning system RIMpro (Relative Infection Measure program) was used for specifying the border value of apple and pear scab infection risk.

The trial with six treatments in three replications was arranged in the autumn 2011 on a moderately susceptible pear cultivar 'Belorusskaya Pozdnaya' (Kārkliņš, 2004). The size of experimental unit (replication) was 18 × 25 m, including 30 trees. Following treatments were made:

1) untreated, non-sanitized control;
2) fungicide applications according to RIMpro (2012);
3) urea application in a tree canopy in autumn (2011) + RIMpro (2012);
4) collection of fallen leaves in spring (2012) + RIMpro (2012);
5) urea application in a tree canopy in autumn (2011) + copper hydroxide in a high dosage in spring (2012) + RIMpro (2012);
6) fungicide applications according to the conventional schedule suggested by the company Syngenta (2012).

A urea (46-0-0) solution (50 g of agricultural grade urea in 950 mL of water) at a rate of 570 L ha^{-1} was applied on 14 October 2011.

Champion 50 WP (a. i. copper hydroxide, 77%), 10 kg ha^{-1} sprayed on 13 April 2012.

Collection of fallen leaves was done on 18 April 2012 before ascospore discharge.

Following fungicide applications were done according to the RIMpro – apple scab warning system or according to the conventional schedule suggested by the company Syngenta (distributer of plant production means) in 2012.

Applications according to RIMpro: Champion 50 WP (a. i. copper hydroxide, 77%), 3 kg ha^{-1} – 26 April; Dithane NT (a. i. mancozeb, 750 g kg^{-1}), 2 kg ha^{-1} – 8 May; Chorus 50 WG (a. i. cyprodinil, 500 g kg^{-1}), 0.3 kg ha^{-1} – 23 May; Score 250 EC (a. i. difenoconazole, 250 g L^{-1}), 0.2 L ha^{-1} – 5 June.

Applications according to the conventional schedule: Champion 50 WP (a. i. copper hydroxide, 77%), 3 kg ha^{-1} – 26 April; Chorus 50 WG (a. i. cyprodinil, 500 g kg^{-1}), 0.3 kg ha^{-1} – 3 May, 10 May, 23 May; Score 250 EC (a. i. difenoconazole, 250 g L^{-1}), 0.2 L ha^{-1} – 31 May, 7 June.

Amount of leaf litter

Assessment of fallen leaves was done on 13 April 2012 in control and treatment with a urea application. Leaves were counted in 0.5 × 0.5 m frame, 1 m distant from a tree, in randomly selected four sites in a treatment.

Amount of pseudothecia

Fallen leaf samples for laboratory analysis were collected on 13 April (2012) - 15 leaves from a non-sanitized control and 15 from a treatment with urea application. From each leaf four discs (each 0.25 cm^2) were cut out. The number of pseudothecia per each leaf disc was determined using a binocular Olympus SZ X7 at four times magnification. Data were analyzed with statistical software R (version 2.15.1), boxplot graphic method (R Core Team, 2012).

Disease incidence

Scab assessments were done on leaves and fruits in the autumn 2011 and in the following vegetation season 2012, on June 7, June 15, June 29, July 5 and August 3. In the summer 2012 a scab incidence (infected objects, %) was assessed on four trees located in the center of each replicate, 25 leaves per tree. All data sets were subjected to analysis of variance using the Genstat 15 statistical package. Data were transformed and then significant F-tests were followed by the Least Significance Difference (LSD)-test for comparing the treatment means.

Weather conditions

The autumn 2011 was comparatively warm, air temperature exceeded long term average. The first sustainable snow cover developed in the middle of January 2012. February 2012 was snowy; in the first decade air temperature was below long term average. Weather conditions from 4 April 2012 were recorded with a portable Lufft weather station placed in apple orchard approximately 2 km distant from the trial site.

The spring in 2012 was late with a comparably low temperature. In the beginning of April the experimental orchard was still covered with snow. The average air temperature and precipitation in 2012, and long-term observations are displayed in Figure 1. The average

amount of precipitation during summer months was over long-time observations; the rain is the main influencing factor for a pear scab development.

Results and Discussion

Application with a urea solution in the autumn 2011 reduced the amount of leaf litter before bud break. An average amount of leaf litter was 127 leaves per 0.25 m^2 in control and 89 leaves in treatment with urea application. For PAD (potential ascospore dose) a calculation number of pseudothecia per lesion is usually used (Gadoury and MacHardy, 1986). In this research, pseudothecia were distributed on the whole leaf surface; therefore, they were counted on leaf discs. The number of pseudothecia per leaf in the trial was high; it reached 250 pseudothecia per one leaf disc in control (untreated) and 160 pseudothecia in treatment with the urea application. Data variance is showed in Figure 2. There was a tendency of pseudothecia reduction in treatment with the urea application, but difference between the number of pseudothecia in the control and in the treatment with urea application was not significant ($p>0.05$), and according to statistical analysis 67% of difference can be explained with the factor 'treatment type'.

The green tip stage (BBCH 07) was observed on 25 April and the first ascospore discharge started nine days later on 3 May. Rainy weather conditions in the end of May (Figure 1.) favored a development of the first scab lesions on pear leaves.

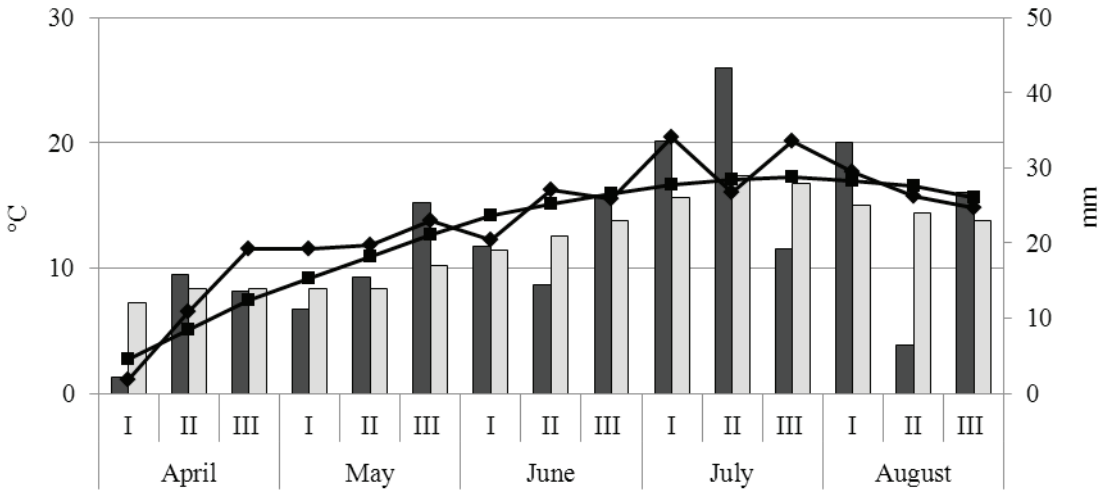

Figure 1. Average air temperature and sum of precipitation:
■ Long-term average, °C; ▲Temperature in 2012, °C; Long-term average, mm; Precipitation in 2012, mm.

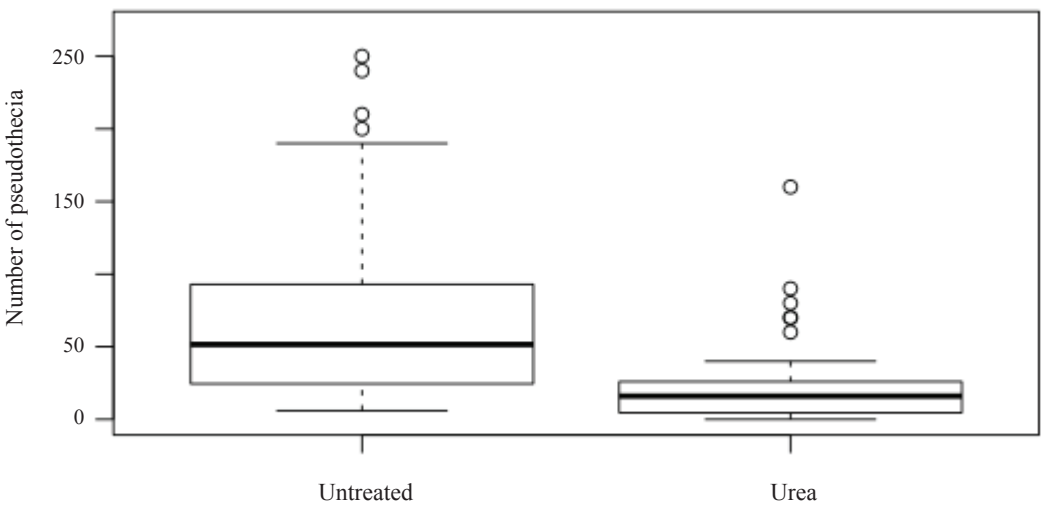

Figure 2. Number of pseudothecia on leaf discs in untreated control and after a treatment with a urea solution.

Table 1

Incidence level of pear scab in sanitation trial in 2012, %

Treatments	Leaves			Fruits	
	June 7	June 15	June 29	July 5	August 3
1. Control	**9.67 a**	7.67 a	6.67 a	5.33 a	10.0 a
2. RIMpro	5.67 b	3.00 a	2.33 b	5.00 a	0.00 b
3. Urea + RIMpro	**1.67 c**	3.67 a	3.00 b	3.00 ab	0.67 b
4. Leaves collection + RIMpro	4.33 bc	3.67 a	2.00 b	3.00 ab	0.00 b
5. Urea + copper + RIMpro	**2.33 c**	2.33 a	1.00 b	1.67 ab	0.33 b
6. Conventional fungicide applications	3.67 bc	3.00 a	1.11 b	0.67 b	0.00 b
$LSD_{0.05}$	2.91	5.78	3.09	3.91	2.69

[a,b,c] – Values marked with the same letter in column, are not significantly different at $p<0.05$.

Pear scab incidence did not exceed 10% in the control. The level of disease did not reach expected level. Nevertheless, statistically significant differences among treatments were observed.

Application with a urea solution in autumn significantly ($p<0.05$) reduced foliar lesions on trees in the first assessment during the primary scab infection period compared with untreated control and treatments followed only RIMpro (Table 1). In the following assessments there was no difference between treatments with urea application and without it. It is explained by the development of conidial stage and regular fungicide applications. Some differences were observed on leaves at the end of June and on fruits in August compared to control. The incidence level, except control, was not different between treatments. There are only some studies that considered the potential of a urea treatment to reduce primary apple scab lesions (Burchill et al., 1965; Sutton et al., 2000). R.T. Burchill et al. (1965) first showed that application of a urea to English orchards in the autumn completely suppressed ascospore production the following spring, and scab lesions on leaves were reduced by 56% and 46%, respectively, compared to the untreated control. Similarly, in France a urea spray applied to trees after harvest, reduced scab in the following spring (Sutton et al., 2000). The main potential of sanitation methods is to reduce the overwintering stage of the scab pathogen in the leaf litter.

Despite the effectiveness of urea in reducing an ascospore survival, the need for spring-summer fungicide applications could not be eliminated in orchards with a high inoculum (MacHardy, 2000) because there is always a strong chance of infection during the growing season if viable spores are present (Holb, 2006a). The main importance of the sanitation is to reduce an infection pressure in orchards with a high inoculum potential and to increase a fungicide efficacy to control scab.

The cultivar 'Belorusskaya Pozdnaya' is moderately resistant (Kārkliņš, 2004). Probably the influence of control measures could be demonstrated more clearly if more susceptible cultivars were grown. The results are preliminary, because the trial demonstrates only one infection period. Investigations should be continued in a pear orchard with a higher disease infection pressure and with special attention to the twig scab control.

Conclusions

1. Urea application in a tree canopy in autumn showed a tendency of pseudothecia number reduction in the treatment with the urea application, but the difference was not significant ($p>0.05$) in comparison with the untreated control.
2. Application with a urea solution in autumn significantly reduced ($p<0.05$) foliar lesions on trees during the primary scab infection period compared with untreated control and treatment followed only RIMpro.
3. The difference between treatments with the urea application and treatment with leaves collection was not significant ($p>0.05$), but the results are preliminary and trial demonstrates only one infection period; thus, further investigations are needed to investigate sanitation practices more in details.

Acknowledgements

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EVALUATION OF YIELD AND GRAIN QUALITY OF OAT CULTIVARS

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Abstract
Oat breeders have improved yielding ability potential of oat (*Avena sativa L.*) cultivars, but set lower standards for biochemical composition of grain. Nowadays the quality of grain for consumers has become important especially in terms of lipids and β-glucan content. Experiments were carried out at State Stende Cereal Breading Institute in the year 2012 to evaluate the oats yield and grain quality. 15 different cultivars of Stende collection were compared by yield and parameters of productivity (test weight and 1000 kernel weight) as well as biochemical parameters (protein, starch, lipid, β-glucan, content). Biochemical parameters were tested by Infratec Analyser 1241. To obtain an equal research background all cultivars were grown in a plant breeding crop rotation field, with similar growing conditions (sowing-time, fertilizer, plant protection activities), which agree with generally accepted technology of oat cultivation in Latvia. Experiments were done in four replications. Plots were laid randomized. ANOVA procedures were used for data analysis. Yield of experimental cultivars varied between 4.28 ± 0.19 - 5.93 ± 0.22 t ha^{-1}, test weight 46.85 ± 0.99 - 52.75 ± 0.44 kg hL^{-1}, 1000 kernel weight 33.70 ± 0.24 - 46.34 ± 0.80 g. Significant differences of tested yield parameters among oat cultivars were observed. The highest protein content was observed for local breed cultivars 'Arta' and 'Mara' 119.5 ± 02.6 and 110.5 ± 1.9 g kg^{-1} accordingly. Low starch content, but high β-glucan content are characteristic for all Latvian cultivars.
Key words: oat, yield, protein, starch, β-glucan.

Introduction

Avena sativa (Linnaeus, 1753) or common oat is one of the cereal crops cultivated in temperate climate zones. It is used both for human and animal nutrition. Nowadays about 70% of the produced oat yield is used for animal feed. However in general the terms demand for oat has considerably decreased. Currently the discussion on oat grain dietetic value and suitability to the production of functional foods is more frequently mentioned in scientific literature. With the development of the techniques of intensive management over crop production demands to oat varieties have changed considerably. Oat breeders through hybridization and selection have improved yielding potential of oat varieties; they have developed oat varieties dwarfed in length and more resistant to lodging. On consumers' side lower standards are set forward regarding biochemical composition of grain: protein, lipids, β-glucan, starch amount in grain, though dietetic value of oats is just due to these traits (Wood, 1997).

Grain yield, test weight and 1000 kernel weight are the most important economic traits mentioned by the oat consumers, as the end-product outcome is due to these traits when processing oat grain. Among the main compounds associated with health-promoting effects in cereals is dietary fiber. Dietary fiber is found only in plant foods. It consists of both soluble and insoluble fiber. Soluble fiber dissolves while insoluble fiber does not dissolve in water. Both types are important for health in different ways (Manthey et al., 1999; Grausgruber et al., 2004). Water-soluble fiber in cereals is composed of non-starchy polysaccharides such as β-glucan. Some of the oat constituents are valuable as ingredients or starting materials for several types of products (Brindzova et al., 2008). Compared to other cereals, oat as well as barley endosperms have relatively higher β-glucan contents. Oat β-glucan has received the most attention and has a number of uses and potential uses. β-glucan is included in the soluble dietary fiber fractions of oat that participates in the glucoregulation and causes a decrease in serum cholesterol levels in humans (Wood, 2007). In comparison with other cereals, oat as well as barley endosperms have relatively higher β-glucan contents (Queenan et al., 2007).

The task of the trial was to compare selected oat cultivars by yield and parameters of productivity (test weight and 1000 kernel weight) as well as biochemical parameters (protein, starch, lipid, β-glucan, content).

Materials and Methods

The field trials were carried out at State Stende Cereals Breeding Institute in 2012. 15 oat cultivars (int. al. 5 local (Latvian) and 10 foreign origin) were used. The soil of the site was sod-podzolic, the humus content – 18 g kg^{-1}, the soil pH KCl – 6.2, the available for plants content of phosphorus P – 42 mg kg^{-1}, and that of potassium K – 59 mg kg^{-1}. The pre-crop was barley. All agro-technical operations were carried out at optimal terms according to the weather conditions during the vegetation period and depending on the

plant development phases. Seed rate was 500 seeds per 1 m^2. Before cultivation of the soil a complex mineral fertilizer was applied: N – 51, P_2 – 30, K_2 – 42 kg ha^{-1}. Variants were arranged in four replications with a plot size 10 m^2 in a randomized block design.

The temperature and moisture conditions provided good oat field germination in 2012 and are represented in Table 1. The mean daily temperature changes were insignificant. Vegetation period was characterized by abundant rainfall and mean values of all months exceeded the long-term observed monthly norm. Harvesting was delayed approximately by ten days because of heavy rainfall in the first decade of August.

Mean samples from all replications (0.5 kg) were taken for testing by Infratec Analyser 1241 (test weight, protein, starch, lipid content) performed at the State Stende Cereals breeding institute. 1000 kernel weight was detected using standard method LVS EN ISO 520:2011. b-glucan content was determined enzymatically following the oat grain procedures of

Table 1

Meteorological data in the experimental period (Stende meteostation data, 2012)

Month	The mean daily temperature, °C			Sum of precipitation, mm		Percentage of monthly precipitation from long term average, %
	Monthly	Long term average	Long term average +/-	Monthly	Long term average	
April	5.6	4.3	1.3	42.6	37.0	115.1
May	11.0	10.2	0.8	58.9	45.0	130.9
June	13.3	14.2	-0.9	86.2	57.0	151.2
July	17.6	16.3	1.3	147.5	87.0	169.5
August	15.6	15.5	0.1	152.4	87.0	175.2

Table 2

Yield and productivity parameters of oat cultivars

Oat cultivars	Yield, t ha^{-1}	Test weight, kg hL^{-1}	1000 kernel weight, g
Latvian origin (n=5)			
Stendes Dārta	5.20±0.33	51.00±0.31	36.41±0.25
Stendes Līva	4.28±0.19	46.85±0.99	33.70±0.24
Māra	4.70±0.23	48.50±0.39	34.91±0.88
Arta	3.85±0.20	51.15±0.35	36.09±0.98
Laima	4.91±0.40	50.38±0.41	35.32±0.31
Mean	4.59b[1]	49.58	35.29b
$RS_{0.05}$	0.42	1.04	1.08
Foreign origin (n=10)			
Rajtar	5.93±0.22	49.80±0.82	37.14±1.19
Corona	5.39±0.05	49.28±0.81	37.32±2.84
Kerstin	5.13±0.15	48.18±0.50	35.10±0.39
Pergamon	5.33±0.02	50.85±0.70	41.48±2.11
Duffy	4.91±0.03	52.75±0.44	35.55±0.99
Freja	5.32±0.00	51.33±0.28	36.15±2.54
Aveny	5.82±0.06	49.85±0.10	38.37±2.25
Scorpion	5.84±0.08	50.90±0.36	46.34±0.80
Kirovec	4.75±0.63	51.40±0.54	36.45±2.70
Vendela	4.50±0.11	48.78±0.98	36.12±1.42
Mean	5.29a	50.31	38.00a
$RS_{0.05}$	0.53	1.06	1.88

[1]Trait means followed by different letters are significant between Latvian and foreign origin cultivars with at the level of $p<0.05$.

the commercial kits from Megazyme (Megazyme International Ireland Ltd.) according to the method developed by McCleary and Glennie-Holmes (1985) (McCleary et al., 1985) and performed at the State Stende Cereals breeding institute. In the procedure, highly purified enzymes were employed. A sample (0.5 g) of flour was weighted and b-D-glucan was depolimerized with lichenase to oligosaccharides and then hydrolyzed to glucose with a specific purified b-glucsidase. The b-glucan content (mg kg^{-1}) was calculated using the glucose quantity found in formula (1):

$$\beta\text{ - glucan} = \Delta E \times F/m \times 270, \quad (1)$$

where

ΔE – the absorbance difference at 510 nm in a UV-spectrophotometer after b-glucosidase treatment – blank absorbance;

m – weight of sample;

F – a factor for conversion of absorbance value to µg glucose

The obtained results were statistically processed by MS Excel program package using the methods of descriptive statistics; arithmetic mean value and standard division were calculated for Latvian and foreign origin cultivars. Mean comparison of both origin cultivars was carried out using the t-test and the p-values less than 0.05 were considered to be statistically significant. ANOVA procedures were used for data analysis.

Results and Discussion

In Latvia oat is mostly studied as raw material for human diet. The parameters, which have been studied, are yield per hectare, test weight, 1000 kernel weight represented in Table 2. Yield varied from 4.28 ± 0.19 - 5.93 ± 0.22 t ha^{-1}. Latvian origin oat cultivars on average characterized with significantly ($p<0.05$) lower yield, test weight and 1000 kernel weight compared with ones of foreign origin. The highest yield was detected for cultivars 'Rajtar', 'Scorpion' and 'Aveny' (accordingly 5.93 ± 0.22, 5.84 ± 0.08 and 5.82 ± 0.06 t ha^{-1}), Latvian origin cultivar 'Stendes Dārta' had the highest yield – 5.20 ± 0.33 t ha^{-1}. Latvian cultivar 'Arta' had significantly ($p<0.05$) lower yield from all cultivars.

Grain test weight characterizes grain kernel filling, and for tested cultivars it varied from 46.85 ± 0.99 - 52.75 ± 0.44 kg hL^{-1}. There was no significant difference ($p>0.05$) between foreign and Latvian origin cultivars. From foreign origin cultivars 'Duffy' had the higher test weight (52.75 ± 0.44 kg hL^{-1}), whereas from Latvian ones it was – 'Arta' – 51.15 ± 0.35 kg hL^{-1}.

1000 kernel weight characterizes the ecological plasticity of cultivar: it depend on meteorology and genetic factors. Variation of this parameter varied from 33.70 ± 0.24 - 46.34 ± 0.80 g. Latvian origin cultivars had significantly ($p<0.05$) lower 1000 kernel weight (accordingly 35.29 g and 38.00 g). Substantially higher 1000 kernel weight was for the cultivar 'Scorpion' – 46.34 ± 0.80 g.

The parameters, which have usually been studied are the following: yield from hectare, test weight, husk content, and crude protein content, but these parameters do not describe oats' nutritive and dietary value, which is an important criterion, describing the quality of food. Oat differs from other cereals by a balanced essential amino acid structure in protein, lipid rich with unsaturated fatty acids, easily available starch and comparatively high amount of β-glucan (Ryan et al., 2007). Plant breeders should pay attention to biochemical meters of cereals, while developing new oat varieties for food production until now. In Latvian oat breeding program the highest crude protein and crude lipid content are the selection criteria to evaluate breeding material (Zute et al., 2010). In his study protein, starch, lipid and B-glucan were tested. Their content is represented in Table 3.

Protein content for tested cultivars varied from 94.8 ± 1.7 - 119.5 ± 2.6 g kg^{-1}. The significantly ($p<0.05$) highest protein content was obtained in Latvian origin cultivars compared to foreign ones (accordingly 109.4 g kg^{-1} and 99.3 g kg^{-1}). The noticeably higher quality parameters are for varieties which are characterized by the lowest yield and its parameters. The greatest protein content showed the cultivar 'Arta' - 119 5 ± 2.6 g kg^{-1}, whereas the lowest - 'Scorpion' - 94.8 ± 1.7 g kg^{-1}: though previously it showed the opposite yield values. Despite the fact that starch content was significantly higher for foreign origin cultivars - 492.3 g kg^{-1}, for Latvian ones it was only – 461.7 g kg^{-1}, and it varied from 452.8 ± 2.9 to 508.8 ± 2.4 g kg^{-1}.

The lipid fraction of the oat grain determines in large measure its energy content and has a significant impact on nutrition (Zhou et al., 1999). Lipid content of selected cultivars varied from 47.3 ± 0.5 - 66.8 ± 1.3 g kg^{-1}. Lipid content of Latvian origin cultivars was significantly ($p<0.05$) higher than that of foreign origin (60.7 and 52.4 g kg^{-1} accordingly). The cultivar 'Stendes Dārta' was characterized by the highest lipid content - 66.8 ± 1.3 g kg^{-1}, the lowest lipid content was detected for cultivars 'Aveny' and 'Vendela' - 47.3 ± 0.5 and 47.3 ± 1.0 g kg^{-1}.

Among the main compounds associated with health-promoting effects in cereals is dietary fiber which is found only in plant foods. β-glucan content for selected cultivars varied from 2.68 ± 0.05 - 3.95 ± 0.10 mg 100 g^{-1}. Latvian origin varieties have significantly ($p<0.05$) higher β-glucan content compared to foreign varieties (accordingly 3.68 and 2.97 mg 100 g^{-1}). The highest β-glucan content was detected for the variety 'Arta' - 3.95 ± 0.10 mg 100 g^{-1}.

Table 3

Quality parameters of oat cultivars

Oat cultivars	Protein g kg^{-1} ± sd	Starch g kg^{-1} ± sd	Lipids g kg^{-1} ± sd	B-glucan mg 100 g^{-1} ± sd
Latvian origin (n=5)				
Stendes Dārta	104.5±2.4	453.0±4.2	66.8±1.3	3.90±0.22
Stendes Līva	107.3±2.2	485.0±3.6	50.8±0.5	3.03±0.17
Māra	110.5±1.9	460.5±3.0	61.8±1.5	3.65±0.26
Arta	119.5±2.6	452.8±2.9	58.0±0.8	3.95±0.10
Laima	105.0±2.2	457.3±2.5	66.0±1.4	3.85±0.10
Mean value	109.4a[1*]	461.7b	60.7a	3.68a
$RS_{0.05}$	3.4	4.9	1.7	0.27
Foreign origin (n=10)				
Rajtar	97.8±2.2	474.0±2.9	61.3±1.7	3.25±0.06
Corona	98.0±2.2	499.0±4.4	50.0±0.0	2.70±0.14
Kerstin	95.0±3.6	500.5±3.9	50.3±1.0	2.95±0.10
Pergamon	100.3±1.0	491.3±6.5	51.3±1.3	3.00±0.22
Duffy	101.5±2.4	493.0±3.6	51.5±0.6	2.95±0.13
Freja	98.8±1.3	468.0±3.8	63.8±1.0	3.73±0.24
Aveny	97.8±1.0	508.8±2.4	47.3±0.5	2.68±0.05
Scorpion	94.8±1.7	494.8±4.8	50.5±0.6	2.78±0.05
Kirovec	106.8±1.7	492.3±2.9	50.8±1.0	2.95±0.10
Vendela	102.0±2.9	501.8±3.4	47.3±1.0	2.73±0.13
Mean value	99.3b[*]	49.2.3a	5.2.4b	2.97b
$RS_{0.05}$	3.1	5.7	1.4	0.19

[1]Trait means followed by different letters are significant between Latvian and foreign origin cultivars with at the level of $p<0.05$.

In the described research, we have found out that the cultivars with highest yielding ability do not have high quality parameters. These high-yielding cultivars with higher quality are acknowledged as perspective in oat breeding program, and it is possible to use them as raw material for new oat varieties further.

Conclusions

1. The experimental testing of yield and its productivity parameters showed that the yield of tested cultivars varied between 4.28 ± 0.19 - 5.93 ± 0.22 t ha^{-1}, test weight 46.85 ± 0.99 - 52.75 ± 0.44 kg hL^{-1}, 1000 kernel weight 33.70 ± 0.24 - 46.34 ± 0.80 g.
2. Yield and 1000 kernel weight were significantly ($p<0.05$) higher for foreign cultivars, for cultivars 'Rajtar' (yield 5.93 ± 0.22 t ha^{-1}), 'Scorpion' (yield 5.84 ± 0.08 t ha^{-1};1000 kernel weight 46.34 ± 0.80 g), 'Aveny' (yield 5.82 ± 0.06 t ha^{-1}) and 'Pergamon' (1000 kernel weight 41.48 ± 2.11 g).
3. Latvian cultivars were with significantly ($p<0.05$) higher quality parameters. The highest protein and β-glucan content was observed for the local breed cultivar 'Arta' 119.5 ± 2.6 g kg^{-1} and 3.95 ± 0.10 mg 100 g^{-1} respectively. The highest lipid content cultivars were – 'Stendes Dārta' and 'Laima' (accordingly 66.8 ± 1.3 and 66.0 ± 1.4 g kg^{-1}).

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INFLUENCE OF ORGANIC PRODUCT EXTRACTS ON THE POTATO YIELD AND QUALITY IN THE CONVENTIONAL GROWING SYSTEM

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Abstract
Agricultural farms in modern production system are specialized;therefore, animal-origin organic fertilizers are not available for many of them. Researches on the use of organic products in agriculture to restrict the use of pesticides and mineral fertilizers have been performed for a long time. Nowadays different organic preparations that are acquired as a result of complex processes are produced, ensuring ready-to-use biologically active substances in them and also activating their properties. One of the purposes of the research was to evaluate the impact of extracts from organic products on the potato (*Solanum tuberosum*) yield and tuber quality in the conventional cultivation system. A field experiment using cultivars 'Borodyansky Rozovij' (early maturity) and 'Lenora' (mid-early maturity) was arranged in the State Stende Cereals Breeding Institute in 2011 and 2012. Peat elixir and vermicompost extract obtained at different temperatures: + 45 °C and + 95 °C, as well as a mixture of these extracts were used for treatment of tubers and plants. The research included 24 treatments in total, including control (without treatment) and a standard potato cultivation technology. Tubers were treated immediately before planting, but plants were treated three times during the vegetation period. Average two-year research results showed that the use of organic product extracts significantly ($p<0.05$) affected the tuber yield in different treatments for both cultivars 'Borodyansky Rozovij' and 'Lenora'. The content of nitrates in tubers, using extracts, did not exceed the allowable level (160 mg kg^{-1}) in any of treatments.
Key words: potatoes, crop, organic product extracts, peat elixir, vermicompost extract, nitrates.

Introduction

According to the data of the Central Statistical Bureau, in the last three years (2010 – 2012) potatoes (*Solanum tuberosum*) are cultivated in Latvia on about 30 000 ha or occupy 2.7% from the total planting area (http://www.csb.gov.lv/statistikastemas/lauksaimniecība-galvenieraditaji30325.html). Potatoes are one of the most important field crops, because of wide opportunities of their use (Skrabule, 2003). To obtain a high potato yield, it should be noted that crop is very demanding to growing management. It is important to obtain not only high yield, but also good quality tubers, and it might be possible by using environment-friendly fertilizers. To obtain a high and good quality crop yield, we need to take care of soil sanitation and soil fertility recovery (Köpke, 2007). Scientists do researches with environment-friendly organic products and their extracts in many places of the world.

Many products of different humic substances are available in Latvia as well as in the world. Their preparation is based on treatment of peat, compost or some other organic material with potassium hydroxide (Purmalis and Šīre, 2012).

Humic fertilizers produced from organic products are not traditional in Latvia; however, interest in them has been increasing during the last years. The most widespread sources for producing of humic substances are peat, brown coal or lignite, coal, leonardite, sapropel, sludge, worm biohumus or vermicompost (Chen et al., 2004; Theunisen et al., 2010). Currently more and more researches are being done in the world regarding the use of organic products in agriculture. New organic products are created by composting organic fertilizers, plant residues and household waste (Ndegwa and Thompson, 2001). Many countries – the USA, Russia, India, Belarus, etc. study the use of earthworms for processing of these organic residues (Aira et al., 2006; Pathasarathi et al., 2007). Latvia is rich in peat resources that may be used in agriculture. Peat is a valuable source of organic matter for agricultural soils and contains 7-61% of humic substances (Kuršs and Stinkule, 1997). To obtain organic products appropriate to modern agricultural technologies, in Latvia extracts from peat and vermicompost are produced, though there are just a few researches on the impact of these products on the yield and development of cultivated plants.

Peat elixir and vermicompost extract obtained at different temperatures: + 45 °C and + 95 °C, as well as a mixture of these extracts were used in this research. A goal of this investigation was to study the impact of extracts, which were obtained from the products of organic origin, on potato yield in the conventional growing system.

Materials and Methods

To study the impact of extracts from organic products on the potato yield, a field experiment in the conventional cultivation system was arranged in the State Stende Cereals Breeding Institute in 2011 – 2012 . The experiment was arranged in 3 replications treatments were arranged randomly. The size of plots was 25.6 m^2, including a yield registration area – 16 m^2. The planting rate was 46 000 tubers per ha. An early-season potato cultivar 'Borodyansky

Rozovij' (Ukraine) and an early-mid-season cultivar 'Lenora' (Latvia) were selected for the research. The experiment was arranged in gleyic sod-podzolic soil, that is characterized by soil acidity pH KCl – 5.34, content of organic substances – 19 g kg^{-1} of soil, content of available nutrients for plants P – 414 mg L^{-1} and K -255 mg L^{-1} of soil in the year 2011, and pH KCl 5.55, content of organic substances – 19 - 21 g kg^{-1} of soil, content of available nutrients for plants P – 447 mg L^{-1} and K – 195 mg L^{-1} of soil in the year 2012. A low content of N, S, Mg, Zn and B was stated in the soil. The amount of nutrients present in the soil was determined in the Laboratory of Plant Mineral Nutrition of the Institute of Biology of the University of Latvia, using methods of G. Riņķis et al. (Ринькис и др., 1987). Before the arrangement of the research, the fields were leveled in April and loosened using a cultivator– a chisel-tiller KR – 4. Before planting potatoes, furrows were made with a furrower with the spacing between furrows - 0.80 m. The potatoes were manually planted in the third decade of May keeping the spacing of 0.3 m between tubers. During the vegetation period, the potato plantation was harrowed twice with a chain harrow – on 8th and 14th day after planting, and it was also loosened twice with a row-crop hoe RKT – 2 on 7th and 14th day after planting. Before planting, complex mineral fertilizer NPK 11:9:21 was given at the rate – 550 kg ha^{-1} (pure matter N – 61 kg ha^{-1}, P – 22 kg ha^{-1}, K – 96 kg ha^{-1}). Before sprouting, the experimental field was sprayed with herbicide Mistral 70 d.g. (metribuzin, 700 g kg^{-1}), dose 0.5 kg ha^{-1}.

According to the methodology, the treatment with organic product extracts was done.

Groups of used organic products:

1. control – without treatment with extracts from organic products;
2. standard cultivation technology, without treatment with extracts from organic products, but with the use of pesticides;
3. treatment of potato tubers with extracts from organic products before planting;
4. treatment of potato tubers with extracts from organic products and pesticide: Maxim 025 s.c. (fludioxonil, 25 g L^{-1}) dose 0.2 L t^{-1} before planting;
5. treatment of plants with extracts from organic products three times per season after sprouting, when the plants reached 10 cm height, before blooming and after the blooming stage;
6. treatment of potato tubers with extracts from organic products before plantating and treatment of plants with these products three times per season in the above mentioned times;

Treatment with organic products:

1. peat elixir – two variants: the product obtained at + 45 °C and + 95 °C (K 45), (K95);
2. vermicompost extract – two variants: the product obtained at + 45 °C and + 95 °C (V45), (V95);
3. mixtures of the two extracts (mixture ratio 1:1) – peat elixir, obtained at + 95 °C and vermicompost extract, obtained at + 45 °C (KV);
4. mixture of vermicompost extracts (mixture ratio 1:1) – vermicompost extract, obtained at + 95 °C, and vermicompost extract, obtained at + 45 °C (VV).

Tubers were treated with the extracts on the planting day using a back-pack sprayer JACTO HD 300, the dose of extracts was 150 mL t^{-1}, but the total spray material consumption was 5 L t^{-1}. The dose sprayed on the plants after sprouting of potatoes, before and after blooming was 1.5 L ha^{-1}. Extracts from organic products were sprayed with a special experimental bike-type sprayer Birchmeier Spray-Matic 10 S. The sprayer is equipped with a flat jet nozzle with the pressure 250 kPa and spray material consumption - 250 L ha^{-1}. Extracts of organic products were sprayed in the evening when the air temperature did not exceed + 20 °C. Before tuber harvesting, tops were mowed with a haulm cutter. The potato yield was harvested in the beginning of September, using a two-row potato digger KTN-2V; potatoes were gathered manually. For the registration of yield 2 medium furrows (16 m^2) were collected from the four planted furrows of one plot. Potato was weighted and yield was converted to t ha^{-1}. To determine one of quality indicators – the content of nitrates in tubers, tuber samples were collected from each treatment and analyzed in the Laboratory of Food and Environmental Investigations of the Institute of Food Safety, Animal Health and Environment BIOR (according to SDA 83 nitrate determination method).

Mathematical processing of data was performed using analysis of variance, Microsoft Excel data processing software.

Meteorological conditions in 2011 and 2012 were characterized by frequent precipitation and moderately warm summers. Excess moisture at the end of summer and the beginning of autumn of 2011 stimulated rotting of tubers in the soil. An earlier development of late blight (*Phytophtora infestans*) was noted in 2012 if compared to 2011.

Results and Discussion

Impact of organic product extracts on tuber yield

An average two-year potato yield of cultivar 'Borodyansky Rozovij' was in the range from 22.26 t ha^{-1} to 31.95 t ha^{-1}, using extracts from organic products (Table). Results of the analysis of variance showed that the use of organic product extracts

significantly ($p<0.05$) affected the tuber yield of 'Borodyansky Rozovij' cultivar in several treatments.

In the control, where potatoes were not treated with extracts from organic products, the average two-year yield of the cultivar 'Borodyansky Rozovij' was 22.26 t ha^{-1}. Yield significantly ($p<0.05$) increased in fourteen treatments from twenty four (Table) when tubers were treated with extracts from organic products. Ten treatments did not provide a significant increase in the yield. Even a slight decrease of tuber yield was observed in treatments 9, 14, 19, if compared to the control, however, the decrease was within the

Table

Impact of extracts from organic products on the average potato yield and the nitrate content in tubers, 2011- 2012 in Stende

Versions	Borodyansky Rozovij			Lenora		
	yield		nitrate content, mg kg^{-1}	yield		nitrate content, mg kg^{-1}
	t ha^{-1}	± control		t ha^{-1}	± control	
1. Control	22.26	0.00	36.00	21.81	0	56.00
2. Standard cultivation	36.77	14.51	37.00	30.84	9.03	73.00
3. Processing of tubers with K (+45 °C)	25.92	3.66	37.50	24.37	2.56	41.00
4. Processing of tubers with K (+95 °C)	29.60	7.34	39.50	30.84	9.03	108.00
5. Processing of tubers with V (+45 °)	23.90	1.64	48.50	23.17	1.36	36.00
6. Processing of tubers with V (+95 °C)	28.11	5.85	38.00	24.15	2.34	74.00
7. Processing of tubers with KV	31.95	9.69	48.00	25.80	3.98	48.00
8. Processing of tubers with VV	29.65	7.39	78.50	26.32	4.51	49.00
9. Processing of tubers with pesticide +K (+45 °C)	22.09	-0.17	45.50	24.06	2.25	61.00
10. Processing of tubers with pesticide + K (+95 °C)	27.65	5.39	45.50	23.70	1.89	49.00
11. Processing of tubers with pesticide +V (+45 °C)	22.34	0.08	50.50	25.76	3.95	86.00
12. Processing of tubers with pesticide +V (+95 °C)	30.00	7.74	63.00	24.84	3.03	36.00
13. Processing of tubers with pesticide +KV	22.27	0.01	48.50	24.68	2.87	44.00
14. Processing of tubers with pesticide +VV	22.08	-0.18	67.50	30.76	8.95	39.00
15. Processing of plants with K (+45 °C)	26.75	4.49	66.50	23.46	1.65	74.00
16. Processing of plants with K (+95 °C)	22.60	0.34	48.00	24.35	2.54	63.00
17. Processing of plants with V (+45 °C)	22.84	0.58	41.50	26.42	4.61	90.00
18. Processing of plants with V (+95 °C)	28.05	5.79	36.00	23.96	2.15	99.00
19. Processing of plants with KV	22.16	-0.10	40.00	30.13	8.32	45.00
20. Processing of plants with VV	28.78	6.52	73.00	30.01	8.20	59.00
21. Processing of tubers and plants with K (+45 °C)	26.37	4.11	72.00	21.78	-0.03	67.00
22. Processing of tubers and plants with K (+95 °C)	28.98	6.72	55.00	22.48	0.67	69.00
23. Processing of tubers and plants with V (+45 °C)	27.76	5.50	49.00	26.40	4.59	96.00
24. Processing of tubers and plants with V (+95 °C)	31.90	9.64	48.50	25.16	3.35	37.00
25. Processing of tubers and plants with KV	24.70	2.44	36.50	30.40	8.59	61.00
26. Processing of tubers and plants with VV	25.17	2.91	92.50	30.48	8.67	50.00
$LSD_{0.05}$	3.10	×	×	3.82	×	×

limits of experimental error. The most substantial increase in the tuber yield, compared to the control, for this cultivar was observed in the treatments, when tubers were treated with a mixture of peat elixir and verimicompost extract (+9.69 t ha^{-1}) before planting, and when both tubers were treated and plants were treated three times during the vegetation period (+9.64 t ha^{-1}). In both treatments (Table, treatments 7 and 24) the yield increased by 43% compared to the control. The treatments when tubers were treated with pesticide and vermicompost extract (+95 °C) were also effective (Table, treatment 12); in this version the tuber yield was 30 t $ha^{-1,}$ i.e. significantly higher than in the control. If plants were treated 3 times during the vegetation period, then a significantly higher yield was obtained by applying peat elixir (+45 °C), vermicompost extract (+95 °C) and a mixture of vermisompost extracts (Table, treatments 15, 18, 20) ($p<0.05$). Significantly ($p<0.05$) lower yield of the cultivar 'Borodjansky Rozovij' was obtained in all treatments with organic extracts if compared with standard growing technology (Table, treatment 2, 36.77 t ha^{-1})

The average two-year yield of potato cultivar 'Lenora', varied from 21.81 t ha^{-1} (in the control) to 30.84 t ha^{-1} (treatments 2 and 4, Table). Results of the analysis of variance proved that the use of organic product extracts significantly ($p<0.05$) increased the yield of the cultivar 'Lenora' in 11 treatments from 24. The cultivar 'Lenora' had significantly higher ($p<0.05$) tuber yeild, when tubers were treated with peat elixir (+95 °C), a mixture of peat elixir and vermicompost extract and with a mixture of vermicompost extracts (Table, treatments 4, 7, 8). The treatment with pesticide on tubers and a mixture of vermicompost extracts has also significantly increased crop of this cultivar (treatment 14, Table, +8.95 t ha^{-1}). While processing plants with organic products, the highest increase in yield was observed, when they were sprayed with a mixture of the two products (Table, treatments 19, 20). A similar increase in the yield was observed, when tubers and plants were treated three times during the vegetation period (Table, treatments 25, 26) ($p<0.05$). The cultivar 'Lenora' similarly to the cultivar 'Borodyansky Rozovij' had a significantly ($p<0.05$) higher yield in the standard cultivation variant (Table, treatment 2) if compared with the control. The average increase in two-year yield in the standard was identical to the one obtained, if tubers were treated with peat elixir prepared at the temperature of +95 °C. Tuber yield did not significantly differ in other treatments with extracts from organic products (Table, treatments 3, 5, 6, 9, 10, 12, 13, 15, 16, 18, 21, 22, 24).

Two-year research results proved that the treatment with extracts from organic products in different ways significantly affected the potato tubers' yield of both cultivars used; however, the cultivars differently reacted to these extracts and treatments with them. Researches performed in various countries all over the world also indicated the positive influence of biological products on the potato yield. Researches performed in Belarus also proved that the use of an eco-gel, biological preparation, has positively affected the tuber yield. The yield in the treatments where biological preparation was used increased by 10 – 30% compared to the control (Кравченко и др., 2009). Researches of some other countries highlight positive influence of vermicompost and its extracts on the growth, development and productivity of plants (Cavender et al., 2003; Gamaley et al., 2001). Research results also specify that high doses of vermicompost extract or vermicompost acquired from pig (*Sus scrofa domestica*) manure, negatively affected the growth of tomatoes (*Solanum lycopersicum)* and cucumbers (*Cucumis sativus)*. The growth of sorghum (*Sorghum bicolor*) reduced, when non-sterilized vermicompost was worked into soil (Cavender et al., 2003). The vermicompost acquired from sewage sludge did not reduce the incidence of diseases (*Phytophtora infestans*), but at the same time negatively affected the growth and development of tomatoes (Szczech and Smolinska, 2001). Thus both, the research data obtained in Stende and conclusions from scientific literature show that the impact of organic products – vermicompost and its extracts on the growth, productivity and plant disease incidence is not always positive.

Impact of organic product extracts on nitrate content in tubers

Section 1 of the Annex to European Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs sets maximum allowable levels for nitrates in spinaches (*Spinacia oleracea*), lettuce (*Lactuca sativa*), processed cereal-based foods and foods for infants and young children. It was clarified that the nitrate content in potato tubers shall not exceed 250 mg kg^{-1}. Results of the two-year study, performing analyses of the nitrate content in tubers of both cultivars – 'Borodyansky Rozovij' and 'Lenora' – (according to SDA 83 method) proved that no treatment caused excess of the permissible nitrate content of 250 mg kg^{-1} (Table). A mixture of vermicompost extracts caused a tendency of nitrate content in all treatments of the cultivar 'Borodyansky Rozovij' to increase, though the permissible level was not exceeded. The cultivar 'Lenora' had comparatively higher nitrate content in tubers in the variant when tubers were treated with peat elixir (+95 °C); however, the tendency to increase is not to be taken into account here either.

Research results in Germany showed that the nitrate content may increase when using soluble mineral fertilizers, but if organic fertilizers are used, the nitrate content level in potatoe tubers is minimum, because organic fertilizers slowly and gradually release nutrients and ensure good supply and nutrition for plants (http://www.food.monitor.de/docs/multimedia/ oekote/Mo310kartoffeln oton.pdf.). Russian studies on the impact of fertilizers on the nitrate content showed that the use of compost significantly reduces the nitrate content, because plant residues bind excess nitrogen that is transformed into a form that may be used by plants (Андрианов и др., 2009). Research results in Belorussia with the use of an eco-gel, biological preparation, also proved that the nitrate content in the treatments with the experimental product complied with the set quality requirements and was within the limits up to 80 mg kg^{-1} (Кравченко и др., 2009). Research data of Lithuanian scientists convincingly inform about a positive impact on the nitrate content in tubers, they reduced in the versions when organic products were used (Недзинскене и Бакшене, 2009).

Although our research demonstrated that the nitrate content in tubers tends to grow if some treatments are applied, but it still does not exceedthe allowable level. Therefore, we may state that extracts from organic products do not reduce the quality of potato tubers.

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Conclusions

Potato cultivars 'Borodyansky Rozovij' and 'Lenora' differently reacted on extracts from organic products and treatment with them, but their use did not cause exceeding of the allowable nitrate level in any of treatments.

1. Peat elixir prepared at + 95 °C significantly affected ($p<0.05$) the tuber yield of both cultivars, when treating tubers before planting. The use of mixtures of extracts from organic products for treatment of tubers also provided a significant increase of yield for both cultivars.
2. The use of peat elixir prepared at +45 °C significantly increased only the yield of 'Borodyansky Rozovij' when tubers were treated, plants were treated three times during the vegetation period, and tubers and plants three times during the vegetation period were treated.
3. The use of vermicompost extract prepared at + 95 °C significantly increased only the yield of 'Borodyansky Rozovij' in different treatments, while this product did not significantly affect the yield of cultivar 'Lenora'.
4. A significant ($p<0.05$) impact of extract mixtures from organic products was observed only in 'Lenora' cultivar, when treating tubers and plants three times during the vegetation period.

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INFLUENCE OF SOWING TYPE, TIME AND SEEDING RATES ON THE BUCKWHEAT (*FAGOPYRUM ESCULENTUM*) YIELD QUALITY

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Abstract
In the last years total area of buckwheat *(Fagopyrum esculentum)* has increased notably in Latvia, including the Eastern part of the countrybut there is a lack of actual information about buckwheat growing, best terms of sowing and seeding rates. This study presents the influence of different sowing times, methods and rates on the seed quality of buckwheat yield – (TGW) thousand grain (nutlet) weight and (HLV) bulk density. A field experiment was conducted on the farm "Arāji", Krāslava region in 2010 and 2012. Buckwheat was sown on six different dates - 15.05, 25.05, 30.05, 05.06, 10.06 and 15. 06. Two types of sowing were used – drill sowing, where 4 seed rates were used - 200, 300, 400, 500 fertile nutlets per m^2, and the column sowing with the three seed rates - 150, 250, 300 fertile nutlets per m^2. It was found out that crop sowing time and type significantly affected buckwheat yield quality. Compensation mechanisms of yield in early terms of sowing when lower seeding rates were used were expressed less than that in later sowing terms. It could be concluded, that in early terms different seeding rates could be used with equal success. In later terms of sowing, smaller seeding rates are more appropriate. After two year investigation the most suitable sowing terms were around 4th and 9th June, where TGW and HLW was the highest. The most inappropriate terms were around 25th and 30th May, because meteorological conditions after sowing in both years were unfavorable that resulted in 0.5 to 1.0 t ha^{-1} lower yields.
Key words: buckwheat, sowing time, sowing method, plant density, yield quality.

Introduction

As an agricultural crop common buckwheat (*Fagopyrum esculentum* Moench) has a lot of advantages: it can be grown on poorer soils, has a short growing period, can be sown at different sowing times as the main or secondary crop. Buckwheat growing is limited due to various problems, such as drought stress (Podolska and Konopka, 2001), frost intolerance, and non-stable yields (Myers, 2002; Ratan and Kothiyal, 2011). Appropriate knowledge on buckwheat biology makes it possible to get high yields. Not just high amount of yield is important, but also the quality of yield, which is affected by several factors. Drought stress decreases the content of some protein fractures – prolamin and glyadin (Podolska and Konopka, 2001). Thousand grain (nutlet) weight TGW differs depending on many factors, but some measurements show, that it could be around 28 g in Korea (Yoon et al., 2004), 22-23 g in Slovenia (Wojcik, 1995), or almost 38 g in Iran (Omidbaigi and Mastro, 2004). The study in Iran also showed, that sowing time influences the TGW, and difference of sowing time in 30 days could affect the TGW and cause increasing of weight per 36% (Omidbaigi and Mastro, 2004). In the study in Korea, it was observed that the value of TGW is not affected depending on the soil moisture condition at sowing date, but it is influenced by water deficit at early growth stages and blossoming (Yoon et al., 2004). Differences in soil type and condition also have influence on TGW, but the difference is small – 24.12 g in good light silt loam, and 21.73 g in light sand soil (Podolska and Podolski, 2004). Other property of buckwheat – bulk density (HLW; g L^{-1} or kg m^{-3}) has rarely been described , only some information is available. The study in Canada shows that bulk density differs between varieties, but there is no significant difference between the compacted density and standard density of the same variety of buckwheat, for instance, the variety 'Koto' – 604.07 g L^{-1} and 599.52 g L^{-1} respectively (Parde et al., 2003). Buckwheat bulk density measurements in India were higher – 750 g L^{-1} (Batham et al., 2013).

The aim of this study was to investigate the influence of sowing type, time and seeding rate to buckwheat yield quality parameters – TGW and HLV in Eastern Latvia.

Materials and Methods

Two-factor field trial (A factor – sowing date, B factor – combinations of sowing rates and types) were arranged on the farm "Araji", Kraslava region (latitude: N 55º 86´, longitude: E 27º 04´) in 2010 and repeated in 2012. In both years the same scheme for trial arrangement was used – a combination of two different sowing types (drill sowing and column sowing) with six different sowing rates (Table 1). Sowing was carried out from the 15th May and repeated after every 5 days (Table 1). The trial was randomly arranged; in total 42 plots were placed in four replications. The plot size was 3 × 15 m. Soil parameters in 2010were the following: silt loam

(organic matter content 22.5 g kg^{-1}, soil reaction pH KCl - 5.8, P – 74.23 mg kg^{-1}, K – 149.36 mg $kg^{-1)}$ in 2012- silt loam (organic matter content 20.17 g kg^{-1}, soil reaction pH KCl – 5.6, P – 69.74 mg kg^{-1}, K – 162.27 mg $kg^{-1)}$ Previous crop was spring barley in both years.

Table 1

Sowing types, rates and dates in this study

Factor – A			Factor – B	
sowing time			Sowing type and seeding rates (nutlets per 1 m^2)	
No.	Date	Difference, days	Drill sowing	Column sowing
1st	15th May	0	200	150
2nd	20th May	+5	300	200
3rd	25th May	+10	400	250
4th	30th May	+15	500	
5th	4thJune	+20		
6th	9th June	+25		

Soil tillage and sowing. Traditional soil tillage with 25 cm deep autumn plowing was done immediately after the harvesting of pre-crop (spring barley). Soil was cultivated three times before sowing in spring. One week before the second cultivation weed treatment with the herbicide Roundup Eco S.C. (glyphosate, 360 g L^{-1}) was done. Prior to sowing and soil cultivation, the necessary amount of nitrogen fertilizer was incorporated. The plots were fertilized with N – 32, P – 14, K – 26 kg ha^{-1}. Buckwheat was sown with mechanical seed-rows-ploughshare 4-m-wide drill – NordsteinLiftomatik, with 12 cm wide line-spacing. Variants sown in columns were composed of two rows with a distance between rows of 12 cm, but that between the columns – 38 cm; sowing depth was 4 cm.

Yield harvesting, TGW and HLW measurement. Yield was harvested with a grain harvester Massey Ferguson 525, header width 3.5 m. After harvesting moisture content with the 'Wille' moisture meter was measured, weighted and cleaned. TGW and HLW of clean nutlets were measured in the laboratory. Nutlets for TGW were counted (500 nutlets twice) with a special equipment 'Count star 2000' (LVS − 273:2000) and then weighted. HLW of nutlets were measured with the standard method (LVST ZM 43 − 95:1995)

Meteorological conditions in 2010 and 2012. Meteorological data were collected from 'Latvian Environment, Geology and Meteorology Centre', Daugavpils hydro-meteorological station (HMS) (Fig.1.) which is the nearest HMS to the farm. For more correct data also local measurements of rainfall with portable precipitation gouge directly on the trial fields were detected.

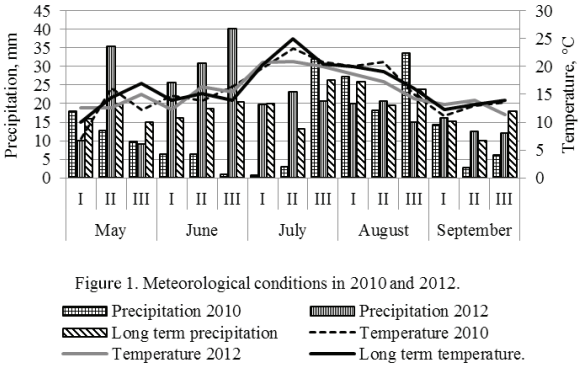

Figure 1. Meteorological conditions in 2010 and 2012.

Meteorological conditions of 2010 growing season can be described as warm and wet with periodic substantial rainfall. When plants reached the technical maturity stage (70 – 75% brown nutlets on the plant), the weather conditions were with the high temperature and extraordinary high amount of precipitations that contributed to the growth of the plants, and the growing season extended. Finally it was difficult to choose an appropriate time for buckwheat harvesting. In 2012, the average air temperature was lower at the sowing time and early stages of buckwheat development, and also low if compared with a long term average temperature, with periodical high amount of rainfall.

Data analysis. Two-factor analysis of variance, correlation and regression analysis methods were used for data processing. In two year trial data the influence of seeding rate, sowing date and type to changes on HLW and TGW parameters were analyzed. Data analyses were done with MS Excel.

Results and Discussion

TGW. Total average weight of thousand nutlets in two different sowing types was not statistically different – 22.72 g when a drill sowing was used and 22.69 g when a column sowing was used in 2010 (Table 2). The average TGW of buchkwheat in 2010 was lower than that in 2012, when the average weight of thousand nutlets was 22.69 at the drill sowing and 22.72 at the column sowing. The highest TGW of buckwheat in 2010 was 23.16 g when the drill sowing was used, at the seed rate of 200 nutlets per m^2, at the second term of sowing – 20 May, 2010 (+5 days to the first date of sowing). The highest TGW (23.12 g) when the column sowing was used in 2010 was observed at the same sowing time using the same seeding rate as at the drill sowing.

Table 2

Influence of sowing time, seeding rate on TGW (g) of buckwheat nutlets at different sowing types in 2010

A_x seeding rate per 1 m^2	Sowing time B_x						
	15.05	20.05	25.05	30.05	4.06	9.06	Average A
Column sowing							$RS_{0.05A}$=**0.64**
A_1 - 150	×	22.94	22.71	22.52	22.34	22.61	22.62
A_2 - 200	×	23.16	22.62	22.50	22.65	22.60	22.71
A_3 - 250	×	23.05	22.81	22.78	22.99	22.51	22.83
Average B $RS_{0.05B}$=**0.59**	×	23.05	22.71	22.60	22.66	22.57	22.72
Drill sowing							$RS_{0.05A}$=**0.30**
A_4 - 200	22.90	23.12	23.02	22.67	22.84	22.36	22.82
A_5 - 300	23.18	22.80	22.35	22.68	22.36	22.16	22.59
A_6 - 400	22.67	22.72	22.83	22.76	22.35	22.50	22.64
A_7 - 500	22.86	22.68	23.00	22.83	22.55	22.41	22.72
Average B $RS_{0.05\,B}$=**0.37**	22.90	22.83	22.80	22.73	22.53	22.36	22.69

Table 3

Influence of sowing time, seeding rate to TGW (g) of buckwheat nutlets at different sowing types in 2012

A_x seeding rate per 1 m^2	Sowing time B_x						
	15.05	20.05	25.05	30.05	4.06	9.06	Average A
Colum sowing							$RS_{0.05A}$=**0.47**
A_1 - 150	×	24.46	24.38	22.92	24.70	25.62	24.42
A_2 - 200	×	24.23	23.73	21.95	23.80	25.26	23.79
A_3 - 250	×	23.46	23.38	21.92	23.70	24.62	23.42
$RS_{0.05\,B}$=**0.68**	×	24.05	23.83	22.26	24.07	25.17	23.88
Drill sowing							$RS_{0.05A}$=**0.37**
A_4 - 200	×	24.66	23.69	23.42	24.90	23.86	24.11
A_5 - 300	×	26.81	23.80	21.54	24.02	23.02	23.84
A_6 - 400	×	24.09	24.37	21.79	23.37	23.95	23.51
A_7 - 500	×	21.71	24.89	24.03	23.87	24.60	23.82
$RS_{0.05\,B}$=**0.74**	×	24.32	24.19	22.70	24.04	23.86	23.82

In 2010, there was a tendency that by increasing the seeding rate, the TGW increases, but the difference was not statistically significant. Also, there is a tendency that the sowing term has a moderate influence on TGW, more positive effect has earlier sowing terms than later ones. The tendency is not related to biological properties of buckwheat, but could be explained with a low amount of precipitation at the beginning of growth period, and high amount of precipitation at the end of the growth period. High amount of precipitation at the end of growth initiated the secondary growth of buckwheat and formation of new, poorly developed nutlets. As described before (Halbrec and Ledent, 2004, Podolska and Mazurek, 2004), new nutlets could be formed, when already 80% are mature, brown, but leaves could not support the transport of active substances, and new nutlets develop smaller and with lower TGW.

In 2012, meteorological conditions were more favorable for buckwheat development, and there was a clear tendency that an increase of the seeding rate leads to a decrease of TGW (Table 3). This tendency is represented in the plots of both sowing types – at the column sowing as well as at the drill sowing.

It is related to the competition of plants in condensed sowings and less developed photosynthetically active leaf area. It has been described (Podolska and Bogusław, 2004) that buckwheat plants in condensed sowings can develop the compensation mechanisms. If more plants are growing per 1 m^2, the number of branches and inflorescences per plant are decreasing, and smaller nutlets with lower TGW are forming. As a consequence, lower HLW and higher huskiness follow. The average amount of TGW is slightly higher in variants with the drill sowing. This tendency was observed in both years, but differences are not statistically significant. This observation is opposite to findingsdescribed before (Кукреш, 1973) stating that the column sowing increases the TGW.

HLW. Statistical analysis confirmed that HLW depends on the sowing date, seeding rate and combinations of those parameters with the sowing type. During the investigation it was found out that a change of HLW of buckwheat nutlets is closely related to growth conditions. Comparison of data from two years of investigation showshow the sowing year influenced the changes of HLW. In 2012, the average HLW was significantly higher than that in 2010 (Table 4, 5).

Variability of data was lower in 2010, if compared with 2012. Overall, the variation of data in both years was within 559 g L^{-1} to 605 g L^{-1} interval. This weight is higher than demanded from the processing industry, stating that it should not be lower than 490 g L^{-1}.

Table 4

Influence of sowing time and rate on HLW (g L^{-1}) of buckwheat nutlets at different sowing types in 2010

A_x seeding rate per 1 m^2	Sowing time B_x						
	15.05	20.05	25.05.	30.05	4.06	9.06	Average
Column sowing							$RS_{0.05}$=**0.91**
A_1 - 150	×	576.7	581.7	566.4	571.8	565.0	572.3
A_2 - 200	×	559.5	583.9	575.6	582.6	579.6	576.2
A_3 - 250	×	567.0	578.3	554.7	581.4	579.9	572.3
$RS_{0.05}$ B=**1.17**	×	567.7	581.3	565.6	578.6	574.8	573.6
Drill sowing							$RS_{0.05}$=**1.08**
A_4 - 200	573.5	580.7	591.4	577.9	580.5	581.9	581.0
A_5 - 300	559.3	574.5	584.4	567.0	577.0	583.3	574.2
A_6 - 400	576.8	580.7	583.5	565.8	582.8	587.5	579.5
A_7 - 500	569.9	582.7	593.5	583.6	583.9	589.2	583.8
$RS_{0.05}$B=**1.32**	569.9	579.6	588.2	573.6	581.0	585.5	579.6

Table 5

Influence of sowing time, seeding rate to HLW (g L^{-1}) of buckwheat nutlets at different sowing types in 2012

A_x seeding rate per 1 m^2	Sowing timeB_x						Average
	15.05	20.05	25.05	30.05	4.06	9.06	$RS_{0.05}$=**1.10**
Column sowing							
A_1 - 150	×	607.7	583.7	597.6	606.4	604.8	600.0
A_2 - 200	×	606.1	601.1	589.3	596.5	590.6	596.8
A_3 - 250	×	601.7	590.5	576.6	605.1	604.5	595.7
$RS_{0.05}$ B=**1.62**	×	605.2	591.8	587.8	602.7	600.0	597.5
Drill sowing							$RS_{0.05}$=**0.87**
A_4 - 200	×	608.4	594.4	597.6	597.7	603.8	600.4
A_5 - 300	×	605.1	595.3	591.3	595.4	595.7	596.6
A_6 - 400	×	597.9	594.8	577.6	596.1	601.5	593.6
A_7 - 500	×	598.2	591.3	578.5	591.0	596.3	591.1
$RS_{0.05}$ B=**1.13**	×	602.4	594.0	586.3	595.1	599.3	595.4

Table 6

Summary and comparison of parameters evaluated in this study in 2010 and 2012

Parameter	Sowing time									
	B_{+5}		B_{+10}		B_{+15}		B_{+20}		B_{+25}	
	2010	2012	2010	2012	2010	2012	2010	2012	2010	2012
Length of nutlet forming period	19	23	17	24	19	26	18	25	20	27
Precipitation, mm	10	27	5	23	7	23	9	18	34	55
TGW, g ($LSD_{0.05}$ = 0.75)	22.83a	24.32b	22.80a	24.19b	22.73a	22.70a	22.53a	24.04b	22.36a	23.86b
HLW, g L^{-1} ($LSD_{0.05}$ = 2.05)	579.6b	602.4 f	588.2d	594.0 e	573.6a	586.3cd	581.0b	595.1 e	585.5c	599.3 f
Yield t ha^{-1} ($LSD_{0.05}$ = 0.55)	0.78 a	1.93 c	0.91 ab	2.25 cd	1.34 b	2.09 cd	2.43 cd	2.57 d	2.24 cd	2.80 d

*different letters at each number indicate significant differences between variances

In 2012, the average amount of HLW was higher than one in 2010. The highest one was in the drill sowing at an early term B+5, and the seeding rate 200 nutlets per m^2 At this sowing time the average weight of hectoliter in the column sowing was even higher.

To examine the reasons why HLW differs significantly between 2010 and 2012, meteorological data were analyzed. In 2010, during the nutlet formation period, the average amount of precipitation for each sowing time was 10 – 20 mm lower than that in 2012. This probably caused the decrease of HLW by 14 g L^{-1} on average in 2010 (Table 6).

When comparing results of vegetative growth period lengths in both years, a significant difference is observed. In 2010, the buckwheat nutlet formation period was 4 – 8 days shorter and it could be affected by meteorological conditions: hot mean air temperature with periodical rainfalls. In June the average temperature of the day was around 23 °C, and the development of plants was dwarfed. Sowing that was done on 30. May was particularly unfavorable for the development of plants and that followed by an unfavorable influence to the nutlet development. At this sowing time the lowest TGW was observed – 22.73 g, but the influence to HLW was not so visible -573.6 g L^{-1}. After two years of investigation it could be assumed that climatic conditions in each year primary influence the nutlet quality parameters.

Conclusions

1. Sowing time, type and rate influenced both – the TGW and HLW of buckwheat. A decrease or increase of those parameters depended on conditions in the trial year, and a clear average tendency after two years still is not observed.
2. TGW of buckwheat sown in the column sowing was similar to that one sown in the drill sowing in both years. Some differences were observed in the values of HLW when both sowing types were compared, but the differences were conflicting in both years; a slightly higher average HLW was observed for the buckwheat grown in the drill sowing in 2010 (+6 g L^{-1}), but in 2012 – a slightly higher average HLW was observed for the buckwheat grown in the column sowing (+2.1 g L^{-1}). In addition, as concerns the agronomical point of view, the mentioned differences are unimportant.
3. Conditions of the trial year showed a clear influence on investigated parameters; more favorable for buckwheat yield and quality parameters – TGW and HLW forming were conditions of the year 2012 when substantially higher values were observed disregarding the sowing term.
4. The highest value of TGW (26.81 g) per trial period was observed in 2012 in the following combination: buckwheat sown on May 20 in the drill sowing at the rate of 300 nutlets per 1 m^2.
5. The highest value of HLW (608.4 g L^{-1}) per trial period was also observed in 2012 in a similar combination: buckwheat sown on May 20 in the drill sowing at the rate of 200 nutlets per 1 m^2.

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PRODUCTION OF BIOETHANOL FROM STARCH BASED AGRICULTURE RAW MATERIAL

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Abstract
Bioethanol can be used for food production and to partially replace fossil fuel. Bioethanol is mainly produced from renewable biomass that contains sugars, starch or lignocellulose. The main raw materials for production of bioethanol are cereals, maize (*Zea mays*), sugarbeets (*Beta vulgaris sacccharifera*) and other plant species. During the trial that took place in State Stende Cereals Breeding Institute during a three year period (from 2009/2010 to 2011/2012) we examined the suitability of grain from winter wheat (*Triticum aestivum* L.), triticale (×*Triticosecale* Wittm) and rye (*Secale cereale* L.) for the production of bioethanol. Three varieties of each species were used in the trial. During the trial period the grain yield, the ethanol outcome (L t^{-1}) and the ethanol yield (L ha^{-1}) were determined. It was established that during three years wheat and triticale provided the highest starch content (more than 700 g kg^{-1}) of the grain as well as the highest ethanol outcome (L t^{-1}). These species provided both high grain yield (more than 9 t ha^{-1} on average) and the highest ethanol yield (3300 – 4665 L ha^{-1}). The choice of variety was also important as both the grain starch content and the grain yield depend on the genotype of the variety.
Key words: winter cereals, starch, yield, ethanol outcome.

Introduction

The economical use of energy resources and the use of renewables in the energy production is a topical issue in the entire world. Intensive use of fossil fuels polluting the atmosphere with gas emissions is mentioned as one of the reasons for rapid climate change. One way to use the renewables is to produce bioethanol that can be used for food production and to partially replace fossil fuel. Bioethanol is mainly produced from renewable biomass containing sugars, starch or lignocellulose (Ethanol production…, 2006). The main raw materials for production of bioethanol are sugar cane (*Saccharum* spp.), cereals, maize (*Zea maize*), sugar beet (*Beta vulgaris sacccharifera*) and other plant species. European Union member states mainly use cereals and sugar beet for that purpose. Sugar and starch processing technologies are mainly used in the production of bioethanol. Technologies for ethanol production from cellulose are used less frequently.

The limiting factor for the production of bioethanol from raw materials containing starch and sugar is the extensive use of water, fertilisers and pesticides in the cultivation of raw materials. An important issue is also the necessity to use these raw materials for food production. For example, in the USA where bioethanol is mainly produced from maize, the price of beef, pork and poultry, eggs, bread, cereals and milk increased for 10 – 20% as a result (Brown, 2008). Taking into account the conditions in Latvia, the cereals with higher starch content in the grain could be more suitable for the production of bioethanol as they are less suitable for food production. Winter cereals: rye (*Secale cereale* L.), wheat (*Triticum aestivum* L.) and triticale (×*Triticosecale* Wittm) provide comparatively high grain yield, but the grain yield is one of the most significant indicators for a high bioethanol yield (L ha^{-1}). According to the data of Serbian researchers triticale and wheat are considered to be the most suitable raw materials for the production of bioethanol (Mojović et al., 2009).

The purpose of the study was to evaluate the suitability of winter cereals: wheat, triticale and rye as the starch based raw materials for the production of bioethanol in the conditions of Latvia. **Work task** resulting from the purpose was to set grain yield and starch content in it as well as the ethanol yield and ethanol outcome from different species of winter cereal crops.

Materials and Methods

The field study was carried out at State Stende Cereals Breeding Institute for three years: 2009/2010, 2010/2011, 2011/2012. The following varieties were examined during the study: winter wheat varieties ’Mulan’, ‘Skalmeje’ and the line ‘99-115’ created at State Stende Cereals Breeding Institute; winter rye varieties ‘Matador’, ‘Placido’ (F1), ‘Dankowskie Nowe’; winter triticale varieties ‘SW Valentino’, ‘Dinaro’ and the line ‘0002-26’, created at State Priekuli Plant Breeding Institute. The study was carried out in sod-podzolic or podzolic-gley soil depending on the year. The fields were chosen according to the crop rotation established at the Institute (Table 1). All the years the soil reaction was slightly too acid for cultivation of wheat, but suitable for the cultivation of rye. The variants were randomly placed in 4 replicates with the harvest reference area of 12 m^2. All three years white mustard (*Sinapis alba*) for green manure was used as a pre-crop. All three years the sowing

Table 1

The characteristics of soil

Year	Type of soil	Granulometric content	pH KCL	Content of organic matter, g kg^{-1}	P, mg kg^{-1}	K, mg kg^{-1}
2009/2010	Sod-podzolic	Clay loam	5.8	24	100	150
2010/2011	Podzolic-gley	Clay loam	5.8	23	82	111
2011/2012	Podzolic-gley	Clay loam	5.3	19	36	105

rate was 200 germinating seeds per m^{-2} for hybrid rye ('Placido'), 400 germinating seeds per m^{-2} for open pollinated rye varieties and triticale, and 450 germinating seeds per m^{-2} for wheat. The sowing was carried out in the second ten-day period of September (18.09.2009, 14.09.2010, 15.09.2011). Before sowing plots were fertilized with NPK fertilizers at the rate: N 12-15 kg ha^{-1}, P 20-26 kg ha^{-1} and K 75-79 kg ha^{-1}.

Ammonium nitrate (N 340 g kg^{-1}) was used as a top-dressing in spring:

- after the renewal of vegetation growth:
  - for winter wheat – 90 kg N ha^{-1};
  - for triticale and rye – 60 kg N ha^{-1};
- at the plant growth stage (GS) 31 – 32 for all examined varieties of winter cereals - 60 kg N ha^{-1}.

Fungicide Osiris e.c. (epoxiconazole, 37.5 g L^{-1}, metconazole, 27.5 g L^{-1}) in the amount of 1.5 L ha^{-1} was used during the vegetation period in order to prevent the diseases of winter wheat and triticale. To keep the weeds at bay the herbicide Mustang s.e. (florasulam, 6.25 g L^{-1}, 2.4 – D, 300 g L^{-1}) in the amount of 0.55 L ha^{-1} (sowing year 2009 and 2010) and Mustang Forte s.e. (florasulam, 5 g L^{-1}, aminopyralid, 10 g L^{-1}, 2.4 – D, 180 g L^{-1}) in the amount of 0.6 L ha^{-1} (sowing year 2011) were used. The retardant Medax Top (prohexadione-calcium, 50 g L^{-1}, mepiquat chloride, 300 g L^{-1}) in the amount of 0.75 L ha^{-1} was used only during the season 2011/2012 when due to increased humidity, there was a risk that the winter cereals would lodge. The crop was harvested at the GS 88–92 on 8 August 2010, 4 August 2011 and 6 August 2012.

After the samples were dried on platform driers, they were cleaned with MINI PETKUS MP100. The yield of winter cereals (t ha^{-1}) was then recalculated at 14% humidity and 100% purity. The quality analysis of grain was carried out at the Grain technology and agrochemistry laboratory of State Stende Cereals Breeding Institute. The content of crude protein (LVS 277:2000) and starch (LVS EN ISO 10520) was determined.

Experimental extraction of bioethanol. The practical extraction of ethanol from all the studied species and varieties took place in the Microbiology and Biotechnology Institute of the University of Latvia. The method was based on the fermentation of saccharised wheat, triticale and rye samples using yeasts *Saccharomyces cerevisiae*, which was followed by the calculation of the ethanol outcome and the fermentation speed.

Saccharification. 20 g of ground cereals sample and 80 g of water were weighed and mixed in the retort. Liquefaction was carried out by using 54 mL alfa-amylase preparation "Talzyme AL90" (JP Biotechnology) for 40 minutes at 90 °C. The pH was adjusted to 5.0 and the mixture was cooled to 60 °C.

Saccharification was carried out by using 400 mL glucoamylase preparation "Talzyme GL60" (JP Biotechnology) for 40 minutes at 60 °C. Liquefaction and saccharification was performed by a constant stirring of the sample.

Fermentation. After saccharification the sample was cooled to 30 °C. *Saccharomyces cerevisae* inoculum in the amount of 2 mL was added. The fermentation was carried out in retorts at 30 °C for 1–2 days. The changes in retort weight were measured during the fermentation. When the weight remained constant (thus indicating the end of fermentation process), the sample was taken and ethanol concentration was determined by chromatography. The ethanol outcome from the theoretical result and the productivity of ethanol formation (g kg^{-1} h^{-1}) were calculated.

The theoretical ethanol outcome g kg^{-1} from grain was calculated from the determined starch content by using formulas (1) and (2):

$$C\ glikoze = \frac{Ccietе \times 180.16}{162.16}, \text{ where} \quad (1)$$

C glikoze – outcome of glycose, g kg^{-1};
C ciete – starch content, g kg^{-1}

$$C\ et.teor = \frac{C\ glikoze \times 2 \times Metanols}{M\ glikoze \times k}, \text{ where} \quad (2)$$

C et. teor. – theoretical ethanol outcome, g kg^{-1};
M etanols – molar mass of ethanol;
M glikoze – molar mass of glycose;
K – dilution factor, 5.

By using formula (3) the practically acquired ethanol outcome % was calculated in comparison to the theoretically calculated ethanol outcome.

$$Cet(\%no\ teor) = \frac{Cet \times 100}{Cet.teor}, \text{ where} \quad (3)$$

Cet (% of the theoretical) – practically acquired ethanol outcome, %, was calculated in comparison to the theoretically calculated ethanol outcome, %

Cet – concentration of ethanol, determined in the fermentation environment, g kg^{-1}

In the laboratory, the ethanol outcome from grains was determined, and the ethanol outcome was calculated in grams from one gram of grains (formula (4))

$$C = \frac{Cet}{Cgraudi}, \text{ where} \quad (4)$$

C – ethanol outcome from grain, g g^{-1}

C_{graudi} – amount of grain in the fermentation environment (grain in g 100 g^{-1} of fermentation environment, in this case – 20 g 100 g^{-1})

The productivity of ethanol formation depends on the concentration of ethanol determined in the fermentation environment and the time of fermentation (formula (5))

$$Cpr = \frac{Cet \times 10}{t}, \text{ where} \quad (5)$$

Cpr – productivity of ethanol formation g kg^{-1} h^{-1}

t – fermentation time, h

Ethanol outcome is expressed in litres by using formula (6)

$$Cl = \frac{C}{0.789} \times 1000, \text{ where}$$

Cl – ethanol outcome, L t^{-1}

0.789 – density of ethanol, g cm^{-3}

Two factor dispersion analysis method was used for the mathematical analysis of data.

The weather conditions during the trial years were varied. During all three years there was adequate humidity and temperature during the germination and tillering in autumn. During the wintering period the most unsuitable conditions were observed in the winter of 2010/2011. The dry spring of this year also affected the development and yield of certain varieties. Hot summers of 2009/2010 and 2010/2011 affected the formation of crude protein in grain whereas the starch content in grain was higher in 2011/2012 when there was sufficient humidity and the temperatures were lower. The increased amount of precipitation affected harvesting in all the years.

Results and Discussion

Starch content in grain. According to the data of scientific literature, the grain of winter cereals is suitable for the production of bioethanol due to the high starch content. According to the data, the starch content is 57-66% in the grain of rye (Boese, 2006), 670-690 g kg^{-1} in the grain of triticale (Krejčirova and Capouhova, 2008), 640-730 g kg^{-1} in the grain of wheat (Ethanol production..., 2006; Kindred et al., 2008; Sedláček et al., 2008; Saunders, 2011). The results obtained during the three years in Stende show that in the conditions of Latvia the starch content was 692.17 – 726.07 g kg^{-1} for winter wheat, 685.50 – 722.07 g kg^{-1} for triticale and 613.10-633.30 g kg^{-1} for rye (Table 2). According to the results of other authors, the starch content in grain depends on both the variety and the climate conditions (Kučerov, 2007; Krejčirova and Capouchova, 2008). Similarly to the descriptions in the scientific literature the impact of variety and the weather conditions during the year of cultivation was also observed in the trial in Stende. The highest starch content in the grain was observed in 2011/2012, when the humidity level was high and temperature range was around the long-term average during the formation of the grain. The starch content of winter wheat was similar in all the trial years, whereas for triticale and rye the grain starch content was substantially higher in 2011/2012 if compared with that of the previous year (Fig. 1).

According to the research data from the Czech Republic, the starch content in triticale grain was 673.3 – 693.9 g kg^{-1} (Krejčirova and Capouchova, 2008). A similar result was obtained in the research of State Stende Cereals Breeding Institute – 703.1 g kg^{-1} on average. Comparing starch content in the grain of different species, the highest average starch content was observed in wheat – 710.4 g kg^{-1}. The average starch content in triticale grains was slightly lower (703.1 g kg^{-1}). The lowest average starch content was observed in rye grain – 627.0 g kg^{-1}; it was 11.7% lower than that of wheat grain. The research of other authors also shows that the starch content in rye grain is lower than that of triticale (Wang et al., 1997).

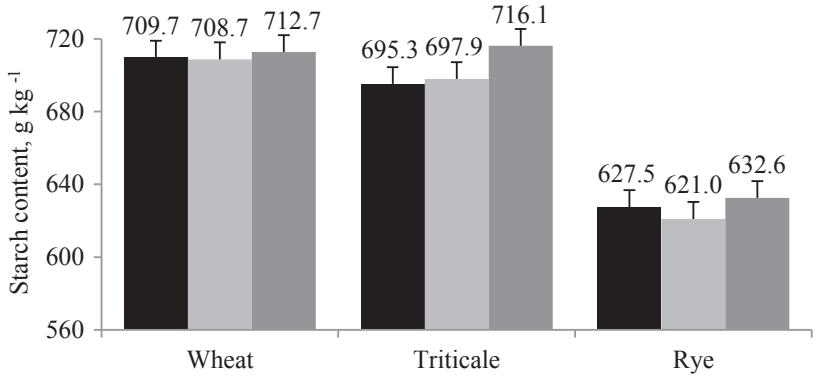

Figure 1. The changes in starch content (g kg^{-1}) of cereals grain in all three trial years depending on the species where 2009/2010 ■ , 2010/2011 ■ , 2011/2012 ■ , LDS $_{0.05}$=9.24.

Table 2

Yield, starch content, ethanol outcome of winter cereals in Stende in the years 2010 – 2012

Variety	Yield, t ha^{-1}	Starch content, g kg^{-1}	Ethanol outcome, L t^{-1}
Wheat			
Skalmeje, min – max average	8.16-10.83 9.50	692.17-718.80 709.77	384.03-435.15 417.97
99-115 average	8.71-9.84 9.27	713.13-726.07 **718.09**	409.38-440.64 **428.67**
Mulan min – max average	8.29-11.48 **9.72**	693.07-721.07 703.13	406.84-426.49 415.50
$LDS_{0.05*}$	0.535	7.88	7.40
Triticale			
SW Valentino min – max average	7.88-11.05 9.18	685.50-712.63 695.28	408.75-439.37 424.24
Dinaro min – max average	8.81-9.31 **9.39**	698.50-722.07 **708.28**	423.32-449.94 **433.67**
0002-26 min – max average	7.32-10.17 8.86	693.80-713.67 **705.69**	403.68-442.33 **426.28**
$LDS_{0.05*}$	0.547	5.89	4.77
Rye			
Placido min – max average	8-42-10.75 **9.74**	626.53-632.37 **628.88**	365.02-409.38 393.32
Matador min – max average	7.30-10.79 8.62	613.10-633.30 623.84	370.09-424.59 **400.08**
Dankowskie Nowe min – max Average	7.41-10.67 8.53	622.20-632.10 **628.38**	366.92-418.25 **396.92**
$LDS_{0.05*}$	0.591	7.85	4.77

*LDS refers to the average results of varieties.

The starch content in grain is a genetically determined feature. The highest average starch content during the three years was observed in the new winter wheat line 99-115 (718.09 g kg^{-1}), for triticale – in the variety 'Dinaro' and line 0002-26, 708.28 and 705.69 g kg^{-1} respectively, for rye – in varieties 'Placido' and 'Dankowskie Nowe', 628.88 and 628.38 g kg^{-1} respectively (Table 2).

Grain yield. One of the preconditions for selection of raw materials for bioethanol production is the high yield of species and varieties. Of all the examined species the highest average three year yield

was obtained from winter wheat – 9.50 t ha^{-1}. Average yield of triticale and rye was slightly lower – 9.14 and 8.96 t ha^{-1} respectively. Certainly, the level of yield is also influenced by the choice of varieties. Evaluating the winter wheat varieties, the highest yield was observed for varieties 'Mulan' and 'Skalmeje', 9.72 and 9.50 t ha^{-1}, respectively. For triticale the highest yield was provided by the variety 'Dinaro' – 9.39 t ha^{-1}, the average yield of 'SW Valentino' was only slightly lower – 9.18 t ha^{-1}. For rye the highest yield was provided by the hybrid variety 'Placido' – 9.74 t ha^{-1}, which is almost identical to the average yield of the wheat variety 'Mulan'. In general, a high average grain yield was obtained from all species and varieties of winter cereals, but in 2010/2011 the winter hardiness of certain varieties was the reason for reduced grain yields.

Ethanol outcome. The practical ethanol outcome is influenced by various factors, including the size of starch granules. The size of starch granules affects the grain processing technology for the production of ethanol. The wheat grain generally contains two types of granules: large disks of the size 15-35 µm and small spheres of the size 1 – 10 µm. Triticale starch granules are disks of the size 12 – 30 µm and small spheres of the size 1 – 10 µm. Rye starch granules are disks of the size 10 – 40 µm (KeShun Liu, 2011). British scientists have concluded that the grain with mixed type of starch granules is the most suitable for production of ethanol (Smith et al., 2006). The scientists found out that the starch content and grain size are not the decisive factors in the extraction of bioethanol; the important factor is the starch reactivity to starch hydrolysis (Mojević et al., 2009). The starch consists of two polymers: amylose and amylopectin. The wheat grains usually contain 20 – 30% amylose and 70 – 80% amylopectin (Šramkova et al., 2009). According to the data of Czech researchers, the amylose content in grains of triticale was 208 – 264 g kg^{-1} depending on the variety, location of cultivation and amount of nitrogen fertiliser (Burešová et al., 2010). In the ethanol processing technologies a higher level of energy is needed in order to gelatinise the amylose. Although winter wheat grain had the highest starch content among all the species, the highest ethanol outcome in the laboratory conditions were obtained from triticale – 428.06 L t^{-1}. The ethanol outcome from winter wheat was 420.72 L t^{-1}. Rye had the lowest starch content in grain and also the lowest ethanol outcome from 1 t of grain – 396.78 L t^{-1} (Fig. 2). According to the research data of S. Wang and his colleagues, the processing of rye in the bioethanol production is an economically sound and good alternative for the extraction of biofuel. According to different research data, rye can produce the bioethanol outcome of 362-409 L t^{-1} (Wang et al., 1997; 1998), and this result is similar to observations during the trial in Stende.

The highest ethanol outcome both acquired in the laboratory and calculated theoretically was obtained from wheat and triticale grain. The estimates show that the ethanol outcome acquired from rye grain is 12% lower than that acquired from wheat. Ethanol extraction in laboratory showed that the ethanol outcome from rye is 6% smaller than that from wheat (Fig. 2). Comparing the theoretically calculated and practically acquired ethanol outcome, it was found out that 82% ethanol was acquired from winter wheat grains, 85% – from triticale grains and 88% – from rye grains, if the comparison is made to the estimate based on the starch content in the raw materials. Depending on the species, the losses in the production process were 12 – 18% in comparison to the theoretical estimate.

Ethanol yield per one hectare (L ha^{-1}) is affected by ethanol outcome (L t^{-1}) and grain yield (t ha^{-1}).

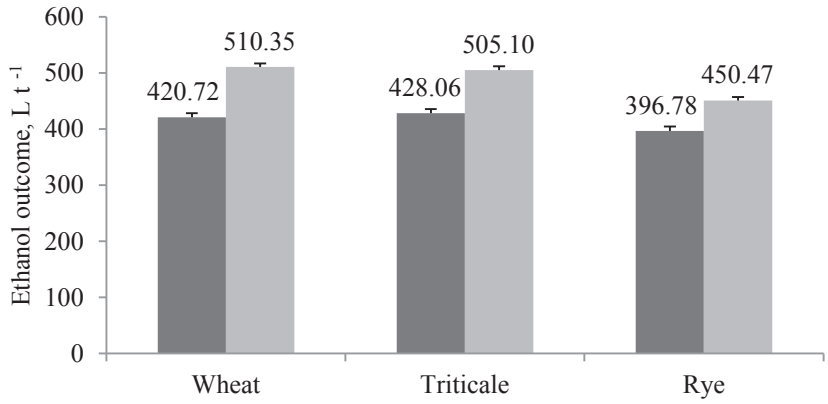

Figure 2. Average ethanol outcome per species, determined in laboratory and calculated theoretically, L t^{-1}, where $LSD_{0.05}$ for practical outcome = 7.40, $LSD_{0.05}$ for theoretical outcome =6.64, practical, theoretical ethanol outcome.

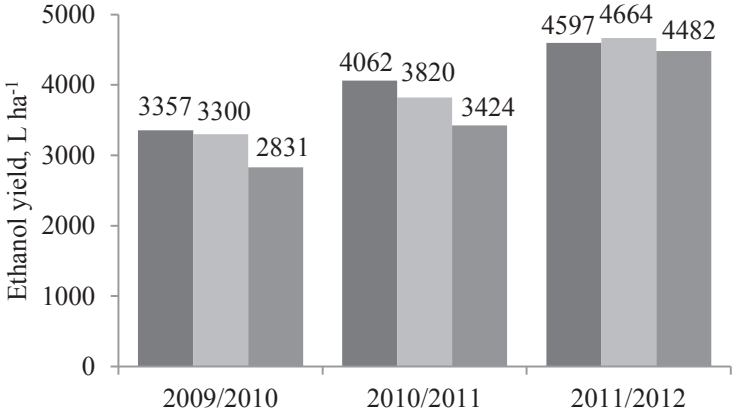

Figure 3. Ethanol yield (L ha^{-1}) depending on the species of cereals and trial year: wheat, triticale, rye.

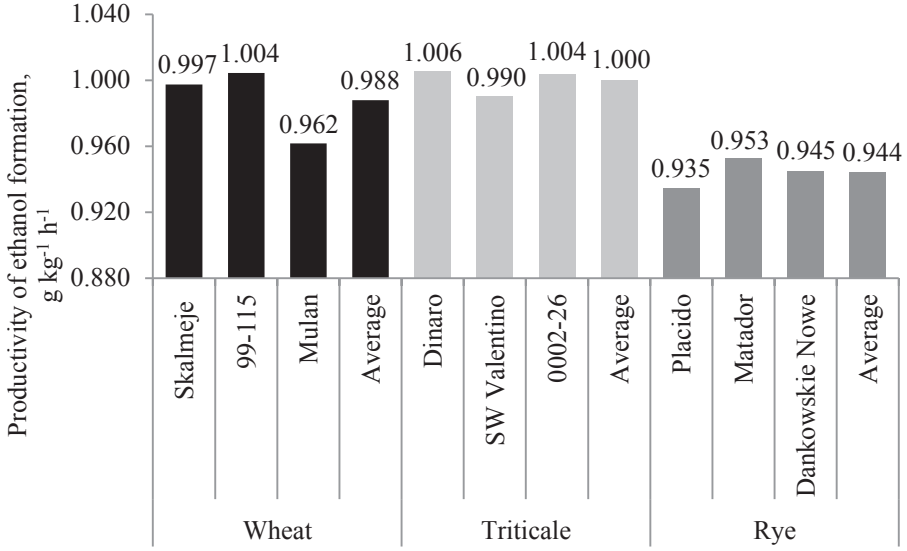

Figure 4. Productivity of ethanol formation in winter cereals, g kg^{-1} h^{-1}.

The lowest grain yield was harvested in 2009/2010, when wintering of cereals was problematical, water scarcity was observed in June during the formation of grain, the temperature in July was higher than the average long-term data, while in August a considerable amount of precipitations encumbered harvesting. Taking into account those circumstances, it was natural to have the lowest ethanol yield (Fig. 3).

The year 2011/2012 on the contrary provided the highest grain yield for all the examined species of winter cereals; consequently, the ethanol yield was considerably higher (4482 – 4597 L ha^{-1}) than in the previous two trial years.

Productivity of ethanol formation. The productivity of ethanol formation is ethanol outcome from the used substrate per hour. The productivity of ethanol formation is affected by starch content and composition of grain as well as the ferments used in the process. Grain fermentation is one of the factors promoting the productivity of ethanol formation. The scientific literature describes the impact of pentozane content. Pentozane is a polysaccharide. The research has proved that rye grain contains higher level of pentozane that forms a soluble compound. It leaves an unfavourable impact on fermentation process. Finnish study in which the impact of fermentation on rye bran was examined proved that fermentation process was also influenced by higher pentoze content (Katina et al., 2007). Pentoze practically does not decompose with *S. cerevisiae* yeast fungi. It means that specific ferments have to be added to substrates in order to ensure higher ethanol outcome in the fermentation process (Juodeikiene et al., 2011).

During our trial the same technology was applied to all the species for the extraction of ethanol in the laboratory. The three year data show that on

average among all examined species by applying this technology the lowest productivity of ethanol formation is observed in rye 0.944 g kg^{-1} h^{-1}. According to the trial results, the average productivity of ethanol formation is higher for triticale and winter wheat – 0.988 and 1.00 g kg^{-1} h^{-1} respectively.

Conclusions

Evaluating three species of winter cereals as starch based raw materials for production of bioethanol, it was found out that winter wheat and triticale are the most suitable species for this purpose. A considerably higher starch content was observed in their grain, wheat and triticale had the higher average grain yield during the trial years and provided higher ethanol outcome per 1 t of grain. The choice of varieties in the framework of one species also had an essential role. Additionally, weather conditions affected the level of grain yield and starch content in grain. The productivity of ethanol formation was lower in rye grain, it might have been affected by the starch content and the impact of ferments on the grain.

Acknowledgements

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METAL UPTAKE FROM CONTAMINATED SOILS BY SOME PLANT SPECIES - RADISH, LETTUCE, DILL

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Abstract
Plants are components of ecosystem that transfer elements from abiotic to biotic environments. Several elements, such as As, Cd, Hg, Pb, can be considered as food chain contaminants. Simultaneously, some essential micronutrients (e.g., Cu, Cr, Ni, Zn) at high concentrations may become toxic to both, plants and animals. To secure the aim to describe the uptake of metals by food crop species such vegetables, as radish *Raphanus sativus* L., leafy lettuce *Lactuca sativa* L. and dill *Anethum graveolens* L., were grown in soils of various grading composition and type. There were five soil types selected. A part of soils was contaminated with copper sulphate solution at different target concentrations, while another part was contaminated with mixture of metal (Pb, Cd, Cu, Zn) compounds. In half of treated soil samples the solution of humic substances was added. Harvested vegetables were dried, and after wet digestion with nitric acid quantitative analysis of metal concentrations was performed by use of atomic absorption spectrometry. The highest concentration of copper was detected in vegetable samples grown in soils with less organics, thereby indicating the importance of soil organic matter to metal transfer routes and accumulation rates in plants. Analysis of lettuce grown in soils contaminated with the mixture of metal compounds revealed that zinc was a metal absorbed more intensively, but metal uptake and accumulation was less intensive from peat if compared with other soils.
Key words: heavy metals; accumulation; contaminated soils; quantitative analysis; vegetables; soil organic matter.

Introduction

Plants are components of ecosystem that accomplish the transfer of trace and major elements from abiotic to biotic environments. Plants take up elements from the primary environmental sources: soil, water and air (Chojnacka et al., 2005). Environmental pollution by heavy metals, for instance with lead and copper, has become one of the major environmental problems due to the human activities in industrial and urban areas. Air pollution, increased amounts of municipal waste and sewage sludge significantly contribute to the release of potential contaminants (Lin et al., 2003). An intensive use of fertilizers and plant protection agents can be assumed as an additional source of metal pollution in agricultural soils (Карпова, 2005; Sobukola et al., 2010; Yusuf et al., 2003). For example, it has been stated that the use of mineral fertilizers may lead to higher concentration of strontium and fluoride in soils, but application of organic fertilizers conveys significant amounts of As, Cu, Ni and Zn into the soils. In addition, phosphorous compounds substantially may affect soil element content by input of several elements, e.g., As, Cd, Cr, F, Ni, Pb, Sr and Zn (Карпова, 2005). In natural conditions soil plays significant role of buffering by protection against excessive translocation of potentially harmful compounds and substances of anthropogenic origin into groundwater aquifers and also plants (Baranowski et al., 2002); therefore, it is very important to take into account various soil factors in studies of heavy metal uptake by plants.

Food crops, such as cereals, vegetables and fruits, are irreplaceable part of human nutrition due to the content of fiber, vitamins, mineral substances, as well as they are important source of naturally occurred trace and major elements in the human diet (D'Mello, 2003). Environmental pollution may affect all plants, including food crops. Some plant species are able to accumulate fairly large amounts of heavy metals without showing stress, and that can result in potential risk for animals and humans through the food chain, hence the contamination of food crops is very important for the society (Heemsbergen et al., 2010). Some elements, such as cadmium, lead and mercury, are likely to be significant contaminants of food supply and may be considered as the most important problem within the interaction of environment and nutrition (Guala et al., 2010). Moreover, heavy metals are not biodegradable, they have long biological half-lives and have the potential for accumulation in different bases, including human and animal organs and tissues leading to adverse effects of health (Sobukola et al., 2010). However, several other elements such as iron, zinc and copper are essential for biochemical reactions in the body, but they may become harmful in high concentrations (Zaidi et al., 2005).

Trace metals can be taken up by plants if they are present in soil as soluble ions in forms of organic or inorganic complexes. For ecological considerations, not only the total concentration, but also the kind of heavy metal species (widely called as element speciation) present in the soil solution is of primary importance, because metal mobility and availability are closely related to the composition of the solution (Alexander et al., 2006). However, availability of elements to plants is a set of complicated biochemical

processes and reactions that are dependent on biogeochemistry and soil properties as well as are influenced by climatic and seasonal conditions.

Within the framework of food chain contamination, the aim of the present study was to investigate the uptake of metals (respectively, Cd, Cu, Pb, Zn) by some food crop species grown in different soil conditions.

Materials and Methods

Contamination of soil and the experiment

Five different soil samples (each about 10 kg) were collected from the northeast region of Latvia, in the southwest of Vidzeme Upland (Vecpiebalga region, Taurene rural municipality, vicinity of Lode manor), during the spring season in 2011. Soil type was identified as follows: S1 - fen peat soil; S2 - sod-podzolic soil / sandy loam; S3 - sod-podzolic soil / sandy with low organic matter content; S4 - sod-podzolic soil / sandy with relatively higher organic matter content; S5 - sod-podzolic soil / sandy clay, considering their representativeness for soils in Latvia (Karklins et al., 2009; Nikodemus, 2011). Piles of selected soil were contaminated by copper sulphate pentahydrate $CuSO_4 \times 5H_2O$ solutions at five different Cu^{2+} target concentrations, respectively, 40, 70, 100, 130 and 200 mg kg^{-1}. It was decided to use copper as the main contaminative metal because it readily forms complex compounds with dissolved organic matter (Inaba and Takenaka, 2005). Other part of selected soils was contaminated with mixture of element compounds in target concentration of 6, 130, 750 and 300 mg kg^{-1} of Cd, Cu, Pb and Zn, respectively. For mixture contamination the following substances were used: cadmium acetate dihydrate $Cd(CH_3COO)_2 \times 2H_2O$, copper sulphate pentahydrate $CuSO_4 \times 5H_2O$, lead (II) nitrate $Pb(NO_3)_2$ and zinc sulphate heptahydrate $ZnSO_4 \times 7H_2O$. Control samples of uncontaminated soils were kept as well. Soil contamination procedure was proceeded as it has been described in literature, i.e., soils were humidified by spraying with calculated amount of certain contaminant water solutions, followed by mixing over several times (Alexander et al., 2006). In addition, half of each portion of contaminated soils was saturated with the solution of humic substances (3 g kg^{-1}). After the contamination, soils were left to equilibrate for two weeks, as well as repeated mixing was done before the filling of soils into the pots.

After pots were loaded with contaminated and control soils, seeds of radish *Raphanus sativus* L. 'Saxa 2', leafy lettuce *Lactuca sativa* L. 'Grand Rapids' and dill *Anethum graveolens* L. 'Mammut' were directly sown into the pots. For every contamination of every soil and vegetable type there were four pots filled, and in every pot 5 seeds of the crop were put. Food crops used in this research were chosen taking into account their relatively rapid growth and moderate requirements for growth conditions. Crops were grown under the open-air conditions, however, when it was necessary, the pots were covered with a plastic shed to protect them from the wind, heavy rainfall or excessive sunlight. The study was done during the summer season of 2011 in the central region of Latvia, in a private field area near Aizkraukle town.

Sampling and analysis

Since the growth of plants was affected by several factors, for instance, climate conditions, high concentration of metals in the soils and unexpected presence of insects, it was not possible to collect all vegetable samples from all the pots. However, crop samples which were evolved enough and not damaged were harvested 35-45 days after the sowing, taking into account the maturity of grown plant species. Vegetables were carefully cleaned, then rinsed thoroughly with deionised water, and edible parts of vegetables were separated. Samples were air dried (lettuce and dill) or dried in oven (radish) and well crashed up (Wang et al., 2003). Until analysis samples were stored in closed disposable plastic bags in a dry and dark place. For trace element analysis it is important to avoid any contact of samples with contaminants, particularly metals. Therefore, a ceramic knife was used to crash up the samples, but sample solutions were kept in polypropylene tubes.

Samples of lettuce, radish and dill were mineralized by wet digestion as it is widely applied for cleavage of biological and environmental samples (Alexander et al., 2006). Sample pretreatment procedure was done as follows: a) 0.1000 ± 0.0020 g of dry sample was weighed on analytical balance (Kern ALJ 220-4); b) 5 mL of concentrated nitric acid (65% w/v, ISO, Scharlau) and 2 mL of concentrated hydrogen peroxide (30% w/v, ISO, Merck) were added (only analytically ultra pure reagents were used); c) after holding overnight, sample solutions were digested by heating at 160 °C temperature on the heating block (Biosan); d) after digestion sample solutions were filled up to 10 mL with ultra pure deionised water (18 MΩ, Millipore). Each sample solution was made in triplicate, as well as blank samples were prepared for every batch of samples. Quantitative concentrations of metals in digested solutions were detected by using atomic absorption spectrometry (AAS). Measurements were done by AAS apparatus (AAnalyst 200, PerkinElmer) with flame atomization. Absorption was measured by background correction. Characteristic values (Table 1) for metal detection with available methodology were determined by using blank samples. Values regarding metal concentration

Table 1

Characteristic values for quantitative metal detection by atomic absorption spectrometer 'AAnalyst 200, PerkinElmer'

Element	Limit of detection, mg kg^{-1}	Level of quantification, mg kg^{-1}	Uncertainty, %
Cadmium (Cd)	0.01	0.04	4.00
Copper (Cu)	0.15	0.50	4.50
Lead (Pb)	0.18	0.59	10.50
Zinc (Zn)	0.32	1.06	6.00

in plant samples were expressed in units of dry mass (mg kg^{-1} DM). Statistical analysis of obtained data was done by using Microsoft Excel software (Microsoft Office 2010).

Results and Discussion

The primary route of trace elements, including metals, and transfer of them into the food chain is realised through the soil-plant interaction within the certain ambient environment. Sustainable micronutrient cycling is an important issue not only in case of essential element transfer but also for the assessment of possible human health risks caused by contamination of daily nutrition with potentially toxic elements (Yang et al., 2007).

Five target concentrations of copper for contamination of soils were applied as it was described in the previous chapter of this paper. Obtained quantitative concentrations of metals in vegetable samples grown in soils contaminated with 70 mg kg^{-1} of Cu were used to describe representation of data within this paper. While copper concentration in radish samples grown in uncontaminated soils on average was only 3.5 mg kg^{-1} DM, the highest value (91 mg kg^{-1} DM) for radish grown in soils contaminated with 70 mg kg^{-1} of copper was detected for samples derived from soil S3 that is sod-podzolic / sandy soil with low organic matter content, but the lowest concentration (10.3 mg kg^{-1} DM) was detected for radish samples grown in fen peat soil (S1) that is soil with naturally significantly high content of organic matter. It is obvious that copper concentration of radish samples grown in contaminated soils with addition of humic substances is much lower than that of radish samples grown in contaminated soils only (Fig. 1).

It was detected that in some cases copper concentration in radish samples grown in soils with addition of humic substances was even four times lower, e.g., for sod-podzolic soil / sandy loam (S2) and sod-podzolic / sandy soil with low organic matter (S3) that affirmed the importance of organic matter in element transfer from soil to plants. Metal transfer from soil to plants is dependent on various chemical

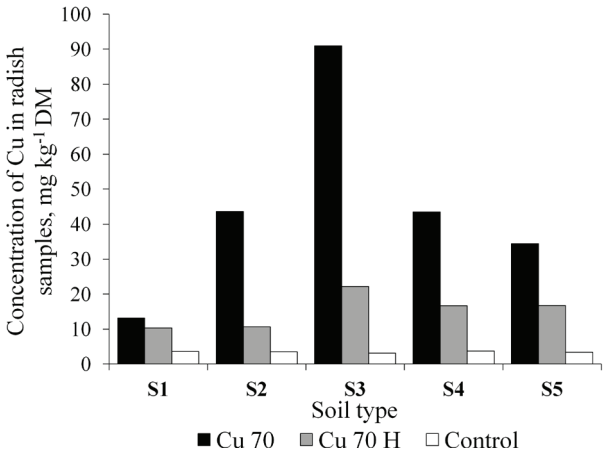

Figure 1. Detected concentrations of copper in radish samples grown in soils contaminated by Cu at target concentration of 70 mg kg^{-1}, with (Cu 70 H) or without (Cu 70) addition of solution of humic substances (S1 - fen peat soil; S2 - sod-podzolic soil / sandy loam; S3 - sod-podzolic soil / sandy with low organic matter content; S4 - sod-podzolic soil / sandy with relatively higher organic matter content; S5 - sod-podzolic soil / sandy clay).

processes such as chelation, absorption and desorption, precipitation, ion exchange and dissolution. Z.L. He with colleagues defined that 'chelation is the process during which trace elements make stable complexes with organic or inorganic ligands' (He et al., 2005). It is supposed that chelation is one of the main factors that affects copper accumulation in lettuce grown in soils amended with solutions of humic substances (Vincevica-Gaile and Klavins, 2012).

It has been proven that natural substances (e.g., humic or fulvic acids) and synthetic solvents (e.g., ethylenediamine tetraacetic acid EDTA or diethylenetriamine pentaacetic acid DTPA) used as chelators may promote metal accumulation in plants and can be used for metal removal from polluted soils (Inaba and Takenaka, 2005). However, soil treatments by humic acids, if compared with synthetic chelators, in case of copper contamination showed the highest potential to reduce copper toxicity by involving metal ions in complex compounds in plants (Inaba and Takenaka, 2005; Heemsbergen et al., 2010). D.A. Heemsbergen with colleagues outlined that copper availability for plants is dependent on speciation of substance applied, i.e., whether it is salt or not, however, copper solubility can be increased by complex formation with dissolved organic matter, but bioavailability in this case can be not changed or even can be reduced (Heemsbergen et al., 2010).

In lettuce samples grown in uncontaminated soils the average concentration of copper was 5.07 mg kg^{-1} DM that is higher than the concentration of copper in radish samples grown in contaminated soils. However, leafy vegetables, particularly, spinach and lettuce, in many studies of metal accumulation in plants have been referred as the most abundant species to accumulate trace metals (Alexander et al., 2006). The highest concentration of copper (19.5 mg kg^{-1} DM) was detected in lettuce samples grown in sod-podzolic sandy soil with relatively low organic matter (S3), but the lowest concentration (9.7 mg kg^{-1} DM) was detected in lettuce samples grown in the same type of soil but with relatively higher organic matter content (S4) (Fig. 2).

The reasons of phytotoxic effects of excessively high concentration of copper have been the subject of many studies in recent years (Guala et al., 2010). However, when absorbed in excess, copper can be considered as a toxic element, especially, for many non-tolerant plants, and can be associated with the disturbance of plant development processes, such as mitosis, inhibition of root elongation, damage of root epidermal cells and root cell membranes (Chojnacka et al., 2005; Lin et al., 2003).

In the present study dill as a leaf vegetable species is considered to be a plant with the lowest tolerance to metals, respectively in case of copper. This assumption was established taking into account the fact that only those dill samples that were grown in soils with addition of solution of humic substances were evolved enough to be harvested within the current experiment. From dill samples grown in soils with addition of organic matter, the highest concentration of copper was detected in samples derived from soils S3 and S4 (Fig. 3). However, similarly as in case of previously described samples of radish and lettuce, the importance of organic matter in element transfer from soil to plants was affirmed.

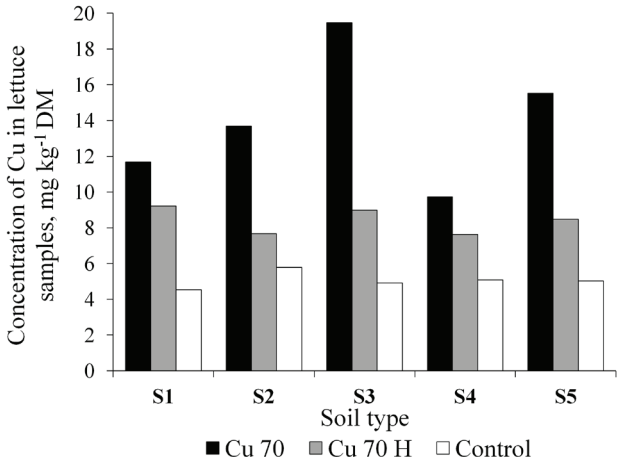

Figure 2. Detected concentrations of copper in lettuce samples grown in soils contaminated by Cu at target concentration of 70 mg kg^{-1}, with (Cu 70 H) or without (Cu 70) addition of solution of humic substances (S1 - fen peat soil; S2 - sod-podzolic soil / sandy loam; S3 - sod-podzolic soil / sandy with low organic matter content; S4 - sod-podzolic soil / sandy with relatively higher organic matter content; S5 - sod-podzolic soil / sandy clay).

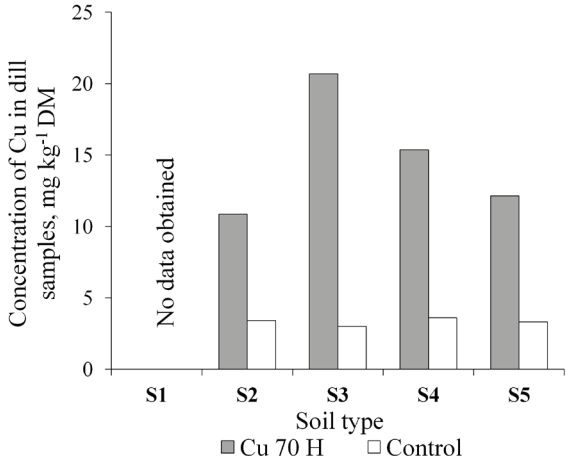

Figure 3. Detected concentrations of copper in dill samples grown in soils contaminated by Cu at target concentration of 70 mg kg^{-1} with addition of solution of humic substances (Cu 70 H)
(S1 - fen peat soil; S2 - sod-podzolic soil / sandy loam; S3 - sod-podzolic soil / sandy with low organic matter content; S4 - sod-podzolic soil / sandy with relatively higher organic matter content;
S5 - sod-podzolic soil / sandy clay).

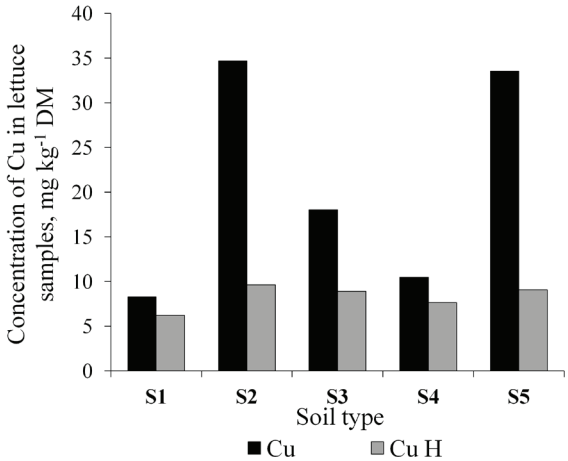

Figure 4. Summarized average concentrations of copper taken up by lettuce grown in soils contaminated by Cu at different target concentrations, with (Cu H) or without (Cu) addition of solution of humic substances
(S1 - fen peat soil; S2 - sod-podzolic soil / sandy loam; S3 - sod-podzolic soil / sandy with low organic matter content; S4 - sod-podzolic soil / sandy with relatively higher organic matter content;
S5 - sod-podzolic soil / sandy clay).

Fig. 4 shows the total average uptake of copper by lettuce grown in soils contaminated by Cu^{2+} at different target concentrations with (Cu H) or without (Cu) addition of solution of humic substances. Obtained results suggested that the uptake of metals is significantly affected by the content of organic matter in soil. However, it has been stated that the most part of copper in soils exists in chemical forms which are not readily available to plants because of the strong binding of Cu^{2+} by organic matter and other soil colloids (Lin et al., 2003); therefore, in this case high concentration of copper in lettuce samples grown in soils with comparatively high content of clay particles (respectively, in soils S2 and S5) cannot be explained by different grading composition of soils and could be affected by other conditions, for instance, soil pH.

In the part of experiment of soil contamination with the salts mixture of elements Pb, Cd, Cu and Zn only lettuce samples were harvested. Obtained results showed different tendencies. It is known that such elements as Co, Cr, Cu, Ni, Pb, Sb, Se and Zn may hyperaccumulate in plant leaves. Within the present study, in lettuce samples grown in soils contaminated with the salt mixture of elements

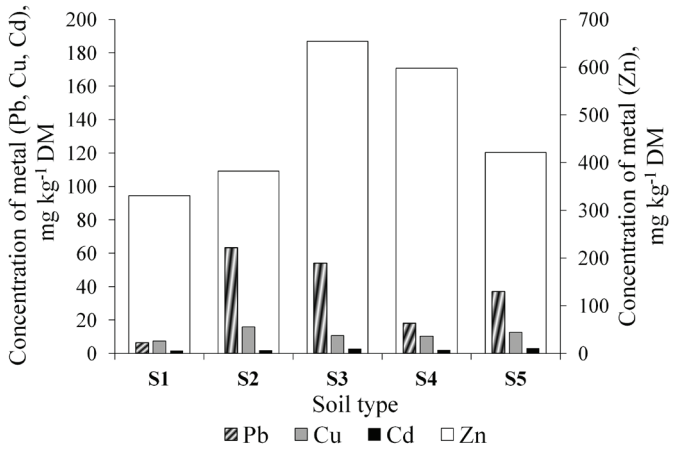

Figure 5. Detected concentrations of copper, zinc, cadmium and lead in lettuce samples grown in soils contaminated by element compound mixture with addition of solution of humic substances (S1 - fen peat soil; S2 - sod-podzolic soil / sandy loam; S3 - sod-podzolic soil / sandy with low organic matter content; S4 - sod-podzolic soil / sandy with relatively higher organic matter content; S5 - sod-podzolic soil / sandy clay).

and with addition of solution of humic substances, obvious hyperaccumulation of zinc was detected (Fig. 5). Furthermore, leafy plants, such as lettuce, are supposed to be efficient hyperaccumulators considering the fact that in case of hyperaccumulation process the most of metals from soils are tended to accumulate in plant leaves (Rascio and Navari-Izzo, 2011). However, taking into account different target concentrations of contaminants, the sequences of uptaken metals in plants were detected as follows: Zn > Cd > Cu > Pb (based on mean results).

Such elements as Cd, Cu and Pb are among the most widespread heavy metal contaminants of agricultural soils and are known to exert toxic effects in animals and plants at elevated concentrations. However, while Cu and Zn are also known as essential plant nutrients, Pb and Cd have no essential biological function, but are taken up by plants from metal enriched soils (Zheljazkov et al., 2006; Zheljazkov et al., 2008). Within the present study the detected different rates of copper, zinc, cadmium and lead uptake showed that not only soil and plant properties, but also chemical properties of elements should be taken into account in case of metal transfer into food chain from soil to plants.

Conclusions

By studying the uptake of metals (respectively, Cd, Cu, Pb, Zn) by food crop species grown in different soil conditions, it was ascertained that the uptake of metals by radish, lettuce and dill is significantly affected by organic matter content in the soils. For instance, the highest concentration of copper was detected in vegetable samples grown in soils with lower levels of organic matter, thus indicating the importance of soil organics in metal transfer routes and accumulation intensity in plants. In lettuce grown in the soils contaminated with the mixture of metal Pb, Cd, Cu and Zn compounds, elevated uptake and accumulation of zinc was observed, thus indicating the significance of not only soil and plant but also particular chemical element properties in case of metal uptake by food crop species. Taking into account soil properties, it was obvious that vegetable samples grown in peat were taking up and accumulating lower amounts of contaminants if compared with other soils. Also, addition of humic substances in any type of soils diminish uptake of metals by plants thereby reducing possible food chain contamination risk. The property of humic substances to bind heavy metals in indissoluble complexes can be developed as a perspective trend for practical use of application of humic substances on metal contaminated agricultural soils or allotment gardens in urban areas.

Among the most important factors that can affect the element transfer from soil to plants, the soil grading composition as well as the selective ability of plant species to accumulate some chemical elements can be accented. Development of the study should involve consideration of certain soil, plant and chemical element properties. Obtained results are useful for overall understanding of possible contamination of food crops with potentially toxic metals.

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IMPACT OF NITROGEN FERTILIZER RATES ON INDUSTRIAL HEMP GROWTH AND DEVELOPMENT

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Abstract
The aim of this study was to evaluate an impact of nitrogen fertilizer rates on industrial hemp's (*Cannabis sativa* L.) growth and development in Latvia. The trial was carried out during 2012 on the Research and Study farm Peterlauki of the Latvia University of Agriculture in the sod calcareous soil. There were three industrial hemp cultivars 'Futura 75', 'Tygra' and 'Felina 32' tested under different fertilizer rates: N0P0K0 – control, N0P80K112 – background fertilizer (in text marked as F), F+N30, F+N60, F+N90, F+N120, F+N150, F+N180 kg ha^{-1}. Weather conditions were proper for good hemp biomass production. Depending on the selected cultivars, the optimal fertilizer rate was in the range of 90 – 150 kg ha^{-1}. Industrial hemp stalk length was significantly ($p<0.05$) influenced by the applied nitrogen fertilizer rate and cultivars. The highest stalk length was observed in the cultivar 'Futura 75' under all nitrogen fertilizer rates. The highest stalk length (3.18 m) had reached under the nitrogen fertilizer rate 150 kg ha^{-1} at 138 growing day from sowing. At the beginning of growing season (June - July) the growth intensity of hemp stalk length is high. Within one month the stalk length grew up for 1.23 meters of cultivar 'Futura 75'. The intensive growth of hemp stalk declines when the flowering stage is reached. Flowering stage occurred in early August, and it was dependent on nitrogen fertilizer rate. Under higher nitrogen fertilizer rate the flowering stage reached later.
Key words: *Cannabis sativa* L., 'Futura 75', 'Tygra', 'Felina 32'.

Introduction

The industrial hemp (*Cannabis sativa* L.) is one of the earliest domesticated and widespread crops all over the world. Industrial hemp is an extraordinarily useful plant that can provide more environmentally friendly products: food, fibre, fuel, medicinal and building products as textiles for apparel hemp, mats for thermal insulation in the construction industry, specialty pulp and paper for technical applications, press-moulded interior panels for the automotive industry, geotextiles for erosion control, needle-punched carpeting, used as animal bedding, seed and oil for food sector, natural body care products, gamma linolenic acid in the cosmetics and pharmaceutical industries, natural THC-based therapeutic drugs, etc. (Bosca et al., 1998).

Nowadays industrial hemp has become very important as a crop for biomass production. It is fast-growing and suitable for Latvia's agro-climate conditions. An interest in possibilities of the hemp growing in Latvia is increasing year by year, and it is considered as one of the most promising renewable biomass sources to replace non-renewable natural resources for manufacturing of wide range industrial products (Adamovičs, 2007).

Latvian hemp sowing areas were registered only in the year 2008 and in the year 2009, 250 ha were grown. In recent years, the amount of industrial hemp growers and cultivated areas have increased in Latvia. According to data provided by Association of Industrial Hemp of Latvia, plantation area of hemp was approximately 600 ha in Latvia in 2012.

Industrial hemp is an herbaceous annual belonging to the family Cannabinaceae. Normally, it is dioecious having both staminate (male) and pistillate (female) plants, each with distinctive growth characteristics. Staminate plants are tall and slender with few leaves surrounding the flowers, while pistillate plants are short and stocky with many leaves at each terminal inflorescence (Ehrensing, 1998). Also, through breeding and selection there have been developed plants that are monoecious with male and female flowers on one stalk. The plants woody stem develops ranging in height from 1.5 to over 2.5 meters and 5 – 15 mm wide in diameter at the soil surface (Adamovičs, 2007; Adamovičs et al., 2012; Grabowska et al., 2005). The cultivation is environmentally friendly with little harmful accumulation or emission of chemical inputs (Struik et al., 2000). The soil structure is improved with its rich leafage suppressed weeds and leaves left on the soil after harvesting (Adamovičs, 2007). Once it has developed a good root system, it can survive most drought conditions (MacKinnon, 1997). The roots of industrial hemp can reach up to 200 cm depth to reach the water table (Amaducci et al., 2008).

The requirement for a well-drained site is necessary as industrial hemp plants are particularly sensitive to wet, flooded, or waterlogged soil (Olsen, 2004). Industrial hemp grows best in humid environment, in temperature between 14 °C and 27 °C. It has a high water requirement, especially during the first six weeks of growth (MacKinnon, 1997). It grows by average of 50 cm a month (Adamovičs, 2007).

Nitrogen is the element that is most widely used in agriculture, and it is the most important element which limits plant growth and development (Masclaux-

Daubresse et al., 2010). Industrial hemp's need for nitrogen is high, especially during the vegetative growth period, and it should be available in the soil in sufficient quantity for a good growth and development (Ehrensing, 1998). Additional nitrogen fertilizer stimulates hemp plant growth in field conditions (Maļceva et al., 2011). A lack of nitrogen will result in a lower yield because steps of growth (internodes' length, canopy area) will be missed, and therefore the efficiency of radiation use is reduced.

In the literary sources it was found that hemp fertilization methodology varies in different countries according to the existing soil and climatic conditions. For example, in the United States quoted nitrogen fertilization rates about 60 kg ha^{-1}, while in EU countries nitrogen fertilization rates vary between 40 – 200 kg ha^{-1} depending on soil composition (Ehrensing, 1998). Recommendations for hemp growing developed in the EU are not considered to be suitable for Latvian climatic and soil conditions. Recommendations of suitable nitrogen fertilizer rates for hemp breeding in Latvia are not developed.

The aim of this study was to evaluate impact of nitrogen fertilizer rates on industrial hemp's (*Cannabis sativa* L.) growth and development in Latvia.

Materials and Methods

The trial was carried out on the Research and Study farm Peterlauki of the Latvia University of Agriculture in the sod calcareous soils in 2012. The content of available P in the soil plough layer was 52 mg kg^{-1}, content of K – 128 mg kg^{-1}, pH KCl 6.7, and organic matter content – 25 g kg^{-1}.

Hemp was sown by sowing-machine on the 4th of May. Seed rate 50 kg ha^{-1} or 250 germinate able seeds per 1 m^2. The trial was randomly spaced, triplicate. The plot size was 7 m^2. In the field rotation, industrial hemp followed previous crop – spring barley.

All tested industrial hemp cultivars are monoecious – male and female flowers are present on the same plant. Cultivar 'Futura 75' is considered as late – maturing in France, 'Felina 32' is considered semi-late in France. Hemp was tested under seven different nitrogen fertilizer application rates: control – N0P0K0; background fertilizer (in text marked as F) – N0P80K112; F+N30; F+N60; F+N90; F+N120; F+N150; F+N180 kg ha^{-1}. Factor A – nitrogen fertilizer rate. Factor B – cultivar.

During growing season the industrial hemp stalk was estimated. Total height of hemp stalk was measured from the soil surface to the tip of plant (±0.5 cm). On average, there were measured 10 plants per plot, seven times during the growing season. The main task of research presented here was to evaluate hemp growth and development under different nitrogen fertilizer rates - how fast hemp stalks grow and reach growth stages – vegetative stage: the first pair of leaves and flowering, and seed stages. No pesticides like insecticides, herbicides, desiccants were used.

Industrial hemp was harvested by a trimmer (leaving the stubble of 5 – 8 cm) on October 10th, 2012.

A statistical evaluation of the data has been made by variance analysis, the LSD test. Correlation and regression analysis methods were used for data processing.

Meteorological data was obtained from Dobele Hydro-meteorological Station (HMS). Precipitations were taken during the growing season near to the trial fields on the farm Peterlauki. Meteorological conditions of the 2012 growing season can be described as not very warm but with large temperature fluctuations (Fig. 1.) and wet with periodic substantial rainfall (Fig. 2.).

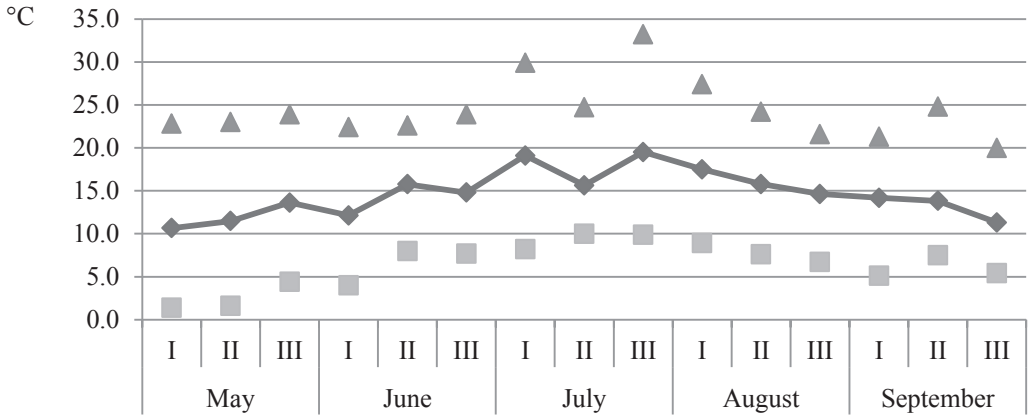

Figure 1. Temperature during the growing season, 2012 (°C):

Average MIN MAX

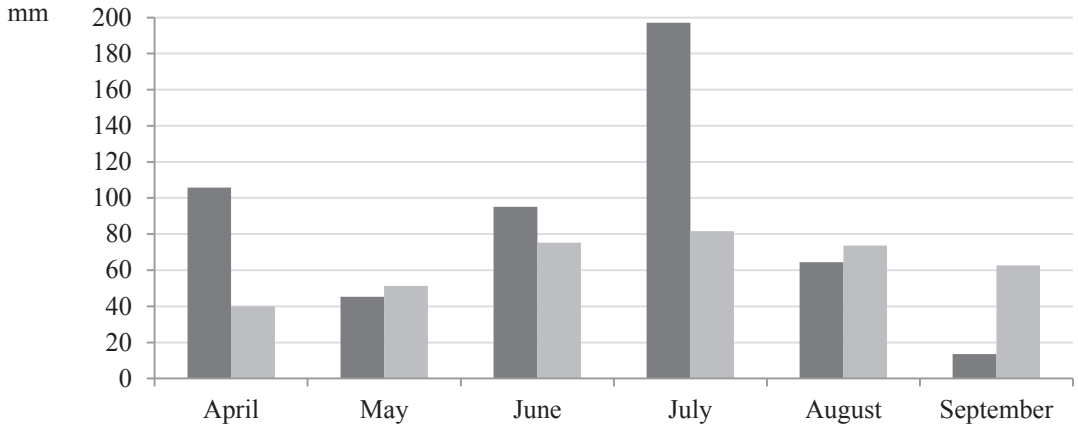

Figure 2. Precipitations during growing season, 2012:

■ 2012 ■ longterm, mm

Results and Discussion

The industrial hemp seeds germinate best when mean daily temperatures are 8 °C – 10 °C (Adamovičs, 2007). In 2012, the month of May was warm with an average temperature 11.9 °C (Fig.1.) and rich with precipitation month sum was 45.2 mm (Fig. 2.). According to the data, presented in Figure 1 and Figure 2, we can see that there was enough wet and good temperature for hemp seed germination in May. Meteorological conditions promoted seed germination. Industrial hemp emerged within a few days after sowing and reached growth stage – the first pair of true leaves (1002) (Mediavilla et al., 1998) in one week after sowing on the 11th of May. There was not any evident difference between the seedlings of different cultivars.

Hemp grows best when mean daily temperatures are between 14 °C and 27 °C (Ehrensing, 1998). Summer was characterized as cold and rich with precipitations, but the growing season temperature was suitable for hemp's growth and development. Hemp requires abundant moisture throughout the growing season, particularly while young plants are becoming established during the first six weeks of growth (Ehrensing, 1998; Grabowska et al., 2009). In the middle of vegetation season from June till July, there were periodical and strong rainfalls, causing water accumulation on the soil surface. It could also explain the high proportion of investigated factors (44.6%) whose effect on the hemp's growth and development has not been studied. According to the observation data of some researches (Olsen, 2004), hemp doesn't grow and develop well and suffer of stagnant water on the soil surface.

According to the information of scientific literature, industrial hemp growth and development is dependent on the applied nitrogen fertilizer rates (Ehrensing, 1998; Masclaux-Daubresse et al., 2010). To obtain a high industrial hemp stalk length, we need to provide plants with the necessary nutrients.

Industrial hemp stalk length was significantly ($p<0.05$) influenced by the applied nitrogen fertilizer rate and cultivars (Tab.). According to the research results, there was seen that plant height gradually increases with increasing N fertilizer rate, compared with the control (N0P0K0), but this growth increase varies between tested cultivars.

The highest stalk length was observed in the cultivar 'Futura 75' under all nitrogen fertilizer rates, compared with other tested cultivars. The highest stalk length (3.18 m) was reached under the nitrogen fertilizer rate F + N150 at 138 growing day from sowing. The stalk length of other cultivars under this nitrogen fertilizer rate was lower, cultivars 'Tygra' and 'Felina 32' – 2.58 meters (Tab.).

The highest stalk length of cultivar 'Tygra' was obtained under nitrogen fertilizer rate N90 (2.60 m), but cultivar 'Felina 32' - under nitrogen fertilizer rate N120 (2.71 m). In researches of Lithuania, the cultivar 'Felina 32' stalk length varies from 2.18 – 2.49 m under fertilizer rates N5P15K30 (Jankauskienė and Gruzdeviene, 2009; 2010).

The lowest stalk length of all cultivars was obtained under control treatment (N0P0K0), where nitrogen fertilizer was not applied, and it demonstrates the need of nitrogen for industrial hemp growing and developing.

According to the obtained data, the choice of industrial hemp cultivar affected the stalk length by 35%, but the applied nitrogen fertilizer rate – by 10%. The interaction of both factors affected the stalk length at about 10%.

Industrial hemp's need for nitrogen was high and increasing the nitrogen fertilizer rate to 150 kg ha^{-1}

Table

Total length of hemp stalk, m

N fertilizer rate (A)	Industrial hemp cultivars (B)			Average (A)
	'Futura 75'	'Tygra'	'Felina 32'	
N0P0K0	2.92	2.39	1.76	2.36
N0P80K112 (F)	3.04	2.41	1.85	2,43
F + N30	2.93	2.41	2.59	2.64
F + N60	2.96	2.41	2.59	2.66
F + N90	3.04	2.61	2.63	2.76
F + N120	3.04	2.58	2.71	2.78
F + N150	3.18	2.58	2.58	2.78
F + N180	3.14	2.57	2.44	2.72
Average (B)	3.03	2.50	2.40	2.64
$LSD_{0.05A}$=0.37; $LSD_{0.05B}$=0.22; $LSD_{0.05AB}$=0.63				

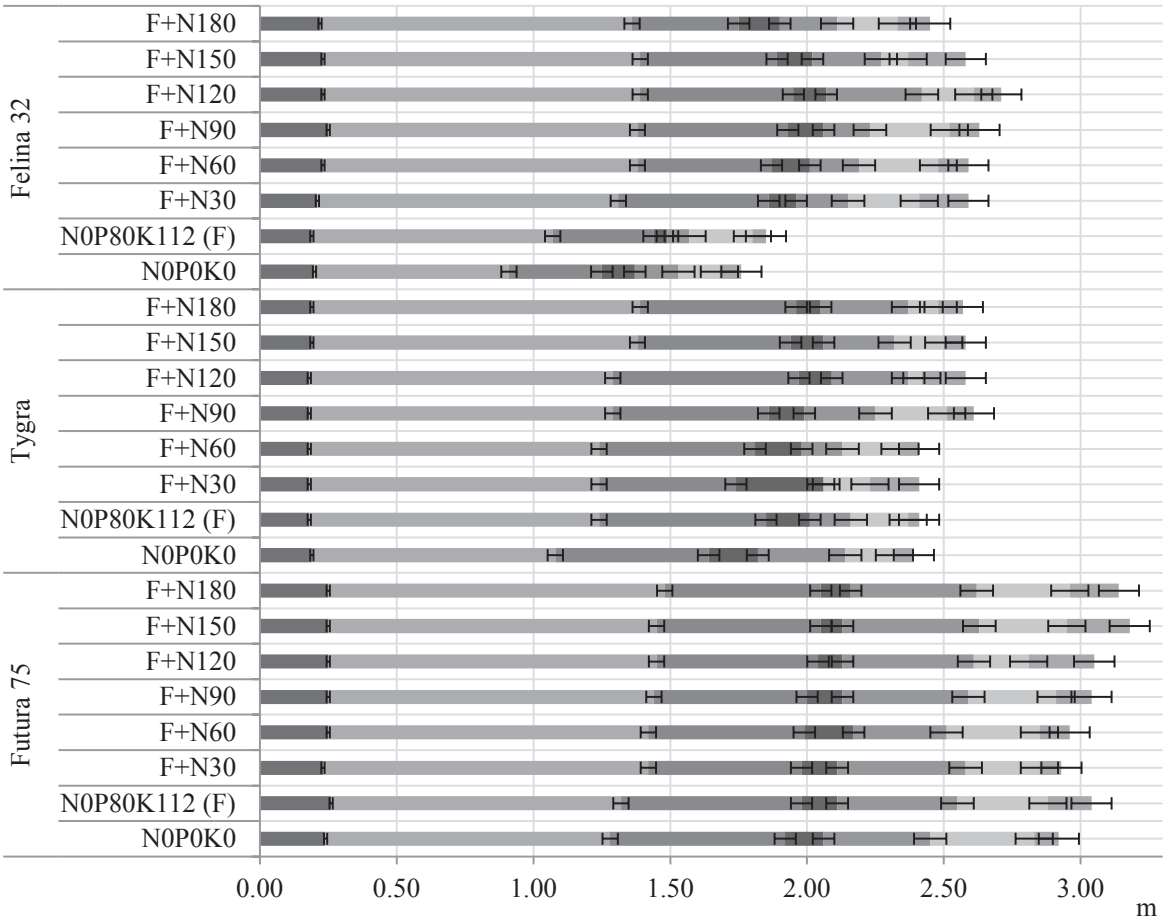

Figure 3. Industrial hemp cultivars' stalk growth dynamic during the growing season under different nitrogen rates, m:

from sown day up to 34th; 34th-60th; 60th - 73rd; 73rd - 83rd; 83rd - 94th; 94th - 104th; 104th - 138th

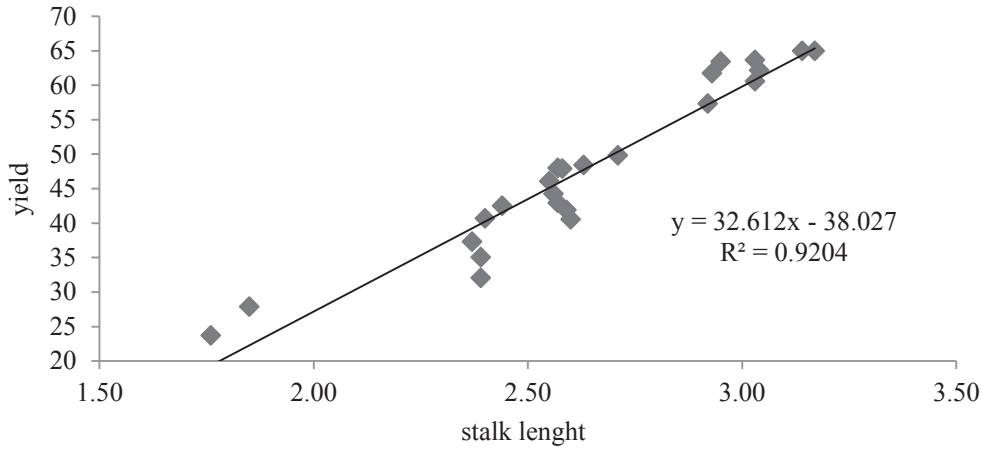

Figure 4. Effect of hemp stalk length on hemp over-green biomass yield.

the stalk length was increased for the cultivar 'Futura 75' during a trial period. But the cultivar 'Felina 32' the highest stalk length reaches under fertilizer rate 120 kg ha^{-1}. The cultivar 'Tygra' reaches the highest stalk length under 90 kg ha^{-1}, but by increasing a fertilizer rate, the stalk length decreases. Thus, we can conclude that with each applied kilo of nitrogen fertilizer the effectiveness increases until it reaches the maximum stalk length.

Assessing the growth intensity of industrial hemp varieties, the fastest growth of all cultivars was observed at the beginning of vegetation period, regardless fertilizer rates (Fig. 3.).

At the beginning of growing season (June - July) the higher growth intensity of hemp stalk length was observed for cultivars 'Futura 75' and 'Tygra' under higher nitrogen fertilizer rates. Within one month the stalk length grew up for 1.23 meters of the cultivar 'Futura 75' and for 1.20 meters of the cultivar 'Tygra' (Fig. 3). It shows that during the first six weeks of vegetative growth period industrial hemp's need for nitrogen is high.

The intensive growth of hemp stalk declined when the flowering stage was reached. Flowering stage occurred in early August, and it was dependent on nitrogen fertilizer rate. Under higher nitrogen fertilizer rate the flowering stage was reached later. During the period of flowering stage, a strong decrease of growth intensity, up to 10 – 15% of all growing season was observed.

The over-green biomass yield of industrial hemp depends on the stalk length during the growing season (Fig.4.).

A significant ($p<0.05$) close linear positive correlation between stalk length and over-green biomass yield ($r=0.96$; $n=24$) was observed, and the relationship is reflected in the regression equation $y=32.612x-38.027$; $R^2=0.92$. It shows that in 92% of cases the changes in yield might be explained by the changes in the stalk length. A decrease in the hemp stalk length will also decrease over-green biomass yield.

Conclusions

1. Industrial hemp stalk length was significantly ($p<0.05$) influenced by the applied nitrogen fertilizer rate (kg ha) and tested cultivars. Depending on the selected cultivars, the optimal fertilizer rate is in the range of 90 – 150 kg ha^{-1}.
2. The highest stalk length was observed in the cultivar 'Futura 75' under all nitrogen fertilizer rates. The highest stalk length (3.18 m) was reached under the nitrogen fertilizer rate of 150 kg ha^{-1} at 138 growing day from sowing.
3. The higher growth intensity of industrial hemp cultivars' stalk length was during first six weeks of growing season (beginning of June - July). Within one month the stalk length grew up for 1.23 meters. The intensive growth of hemp stalk declined when the flowering stage was reached (early August). Under higher nitrogen fertilizer rate the flowering stage was reached later.
4. The industrial hemp stalk length has a significant impact on the obtained hemp yield. By increasing the stalk length, the yield increased, too.

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DISCUSSION ON GROUND BEETLES AND ROVE BEETLES AS INDICATORS OF SUSTAINABLE AGRICULTURE IN LATVIA: REVIEW

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Abstract
Ground beetles (Coleoptera: Carabidae) and rove beetles (Coleoptera: Staphylinidae) as predators of many pests and weeds in every crop are significant elements of integrated pest management. Worldwide studies show that ground beetles reflect different soil tillage methods, crop rotation, chemical and genetic pollution, usage of fertilizers and landscape fragmentation. All these factors are the parameters based on which it is possible to assess agriculture whether it is sustainable or not. Ground beetles also can indicate different farming systems and potentially serve as keystone indicators of pest abundance. Thus ground beetles can be good indicators of sustainable agriculture, but rove beetles have a good potential to do it. Researches on crop dwelling ground beetles and rove beetles have been done infrequently in Latvia. Mainly these are faunistic studies not paying attention to agricultural environmental factor effect to ground beetles and rove beetles. For using ground beetles and rove beetles as indicators of sustainable agriculture in Latvia, studies on these beetles reaction to different farming activities should be done. These studies must occur in different crops and different places of country, because ground beetle and rove beetle reflection to changes of agricultural environmental factors may be crop- and site - or even field-specific.
Overview of literature on ground and rove beetles' ecology in agroecosystems recorded in Latvia, other European countries and Northern America has been used for this study.
Key words: Carabidae, Staphylinidae, beneficial insects, integrated pest management.

Introduction

It is possible to find many definitions that define sustainable agriculture. C. A. Francis and M. B. Callaway (1993) summarize that sustainable agriculture is an integrated system including economy of resources, maintenance of productivity, reduction of environmental degradation and promotion of short- and long-term profitability. According to M. Kogan and P. Jepson (2007) sustainable agriculture and integrated pest management (IPM) are complementary concepts. IPM is a system of ecologically safe plant protection, and biological pest control is important element of it (Kapitsa, 2012). Many researches show that ground beetles (Coleoptera: Carabidae) and rove beetles (Coleoptera: Staphylinidae) play significant role as predators of pests in different crops. Thus, ground beetles and row beetles are inalienable elements of IPM and sustainable agriculture.

Ground and rove beetles are two beetle families containing many species living in Central Europe (Freude et al., 1964, 1974, 1976). More than 300 ground beetle species and more than 600 rove beetle species are found in Latvia (Barševskis, 2003; Telnov, 2004). Almost all ground beetles and many rove beetles are soil dwelling or epigeic insects. Ground beetles mostly are carnivores, but there are many species which can be classified as herbivores or omnivores. Carnivorous ground beetles feed on different invertebrates: insects, spiders, slugs, snails etc. Herbivorous ground beetles feed on pollen, small sized seeds or sprouts of different plants. Omnivorous ground beetles feed on food objects most readily available in their immediate habitat. In fact, carnivorous ground beetles can become temporal herbivores when there is lack of prey (Riddick, 2004). Almost similar situation is among species of rove beetles – many species are predators, but also many species are herbivores, fungivores, coprovores or omnivores. Some groups of rove beetles feed on decomposing fungi, plant or animal material, but species included in *Aleochara* genus are parasitoids in fly pupas (Frank and Thomas, 2004).

Review on ground beetles occurring in agrocenoses in Latvia is done by A. Bukejs et al. (2009). Researches on ground beetles were done in 15 crops. Mostly species compositions and dominance structure, but in some cases influence of pesticides on ground beetles, is analysed. In Latvia, here are no researches on influence of different agrotechnical activities to ground beetles done. There are only few faunistic researches on rove beetles occurring in agrocenoses done in Latvia, e.g., studies on rove beetles living in strawberry fields (Cibuļskis and Petrova, 2002; Petrova et al., 2006).

Objectives of this study are as follows:

1. To discuss ground and rove beetle role in IPM and possibilities to use these beetles as indicators of sustainable agriculture in Latvia.
2. To discuss necessary researches on ground and rove beetles living in agrocenoses in Latvia.

Materials and Methods

Monographic method has been used for this study. Available literature on ground beetle and rove beetle ecology in agroecosystems recorded from Latvia, other European countries and Northern America had

been used for the study. Check-list of Latvian beetles (Telnov, 2004) has been used for nomenclature of beetle species.

Results and Discussion

Ground and rove beetles as plant protectors

Different studies show that carnivorous ground and rove beetles as pest and weed controllers are important elements of agroecosystems. For example, these beetles can significantly decrease abundance of leaf beetles (Chrysomelidae), aphids (Aphidodea) and slugs in cereals (Sunderland and Vickerman, 1980; Sotherton et al., 1984; Sunderland et al., 1987; Winder et al., 1994; Wiltshire and Hughes, 2000; Lang, 2003; Schmidt et al., 2003). Rove beetles are widespread in every agroecosystem where they mostly feed on aphids and fungi – causal agent of mildew, but *Aleochara* spp. rove beetles are parasitoids of fly pupas, i.e., they are significant controllers of *Delia* spp. flies in cruciferous fields (Petrova et al., 2006; Balog et al., 2008, 2009).

Retrospectively, an opinion on herbivorous and omnivorous ground beetles as beneficial insects has been changed since the middle of the 20th century. For example, E. Ozols (1963) calls *Harpalus rufipes* as strawberry pest, but *Harpalus affinis, Amara apricaria, Bembidion lampros, Bembidion properans, Poecilus cupreus* and *Pterostichus melanarius* – as cereal pests which should be controlled. More precise studies show that these ground beetle species mostly act like pest and weed predators. K. D. Sunderland (1975) made study on diet of predatory arthropods in cereal crops. This study was based on gut dissection of different insect species and one centipede species. Results showed that *Bembidion lampros* is absolute carnivorous species feeding mostly on springtails (Collembola), aphids and dipterans (Diptera). But *Harpalus rufipes* is omnivorous species – guts of two thirds of individuals contained remains of different insects, mostly aphids, beetle adults and beetle larvae. One third of *Harpalus rufipes* guts contained unidentified plant material. Similar study of K. D. Sunderland and G. P. Vickerman (1980) showed that *Bembidion lampros* and *Pterostichus melanarius* together with fore more ground beetle species feed on aphids independently of aphid density in cereal fields. Even *Amara* spp. ground beetles, which are considered to be herbivores, feed on aphids, when aphids are particularly abundant (>100 m^{-2}) in cereal fields. S. Skaldere (Скалдере, 1981) observed that *Amara aenea* and *Harpalus affinis* feed on aphids in barley (*Hordeum vulgare*). One more study on ground beetles feeding on aphids in cereals shows that *Bembidion lampros, Pterostichus melanarius* and *Amara aenea* are significant aphid predators before start of flowering of cereals (Sunderland et al., 1987).

Pterostichus melanarius is mentioned as a significant predator of slugs in winter wheat (*Triticum aestivum*) (Wiltshire and Hughes, 2000); of blueberry maggot *Rhagoletis mendax* in highbush blueberries (*Vaccinium corymbosum*) (Renkema et al., 2012) and of Colorado beetle *Leptinotarsa decemlineata* in potato (*Solanum tuberosum*) fields, along with *Poecilus cupreus* and *Harpalus rufipes* (Koval, 1999). Studies in oilseed rape (*Brassica napus*) show that *Poecilus cupreus, Harpalus affinis* and *Harpalus rufipes* are predators of brassica pod midge *Dasineura brassicae* and pollen beetle *Meligethes* spp. larvae. *Harpalus affinis* and *Harpalus rufipes* also feed on rape seeds, but predation on pest larvae is more noticeable (Schlein and Büchs, 2006a). Studies on *Amara similata* feeding habits show that this ground beetle species can be a significant controller of oilseed rape pod midge larvae. Microcosm study proved that *A. similata* distinguishes uninfested pods from infested ones which are preferred (Schlein and Büchs, 2006b). If *A. similata* cannot find enough infested rape pods, it starts to feed on various plants, also rape, seeds (Schlein and Büchs, 2006a, 2006b).

Herbivorous and omnivorous ground beetles are weed controllers in various crops. Study of M.J. Ward et al. (2011) shows that herbivorous ground beetles reduce weed density of 60-80% during vegetation season. Especially effective weed reduction by ground beetles had been observed in maize (*Zea mays*) fields. After soil tillage, ground beetles and other seed predators consume 22-28% of weed seeds. This is 78-90% of total seed predation rate in crops (Cromar et al., 1999).

Worldwide studies show that even herbivorous and omnivorous ground beetles are significant predators of pests and weeds in agroecosystems. In few cases they feed on crop seeds or sprouts. It means that herbivorous and omnivorous ground beetles along with carnivorous ground beetles and rove beetles are beneficial insects and important elements of IPM in agroecosystems.

Ground and rove beetles as indicators of sustainable agriculture

Comparably large species diversity and density of individuals in agroecosystems, ability to react on different husbandry activities and good knowledge on their ecology are main factors which allow using ground beetles as indicators of sustainable agriculture. Ground beetles poorly indicate overall biodiversity of invertebrates in various habitats. On the other hand, ground beetles reflect human-caused disturbances such as soil tillage, crop rotation, chemical and genetic pollution, usage of fertilizers and landscape fragmentation. All these factors are the parameters based on which it is possible to assess agriculture whether it is sustainable or not. Ground beetles also

can indicate different farming systems and potentially serve as keystone indicators of pest abundance (Holland and Luff, 2000; Rainio and Niemelä, 2003; Koivula, 2011; Cameron and Leather, 2012).

Proper soil tillage and crop rotation are important components of IPM. In regard to ground beetles, soil tillage and crop rotation should be discussed complementary, because both these factors depend on each other. Ground beetles react both to crop type and soil cultivation method. Bigger diversity and abundance of ground beetles are observed in winter cereals than in spring root crops (Holland and Luff, 2000). This is due to soil surface loosening in root crops during vegetation season. Soil loosening provides direct mortality of ground beetles up to 51%, and this effect remains within 18 days after performed activity. On the other hand, soil loosening does not affect rove beetle diversity and abundance in crops (Thorbek and Bilde, 2004). According to this, it is possible to say that species composition and abundance of ground beetles in crop may depend on fore-crop in the same field. Soil tillage is ecological disturbance which eliminates large sized ground beetles out of agroecosystem. For example, *Carabus* spp. beetles do not inhabit very intensively tilled fields (Holland and Luff, 2000; Cole et al., 2005). On the other hand, intensively tilled fields provide patchiness of vegetation suitable for small and medium sized ground beetles which are so called visual hunters. For example, density of *Anchomenus dorsalis* and similar species increases within intensively tilled crop fields (Cole et al., 2005). Non-inverse soil tillage maintains organic layer on soil surface and promotes composition of weeds. As a result, abundance of ground beetles increases – herbivorous species are attracted by weed seeds and sprouts, but carnivorous ground beetles are attracted by phytophagous invertebrates feeding on weeds. Weeds also affect soil microclimate positively for ground beetles (Holland and Luff, 2000; Thorbek and Bilde, 2004). Other studies show that ground beetles do not react to soil tillage intensity. For example, J. P. Twardowski (2006) and N. S. Mason et al. (2006) reports that ploughing and non-inverse soil tillage make almost similar effect to ground beetle assemblages in winter oilseed rape. But S. Belaoussoff et al. (2003) accent that, in general, soil tillage does not make statistically significant effect to the ground beetle diversity in farmlands. Also, different studies on ploughing and non-inverse soil tillage effect to rove beetles show different result. According to P. Thorbek and T. Bilde (2004), the rove beetle diversity and abundance are not affected neither by ploughing nor by non-inverse soil tillage. On the other hand, N. S. Mason et al. (2006) report that during July rove beetles have been more abundant in non-inverse tilled crops than in ploughed ones.

Ground beetles have been implicitly affected by herbicides and fungicides. These chemicals directly reduce food resources for herbivorous species. Chemical weed elimination from agroecosystem also causes rapid changes to soil microclimate and absence of additional food (weed herbivores) for carnivorous ground beetle species (Holland and Luff, 2000; Koivula, 2011). The study of R. A. Chiverton and N. W. Sotherton (1991) shows that activity and cereal aphid consumption of carnivorous ground beetles *Anchomenus dorsalis* and *Pterostichus melanarius* increase as a result of herbicide spraying in spring barley. This may be beneficial effect, but on the other hand, fertility of ground beetle females decreases as a result of lack of additional prey. Caused by herbicide spraying, higher activity of big sized ground beetles can promote predation of small sized ground beetles (Navntoft et al., 2006). Overall, it is possible to conclude that herbicide usage negatively affects long-term density of ground beetles within all trophic groups. Insecticides affect ground beetles directly causing their death. Ground beetle populations in agroecosystem react to insecticides by decreasing their abundance (Holland and Luff, 2000, Koivula, 2011). The study of O. R. Aleksandrowicz (2002) shows that insecticides also cause changes in dominance structure of ground beetle species. Dominants and subdominants may become recedents and subrecedents, but some previously recedent species may become dominant or subdominant. R. Cinītis (1975), J. M. Holland and M. L. Luff (2000) and M. J. Koivula (2011) maintain that insecticides make short-term effect due to habitat fast re-colonization by ground beetles. On the other hand, O. R. Aleksandrowicz (2002) reports that the effect caused by insecticides may last almost two months. Sometimes ground beetles do not indicate usage of insecticides. It happens when insecticides have been sprayed on crop canopy, not hitting the ground (Holland and Luff, 2000; Koivula, 2011). There is less data on insecticide caused effect to rove beetles. It is clear, that rove beetle abundance within crop also decreases due to the usage of insecticides, but this decrease is not statistically significant (Aleksandrowicz, 2002).

M. J. Koivula (2011) mentions that ground beetles can implicitly indicate genetically modified crops or so called genetic pollution in agroecosystem. Pesticide usage rate in genetically modified crops is noticeable high, and it affects ground beetle assemblage and abundance. Opposite opinion had been expressed by D. A. Bohan et al. (2005). According to it, ground beetle species richness does not differ between genetically modified and conventional winter oilseed rape crops. In general, there is lack of experience on ground beetles as indicators of genetically modified crops, thus, researches are needed.

The usage of fertilizers can affect ground beetles in different ways. Organic fertilizers change soil surface; it affects overwintering, burrowing and oviposition. Organic fertilization also promotes presence of earthworms and other saprophagous invertebrates which are prey for ground beetles. Both organic and inorganic fertilizers promote weed assemblages and more dense plant leaf cover over the ground. It all changes soil microclimate (soil is more shaded and humid), creates more shelters for epigeic invertebrates and attracts more herbivores (Holland and Luff, 2000). E. Diehl et al. (2012) accent that weeds foster ground beetles by resource- and structure-mediated effects. Attraction of herbivorous invertebrates is resource-mediated factor which fosters ground beetles more significantly than microhabitats created by weeds (structure-mediated factors) in crops. Inorganic crops are more homogeneous in their density and growth rates than organic crops. It means that organic crops provide more diverse environmental conditions suitable for wider ground beetle species diversity (Holland and Luff, 2000).

Ground beetle diversity and abundance are indicators of landscape fragmentation and heterogeneity. Unmanaged field margins, hedgerows, neighbouring different ecosystems etc. increase ground beetle species diversity in the crop field (Holland and Luff, 2000; Weibull et al., 2003). On the other hand, such linear formations as roads (even thin earth roads) and ditches are hardly surmountable biogeographic barriers for ground beetles (Holland and Luff, 2000). It means that intensively cultivated crop field surrounded by roads and ditches contains lower diversity ground beetle assemblage. Available information on rove beetles shows that they do not indicate landscape heterogeneity. A.-C. Weibull et al. (2003) did not find any correlation between landscape heterogeneity and rove beetle species richness.

Farming system affects ground beetle species diversity, but not abundance of individuals. Conventional crops mostly provide the lowest ground beetle diversity comparing to integrated, organic and biodynamic crops (Holland and Luff, 2000). On the other hand, studies in Sweden show that ground beetle species richness is higher within conventional crops than organic ones. It is explained with inorganic fertilizer usage providing suitable conditions for herbivorous species in conventional crops (Weibull et al., 2003). Ground beetle assemblages contain less herbivorous species within integrated system crops due to farming practices decreasing weed density. Many factors within each farming system determine ground beetle species assemblage of crop field. These factors might be unique within every single crop field (Holland and Luff, 2000). The study of A.-C. Weibull et al. (2003) did not find correlation between rove beetle species richness and farming system.

Ground beetles have potential to serve as keystone indicators in crop fields. Many studies show that ground beetles significantly reduce pest and weed density. Presence and definite density of ground beetle species can indicate decreased pest and weed amount. However, more studies should be done in this aspect (Koivula, 2011).

Necessary researches on crop dwelling ground and rove beetles in Latvia

Researches on ground beetles in Latvian agroecosystems had been done infrequently. Mostly ground beetle fauna in different crops had been analysed (Bukejs et al., 2009). There are also some studies on ground beetle activity changes during twenty-four hours, but some studies report how insecticides affect ground beetles abundance and assemblages within crop fields (Цинитис, 1962; Цинитис и Вилкс, 1962a, 1962b; Cinītis, 1975). Effects of other farming activities had not been studied. Knowledge on rove beetles is similarly poor. As mentioned above, seldom data on rove beetle fauna of agrocenoses are available. It means that it is not possible to use ground and rove beetles as indicators of sustainable agriculture in Latvia right now due to lack of knowledge. But, as it was discussed previously, ground beetles can serve as good indicators of IPM and sustainable agriculture. Also, rove beetles have great potential to do it.

To use ground and rove beetles as indicators of sustainable agriculture in Latvia, a lot of studies should be done. The study of A. Bukejs et al. (2009) shows that ground beetle fauna can be significantly different within different crops and regions of country. It is possible to speculate that rove beetle fauna differs similarly. Previous discussion highlighted that response of ground beetles and rove beetles to farming activities can differ depending on the crop. Also sometimes one husbandry activity, for example, similar soil tillage caused opposite response of ground beetles and rove beetles within different study sites. Thus, it is possible to say, that indication of sustainable agriculture by ground beetles and rove beetles is crop- and site- specific or maybe even field-specific, because theoretically many environmental factors (soil, neighbouring habitats, historical usage of agrochemicals, meso and macro relief etc.) can be unique in every single field. It means that researches on ground beetle and rove beetle reaction to different farming activities, such as discussed in previous subsection of this paper, should be done in different regions of Latvia to cover different environmental and farming factors as more as possible. First step to reach this objective was done in 2012, when researches on ground and rove beetles as indicators for sustainable

soil use in winter wheat in Zemgale started. Main objectives of this research are to compare how ground beetles and rove beetles react to soil ploughing and non-inverse tillage and different crop rotation schemes (Gailis and Turka, 2012).

Conclusions

1. Ground beetles and rove beetles are beneficial insects as significant predators of many pests and weeds in any crop; thus, they are important elements of integrated pest management.
2. Worldwide studies show that ground beetles can serve as indicators of sustainable agriculture, but rove beetles have a potential to do it. Reaction of beetles to environmental changes is crop- and site- or even field-specific.
3. Lack of knowledge does not allow using ground and rove beetles as indicators of sustainable agriculture in Latvia right now.
4. Researches on ground beetle and rove beetle reaction to different soil tillage, crop rotation, usage of agrochemicals and other agricultural environmental factors should be done in different crops in different regions of Latvia.

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GOAT MILK COMPOSITION VARIABILITY AFTER KID WEANING

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Abstract
The aim of the research was to evaluate the variability of milk yield and composition for goats with different kid suckling periods. The research was carried out in 2011 and 2012 on a farm where goats of Latvian breed were reared. In both years goats kidded in February and March. Each group included 10 animals. In the first group the kids were weaned on the first day of kidding (A1), in the second – on the 30th day (A30) but in the third – on the 60th day (A60) after kidding. The amount of the milk yield was measured in five successive days after the kid weaning. Milk samples were analysed in an accredited milk laboratory. The highest milk yield was obtained from the goats when kids were weaned immediately after the birth, and samples were taken starting with the 6th day of lactation (2.10 + 0.05 kg), but the lowest from the goats with suckling period of 60 days – 1.68 ± 0.03 kg. The average milk fat and protein content of the goats from the group A1 (53.6 ± 0.92 and 41.6 ± 0.66 g kg^{-1}), was significantly higher than from the groups A30 and A60 ($p<0.05$). The average variability of milk yield proved to be from 4.9% in the group A1 to 10.5% in the group A30. The lowest fat content variability was observed for goats of the group A1 in both years – 8.9% and 10.7%, but the highest 20.5% in the group A60 in the first year. The highest variability of milk protein content was observed in the group A30 – 14.8%.
Key words: dairy goat, daily milk yield, fat and protein, variability.

Introduction

In Latvia, goat breeding as a branch of agriculture started to develop in the 90s of the 20th century. Basically Latvian breed goats (Latvian local – LVK) are reared as dairy goats in all regions of Latvia. It constitutes up to 45% of the total number of dairy goats. The breed was developed at the end of the 19th century by crossbreeding local goats with Russian and Megrel breed bucks introduced in Latvia from Russia and the region of Vitebsk (Latvijas kazkopības biedrība, 2012) The most essential trait of LVK is high reproductivity – fertility 300 – 350% and kid rearing till the weaning age. Depending on feeding and keeping conditions an average milk yield of LVK goats is no less than 450 – 700 kg, with fat content 38.0 – 50.0 g kg^{-1}, and protein content 30.0 – 35.0 g kg^{-1} (Piliena and Jonkus, 2010).

The average milk yield from goats recorded in 2012 was 522 kg with the average fat content 3.81% and protein content 3.14%. An important problem in goat recording is a precise inventory and assessment of goat milk productivity, because it singles out the best animals for an aim oriented goat selection. Goat milk productivity recording in Latvia is not carried out uniformely because kid suckling periods vary from farm to farm. Traditionally it lasts till aprox. the 60th day of lactation. Many European countries practise weaning some hours after kidding and kids are fed artificially. The first milk recording shows important variability of milk composition from goats with the suckling period to 2 months. Milk from individual goats with long suckling period in the first days of recording showed a very low milk fat content which does not comply with the International Committee for Animal Recording (ICAR) rules stating that the variability of milk composition should not decrease lower than 20.0 – 90.0 g kg^{-1}, but protein content 10.0 – 70.0 g kg^{-1} (ICAR, 2012).

The aim of the research was to evaluate the variability of milk yield and milk composition for goats with different kid suckling periods.

Materials and Methods

Productivity traits of Latvian breed goats were analysed in two year period: 2011 and 2012. 60 dairy goats were included in the research. They were divided into three groups, 10 animals in each. The average age of goats was 4.7 lactation, and there was no significant difference among the groups. In both years goats included in the research kidded in the period from the beginning of February to the end of March. In the first group the kids were weaned on the first day of birth (A1), in the second group – on the 30th day A30) and in the third group – on the 60th day after birth (A60).

During the research the milk yield (kg), the fat content (g kg^{-1}) and protein content (g kg^{-1}) were analysed as well as somatic cell count (thousand mL^{-1}). Milk productivity and quality in all groups were recorded for five susessive days after the kid weaning. After weaning the goats were milkedtwice a day. In the group A30 the check up was done on the 31st, 32nd, 33rd, 36th and 38th day, but in the group A60 – on the 61st, 62nd, 63rd, 66th. and 68th day of lactation. In the group A1 milk yield was measured starting from the 6th day of lactation when according to the rules of the International Committee for Animal Recording (ICAR) the milk recording is allowed. Also, for the goats of this group the milk check up was carried out for five days – on the 6th, 7th, 8th, 9th and 11th day of lactation. The milk yield was measured with electronic scales. The milk samples were analysed in Sigulda Artificial Insemination Station, where fat and

protein content was identified according to the method of ISO 9622:1999 with the device Milko-Skan 133. In winter, goats of all groups were kept in stables and fed on hay and haylage, but in summer they grazed in cultivated pastures. From the annual energy value 30.0% is concentrate, but grass is 70.0%. In dry matter there are 20% crude protein, and it includes 6.35 MJ kg^{-1} neto energy for lactation (NEL). Every day the goats received 0.5 kg concentrate with 20% of the digestible protein.

The statistical analyses were performed using SPSS program package and MS EXCEL for Windows. Data in tables and figures are presented as the least square mean ± standard error of means. The coefficient of variation (CV) was used to describe the traits variability. The results were analyzed using two-factor analysis of variance to determine the factors year and the weaning group of the milk productivity traits. Statistical differences with *p*-values under 0.05 were considered as significant.

Results and Discussion

The average milk yield from all the research groups of goats in a standard lactation in 2011 was 435 kg, milk fat content – 44.5 g kg^{-1} un protein content – 32.0 g kg^{-1}, but in 2012 the average milk yield was 431 kg, milk fat content – 45.8 g kg^{-1} and protein content – 32.2 g kg^{-1}, the average somatic cell count was 322 thousand mL^{-1}.

To find out the variability of goat milk yield and composition, the average milk productivity traits were assessed in five successive days after kid weaning (Table 1).

In the period of research the highest 24 hour (daily) milk yield was obtained from the A1 group goats in 2011 – 2.52 kg, which was significantly higher than the yield from the groups A30 and A60, respectively – 1.82 and 1.65 kg. The next year the 24 hour (daily) milk yield was taken from goats when kids were weaned on the 60th day of suckling – 1.71 kg, but it was only 0.03 kg higher than from the A1 group goats. The 24 hour (daily) milk yield from the group A30 was significantly less – 1.51 kg ($p<0.05$).

The fat and protein content varied significantly among the groups in both years when the research was carried out. The highest milk fat content in both years was obtained from the goats when kids were weaned on the first day after birth and were fed artificially, 53.6 and 53.5g kg respectively, but the lowest milk fat content was obtained from the goats which had suckled kids for 60 days (30.2 and 27.6 g kg^{-1}). The fat content characteristic for the breed (45.0 and 45.7 g kg^{-1}) was obtained from the goats when kids were weaned on the 30th day of suckling. Similar tendency was observed for the variability of milk protein content among the research groups. The average milk protein content 43.2 and 40.7 g kg^{-1} obtained from the group A1 was significantly higher than the milk protein content from A30 and A60. As the scientific data prove, goat milk yield grows till the second week of lactation. Provided that the feeding and keeping conditions are set correctly, the maximum milk yield is maintained till the 10th week of lactation (Bömkes, 2004). After that the milk yield gradually decreases by approx. 10% (Gall, 2011).

Table 1

The average goat milk productivity in research days

Group	Kidding Year	Milk yield kg day	Min	Max	Fat content, g kg^{-1}	Min	Max	Protein content, g kg^{-1}	Min	Max
A1	2011	$2.52±0.02^{a}$	2.3	2.9	$53.6±0.92^{a}$	36.3	63.5	$42.4 ±1.41^{a}$	33.0	60.3
	2012	$1.68±0.04^{A}$	1.0	2.4	$53.5±1.61^{A}$	38.2	72.4	$40.7±0.81^{A}$	30.1	35.1
	Average	2.10±0.05	1.0	2.9	53.6±0.90	38.2	72.4	41.6±0.66	30.1	60.3
A30	2011	$1.82±0.05^{b}$	1.3	2.7	$42.0±1.16^{b}$	20.4	54.0	$36.2±0.88^{b}$	25.3	49.3
	2012	$1.51±0.02^{B}$	1.1	1.9	$45.7±1.50^{B}$	18.8	75.6	$38.0±0.64^{B}$	30.9	50.3
	Average	1.66±0.33	1.1	2.7	43.9±0.91	18.8	75.6	37.1±0.56	25.3	50.3
A60	2011	$1.65±0.45^{c}$	1.1	1.6	$30.2±0.97^{c}$	16.3	44.4	$28.0±0.82^{c}$	21.0	41.8
	2012	$1.71±0.03^{A}$	1.2	2.2	$27.6±0.43^{C}$	21.2	34.6	$30.2±0.29^{C}$	23.4	34.0
	Average	1.68±0.03	1.1	2.2	28.8±0.54	16.3	44.4	29.1±0.44	21.0	41.8

$^{a;b;c}$ – differences between groups with different letter are significant in the year 2011 ($p<0.05$).
$^{A;B;C}$ – differences between groups with different capital letter are significant in the year 2012 ($p<0.05$).

The frequency of milking influences both milk productivity and quality. The scientists in Turkey found out that goats milked 4 times a day yield 2.05 kg milk with the fat content 29.5 g kg^{-1}, protein content – 30.8 g kg^{-1}, but goats milked 2 times a day yield 1.8 kg with fat content 30.8 g kg and protein content 31.9 g kg^{-1}. By increasing frequency of milking, fat content got lower (Koyuncu and Pala, 2008). They also stated that the milk composition is influenced by machine milking (Cetin et al., 2010). Goats from research gruops were milked twice a day at 7 a.m. and 5 p.m.

Another research showed that the productivity and quality of milk during the suckling period are influenced by the amount of fodder in 24 hours. If the amount of fodder is increased during the suckling period by 15%, milk yield among research groups was more than 116.2 kg in comparison with the check-up group, but if the amount was increased by 20%, the milk yield was more than 171.7 kg (Abdelhamid and Abdel-Khalek, 2012). Goats after kidding let kids suckle as often as they wanted for the first 4 weeks 6 – 8 times a day (Jensen, 2009; Rahmann, 2010).

Significant variability of milk yield and composition were observed for individual animals (Figure 1).

In all three groups there were goats with very low (daily) milk yield in 24 hours – only 1.0 kg or a little more than one kilogram (1.1 – 1.3 kg). The maximum yield (2.9 kg^{-1}) in 2011 was obtained from the goat in the group A1. The average variability of milk yield during the whole research period was from 4.9% in the group A1 to 10.5% in the group A30. The variability of fat and protein content during the research was higher than variability of milk yield. The observed minimum milk fat content was 16.3 g kg^{-1} in thegroup A60, but maximum 75.6 g kg^{-1} in the group A1. The variability of milk fat content assessment showed that the lowest values of coefficient of variation showed the group A1 in both years of research – 8.9% and 10.7%, but the highest coefficient of variation of milk fat contetnt variability – 20.5% was observed in the group A60 in the first year of research. Permanently high variability of fat content was observed in the group A30 in both years (16.0 and 17.3%). The individual minimum variability of milk protein content was 21.0 g kg^{-1} in the group A60 and the maximum – 60.3 g kg^{-1} was observed in the group A1 in 2011. The highest variability of milk protein content from all the groups was noted in 2011 in the group A30 – 14.8%.

Analysing the total milk yield of a lactation from the research groups of goats, we found out that the milk yield in the first year in all groups was higher than in the second year although the difference was not statistically valid. The average milk yield obtained in a standard lactation significantly varied among the goat groups in both years of research. The highest milk yield was obtained from goats in the group A1 in both years – 455.6 kg and 455.3 kg ($p<0.05$; Table 2).

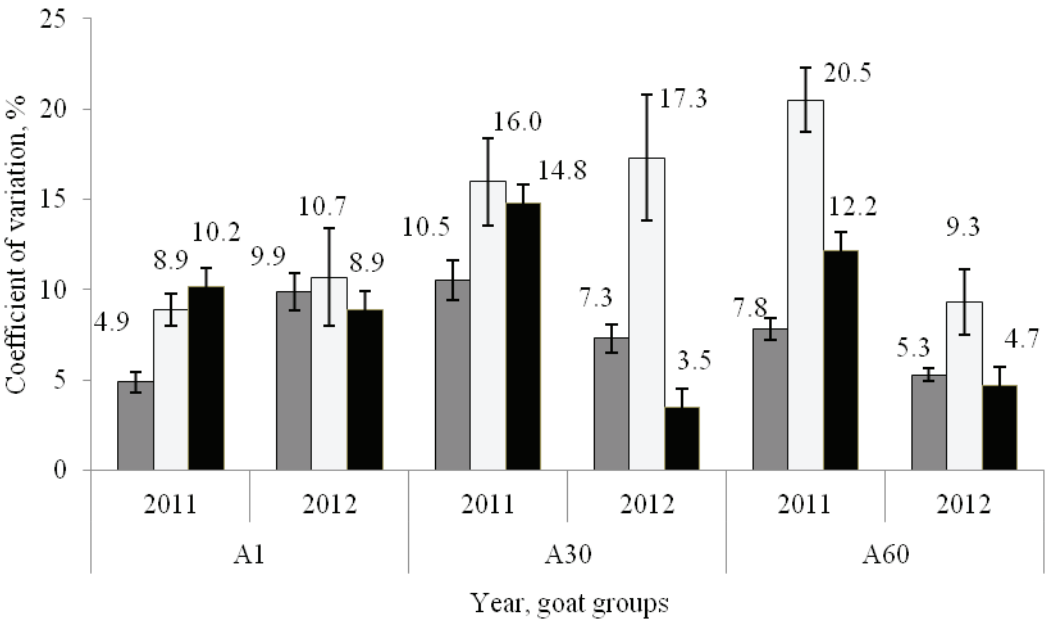

Figure 1. Daily milk productivity variability in goat groups.
■ – Milk yield, ■ – Fat content, ■ – Protein content.

Table 2

Average milk productivity in a standard lactation of goats research group

Group	Kidding year	Milk yield, kg	Fat content, g kg^{-1}	Protein content, g kg^{-1}	SCC, thousand mL^{-1}
A1	2011	455.6±2.91^{a}	44.1±0.61^{a}	31.9±0.22^{a,b}	366±25.75^{a}
	2012	455.3±2.23^{A}	47.4±0.70^{A}	32.7±0.21^{A}	266±31.30^{A}
	Average	455.5±1.82	45.7±0.52	32.3±0.11	316.5±20.78
A30	2011	443.4±4.12^{b}	46.5±0.70^{b}	32.4±0.33^{a}	325±31.88^{b}
	2012	434.7±1.58^{B}	45.0±0.51^{B}	31.7±0.16^{B}	157±14.48^{B}
	Average	439.0±2.23	45.8±0.42	32.1±0.22	241.4±19.34
A60	2011	406.6±7.51^{c}	42.8±0.61^{c}	31.7±0.24^{b}	493±83.48^{c}
	2012	405.5±5.92^{C}	44.9±0.62^{B}	32.0±0.17^{B}	272±28.96^{C}
	Average	406.0±4.75	43.9±0.44	31.9±0.11	382±45.33

$^{a;b;c}$ – differences between groups with different letter are significant in the year 2011 ($p<0.05$).
$^{A;B;C}$ – differences between groups with different capital letter are significant in the year 2012 ($p<0.05$).

The goat milk fat content in 2011 significantly varied among the research groups. The highest average fat content in 2012 was 47.4 g kg^{-1}, and it significantly exceeded the average fat content characteristic for the population of LVK goats. Also, the milk protein content – 32.7 g kg^{-1} – was significantly higher for this group of goats. The same can be stated about the average somatic cell count among the groups. In 2012, the milk had higer quality, because the average somatic cell count was significantly lower (156 to 272 thousand mL^{-1}) than in 2011 (325 to 493 thousand mL^{-1}).

A group of scientists in Croatia has found out that Saaneen breed goats which had the shortest period of kid suckling – 32 days – during a lactation produced the highest milk yield – 724.40 kg. Also, the average day yield is the highest for these goats – 2.76 kg, and they produced the most milk fat and protein kilograms (20.16 and 18.64 kg respectively). Alpine goats had the longest kid suckling period – 51 day as well as the longest lactation – 259 days. They had the highest milk fat content in a lactation – 35.5 g kg^{-1}, but the highest milk protein content was obtained from German Improved White (VBD) goats – 32.3 g kg^{-1}, which had 45 day long kid suckling period (Mioč et al., 2007).

During our research LVK breed goats in a standard lactation, which according to ICAR is from 240 to 305 days long, had lower average milk yield (405.5 to 455.6 kg), but significantly higher milk fat content (42.8 to 47.4 g kg^{-1}) and similar milk protein content (31.7 to 32.7 g kg^{-1}) as in Croatia, because high milk yield is a characteristic trait for Saanen goats.

Conclusions

The highest (daily) 24 hour milk yield was obtained from goats when kids were weaned after the birth, and milk samples were taken starting from the 6th day of lactation – 2.10 ± 0.05 kg, but the lowest – from goats with the kid suckling period of 60 days – 1.68 ± 0.03 kg

A1 group goats showed significantly higher average milk fat content – 53.6 ± 0.92 g kg^{-1}, whereas the group A30 – 43.9 ± 0.91 g kg^{-1}, and A60 – 28.8 ± 0.54 g kg^{-1}.

Significantly higher average milk protein content was obtained from the group A1 goats 41.6 ± 0.66 g kg^{-1}, from the group A30 – 37.1 ± 0.56 g kg^{-1} and from the group A60 – 29.1 ± 0.44 g kg^{-1} ($p<0.05$).

The variability of milk yield ranged from 4.9% in the group A1 to 10.5% in the group A30.

The lowest goat milk fat content variability (8.9%) was observed in the group A1, while the highest (20.5%) in the group A60. The highest milk protein variability (12.2%) had the group A60, while the lowest (3.5%) – goats from the A30 group.

Ssignificantly higher average milk yield in a standard lactation was obtained from the first group of research goats – 455.6 ± 1.82 kg, but the lowest – from the group three goats – 406.1 ± 4.76 kg ($p<0.05$).

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ESTIMATION OF GENETIC PARAMETERS FOR GROWTH TRAITS OF SHEEP POPULATION IN LATVIA

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Abstract
The aim of the present study was to estimate variance and covariance components and genetic parameters for birth, weaning and yearling weights. The data were collected from lambs who born in period from 2007 to 2010 years and located in 58 pure-bred sheep herds in Latvia. Records of 11310 lambs from 160 rams and 4180 ewes for birth and weaning weight and on 3194 lambs from 134 rams and 2058 ewes for yearling weight were used in this study. The total number of individual pedigree was 18932. The fixed effects in the model were sex and type of birth, birth year and month and age of dam, because all growth traits were significantly affected by these fixed effects ($p<0.01$, $p<0.05$). Genetic parameters for growth weights were estimated by Restricted Maximum Likelihood (REML) procedure fitting three animal models including various combinations of maternal and herd effects. Based on the most appropriate fitted model which included additive genetic, maternal additive genetic and herd effect, direct heritability's were estimated to be 0.26 ± 0.01, 0.29 ± 0.63, 0.29 ± 2.63, but maternal heritability's 0.14 ± 0.00, 0.04 ± 0.19, 0.04 ± 0.94, respectively for birth, weaning and yearling weights. The results showed maternal and strong herd influence in this study, therefore inclusion of maternal and herd effects into the model for growth traits is necessary.
Key words: genetic parameters, growth traits, sheep.

Introduction

The total sheep population in Latvia is 83743 heads and Latvian dark-head sheep breed is the main sheep breed with a population of 37773 (Agricultural Data Centre, 2013). This local sheep breed belongs to the mutton-wool type, the sheep are white color with dark head and legs and animals are well adapted to the local conditions. In Latvia, sheep breeding becomes a significant branch of agriculture, because there are favorable conditions created for raising sheep breeding and it is a complementary component of a mixed farming system. Similarly, the high fertility and short generation intervals make it popular among breeders.

Estimated breeding value is a tool for the genetic improvement of animals therefore the aim of genetic evaluation is to provide an accurate estimate of the breeding value of an animal from the given performance and pedigree information. The performance of an animal is affected by several genetic and environmental factors. The most important environmental factors are sex, type of birth, birth year and age of dam. The significant influences of environmental factors on body weight at the various ages can be explained in part by differences in years, male and female endocrine system, limited uterine space and inadequate availability of nutrients during pregnancy, competition for milk between the twins, maternal effects and maternal ability of dam in different ages (Mohammadi et al., 2010b). Various environmental factors have been studied in several investigations (Hussain, 2006; Thiruvenkadan et al., 2008; Tariq et al., 2010; Mokhtari and Rashidi, 2010; Savar-Sofla et al., 2011).

Direct genetic effects, maternal genetic effects and environmental factors are random factors which affect both the lamb and its dam. Growth traits, in particular until weaning is not only influenced by genes of the individual for growth and environmental under which it raised but also by the maternal genetic composition and environment provided by the dam (Lewis and Beatson, 1999). Dam's genes for growth traits affect the environment experienced by the offspring through milk production and mothering ability (Lotfi Farokhad et al., 2010). Numerous studies have shown that both direct and maternal genetic influences are of importance for lamb growth (Bahreini Behzadi et al., 2007; Mohammadi et al., 2010a; El-Awady et al., 2011; Savar-Sofla et al., 2011; Ghafouri-Kesbi and Baneh, 2012). Hence, to achieve optimum genetic progress in a selection program both the direct and maternal components should be taken into account (Meyer, 1992).

Knowledge of genetic parameters for weight traits is needed to determine optimal breeding strategies to increase the efficiency of sheep production in Latvia. The aim of this study were to estimate the variances and covariance's for direct genetic effect, maternal genetic effect and herd effect on lamb weights and to determine the most appropriate model of analysis for three growth traits of Latvian dark-head lambs.

Materials and Methods

Pedigree and performance data of the Latvian dark-head lambs used in this study were obtained from the state agency 'Agricultural Data Centre' which is responsible for the data processing of the sheep recording results. Lambs were born during

the period from 2007 until 2011 and located in 58 pure-bred sheep herds. Three traits were considered: birth weight, weaning weight (weight at 70 days of age) and yearling weight. Characteristics of data structure are summarized in Table 1. Data set used for analyses consisted of 11310 records for birth and weaning weights and 3194 records for yearling weight. Recording data collected from lambs located in 58 herds (in average 195 lambs in one herd). The lambs were the progeny of 160 rams and 4180 ewes for birth and weaning weights and 134 rams and 2058 ewes for yearling weight. The pedigree links were considered for all animals with performance records. Total numbers of individuals in the pedigree were 18932 and all the pedigree information was utilized in the estimation of genetic parameters using animal model. Means of birth, weaning and yearling weighs were 4.0 kg, 21.7 kg and 47.6 kg, respectively.

The General Linear Model (GLM) procedure of SAS statistical package (SAS, 2003) was used to test the significance of the fixed effects of sex (male and female) and the type of birth (single, twins, triplets and quads), birth year (3 levels: 2007 and 2008, 2009, 2010), birth month (4 levels: January, February, March, April-December) and age of dam (4 levels:1 and 2, 3, 4, 5 years old and older).

Variance and covariance components and genetic parameters were estimated by Restricted Maximum Likelihood using VCE-6 software package (Groeneveld et al., 2010). Three different single-trait animal models were fitted for each trait by ignoring or including maternal genetic effect and herd effect (Table 2).

Model 1 was a model with direct additive genetic effect as the only random effect. Model 2 included an additive maternal genetic effect fitted as second random effect, ignoring direct – maternal genetic covariance. Model 3 included direct additive genetic, maternal additive genetic and herd effect, ignoring direct – maternal genetic covariance. A log likelihood ratio test was used to choose the most suitable random effects model for each growth trait.

Results and Discussion

All traits were significantly affected by sex and type of birth, birth year and month and age of dam ($p<0.01$, $p<0.05$). As it is expected, male lambs and single born lambs were heavier (+0.16, + 0.94 and + 15.28 kg for male lambs and + 0.71, + 3.48 and + 1.07 kg for single born lambs, respectively for birth, weaning and yearling weights) than lambs born as female and lambs born from larger litters (Table 3). The difference between the two sexes increased with age of lamb, probably because of increasing differences in the endocrine system between males and females. The differences of traits related to type

Table 1

Characteristics of the data structure

Character	Birth weight	Weaning weight	Yearling weight
Number of lamb records	11310	11310	3194
Number of rams	160	160	134
Number of ewes	4180	4180	2058
Ratio of rams and lambs	70.7	70.7	23.8
Ratio of ewes and lambs	2.7	2.7	1.6
Ration of rams and ewes	26.1	26.1	15.4
Mean (kg)	4.0 ± 0.01	21.7 ± 0.05	47.6 ± 0.14
Standard deviation (kg)	0.71	5.41	7.91

Table 2

Description of animal models fitted

Model [1)]			(Co)Variance components estimated [2)]
1	$y = Xb + Z_1a + e$		σ^2_a, σ^2_e
2	$y = Xb + Z_1a + Z_2m + e$	Cov (a, m) = 0	σ^2_a, σ^2_m, σ^2_e
3	$y = Xb + Z_1a + Z_2m + Z_3h + e$	Cov (a, m) = 0	σ^2_a, σ^2_m, σ^2_h, σ^2_e

[1)] y: vector of records on the different traits; b, a, m, h and e: vectors of fixed direct additive genetic, maternal additive genetic, herd and the residual effects; X, Z1, Z2 and Z3: corresponding design matrices associating the fixed, direct additive genetic, maternal additive genetic and herd effects.

[2)] σ^2_a: direct additive genetic variance, σ^2_m: maternal additive genetic variance, σ^2_h: herd variance, σ^2_e: residual variance.

Table 3

Least square means and standard errors for all traits

Factors	Classes	Birth weight, kg	Weaning weight, kg	Yearling weight, kg
Sex	male	$4.00^{a} \pm 0.01$	$22.29^{a} \pm 0.08$	$62.72^{a} \pm 0.66$
	female	$3.84^{a} \pm 0.01$	$21.35^{a} \pm 0.07$	$47.44^{a} \pm 0.19$
Birth type	1	$4.29^{a} \pm 0.01$	$23.83^{a} \pm 0.09$	$55.47^{a} \pm 0.45$
	2	$3.89^{a} \pm 0.01$	$21.29^{a} \pm 0.05$	$54.40^{a,b} \pm 0.33$
	3 end more	$3.58^{a} \pm 0.02$	$20.35^{a} \pm 0.15$	$55.36^{b} \pm 0.52$
Birth year	2007/2008	$3.86^{a1,a2} \pm 0.01$	$25.16^{a1,a2} \pm 0.09$	$57.30^{a1,a2} \pm 0.40$
	2009	$3.94^{a1} \pm 0.01$	$20.25^{a1} \pm 0.09$	$54.31^{a1,b} \pm 0.40$
	2010	$3.97^{a2} \pm 0.01$	$20.06^{a2} \pm 0.09$	$53.63^{a2,b} \pm 0.41$
Birth month	1.	$3.92^{a1,a2} \pm 0.01$	$21.84^{a1,b} \pm 0.09$	$54.75^{a1} \pm 0.40$
	2.	$3.87^{a1,a3,b} \pm 0.02$	$20.97^{a1,a2} \pm 0.11$	$53.70^{a1,a2} \pm 0.45$
	3.	$3.97^{a2,a3,a4} \pm 0.01$	$22.91^{a1,a2} \pm 0.10$	$56.58^{a1,a2} \pm 0.42$
	4.	$3.92^{a4,b} \pm 0.01$	$21.58^{a2,b} \pm 0.10$	$55.29^{a2} \pm 0.42$
Lambing age of dam	1.	$3.75^{a1,a2,a3} \pm 0.01$	$21.25^{a} \pm 0.10$	$52.98^{a1,a2,a3} \pm 0.44$
	2.	$3.91^{a1,a4,a5} \pm 0.01$	$21.88^{a} \pm 0.10$	$54.02^{a1} \pm 0.45$
	3.	$4.03^{a2,a4} \pm 0.02$	$22.56^{a} \pm 0.11$	$54.61^{a2} \pm 0.47$
	4.	$4.04^{a3,a5} \pm 0.01$	$22.23^{a} \pm 0.09$	$54.32^{a3} \pm 0.43$

Within each factor, mean values with the same superscript letters are significantly different at $p<0.01$ (^{a}) or $p<0.05$ (^{b}). The figure (1, 2, 3, 4, 5) at the superscript letters are indicates to compared factor mean values.

Table 4

Estimates of variance components and genetic parameters for body weights using single-trait analysis

Trait	Model	σ^2_a	σ^2_m	σ^2_h	σ^2_e	$h_a^2 \pm$ S.E.	$h_m^2 \pm$ S.E.	-2logL
Birth weight	1	0.296	-	-	0.156	0.66 ± 0.01	-	19869.62
	2	0.221	0.071	-	0.149	0.50 ± 0.01	0.16 ± 0.01	19618.11
	3	0.131	0.069	0.109	0.189	0.26 ± 0.01	0.14 ± 0.00	19233.25
Weaning weight	1	12.888	-	-	7.452	0.63 ± 0.58	-	17236.86
	2	11.480	1.466	-	7.201	0.57 ± 0.61	0.07 ± 0.20	17174.69
	3	7.509	1.065	8.802	8.848	0.29 ± 0.63	0.04 ± 0.19	16532.70
Yearling weight	1	35.876	-	-	10.607	0.77 ± 2.31	-	14121.85
	2	34.388	3.137	-	8.900	0.74 ± 2.35	0.07 ± 1.06	14112.98
	3	17.837	2.372	25.009	16.463	0.29 ± 2.63	0.04 ± 0.94	13853.07

σ^2_a: direct additive genetic variance, σ^2_m; maternal additive genetic variance, σ^2_h: herd variance, σ^2_e: residual variance, h_a^2: direct heritability, h_m^2: maternal heritability, -2logL: Log likelihood values

of birth might be because of limited uterine space and competition in milk suckling.

As well as, lambs born in March (3.97, 22.91 and 56.58 kg, respectively for birth, weaning and yearling weights) were heavier than lambs born in other period. Variation in birth weight across years indicated that the feeding, management and environmental conditions affect the ewes during pregnancy (Hussain, 2006). The ewes those conceived during September to November months had lambing during January and February, favorable environmental conditions with good availability of the fodder during the gestation period, which might have been contributed to higher body weight at birth (Thiruvenkadan et al., 2008).

Also lambs born from adult ewes had higher weights than those born to younger ewes (difference + 0.29, + 1.31 and + 1.63 kg, respectively for birth, weaning and yearling weights). The significant effect of dam's age could be due to differences in maternal behavior, uterus space and milk production of ewes in different ages.

Effects of these environmental factors has been reported significantly in breeds like Moghani (Savar-Sofla et al., 2011), Mengali (Tariq et al., 2010), Kermani (Mokhtari and Rashidi, 2010), Mecheri (Thiruvenkadan et al., 2008), Thalli (Hussain, 2006).

The estimates of direct heritability (h_a^2) for traits studied were in the range from 0.26 to 0.66 for

birth weight, from 0.29 to 0.63 for weaning weight and from 0.29 to 0.77 for yearling weight (Table 4). Model 1, which ignored maternal and herd effects, resulted in larger estimates for direct additive genetic variance (σ^2_a) and direct heritability (h_a^2) compared with other models. It is agreement with M.R. Bahreini Behzadi et al. (2007) who reported high heritability's for birth (0.62 ± 0.07) and weaning weights (0.59 ± 0.08) in Kermani sheep using model with direct additive genetic effect as the only random effect. K. Meyer (1992) showed that models not accounting for maternal genetic effects could results in substantially higher estimates of additive direst genetic variance and, therefore, higher estimates of h_a^2. If maternal effects are present, but not considered, the estimate of additive genetic variance will include at least part of the maternal variance.

With Models 2 and 3, the addition of maternal additive genetic and herd effects, reduced the estimates of both σ^2_a and h_a^2, compared with Model 1. Therefore, estimates of direct heritability will decrease when maternal and herd effects are included. Model 3, which included a herd effect, showed smaller estimates of σ^2_a and h_a^2, than did Models 1 and 2. The herd effect was determined to be more important than maternal genetic effect for all traits of the Latvian dark-head lambs. It was determined that on the basis of the log likelihood ratio test results, Model 3 was the most appropriate model for all traits.

Heritability is one important component used to predict genetic progress from selection to improve a trait. Using the most appropriate model, direct heritability's of birth, weaning and yearling weights were estimated 0.26 ± 0.01, 0.29 ± 0.63 and 0.29 ± 2.63, respectively. This heritability's are in the range of those presented by I. Komlosi (2008) for weaning weight in Texel sheep, M.R. Bahreini Behzadi et al. (2007) in Kermani for weaning weight, F. Ghafouri-Kesbi and H. Baneh (2012) in Makooei for birth and weaning weights. The estimates were higher than those reported by M.M. Tariq et al. (2010) in Mengali for weaning weight, P. Akhtar et al. (2008) in Hissardale for yearling weight, S. Savar-Sofla et al. (2011) in Moghani for birth and weaning weights, M.R. Bahreini Behzadi et al. (2007) in Kermani for birth and yearling weights, K. Mohammadi, A. Aghaei et al. (2010a) in Arabi for birth and weaning weights. Estimates of the present study were lower than those of M.M. Tariq et al. (2010) in Mengali for birth weight, H.G. El-Awady et al. (2011) in Egyptian Rahmani for birth weight. The large standard errors associated with the heritability estimates for yearling weight are possible the results of the smaller sample size used in this study.

For all traits, estimates of maternal heritability were lower than the estimates of direct heritability. Using the most appropriate model, maternal heritability's of birth, weaning and yearling weights were estimated 0.14 ± 0.00, 0.04 ± 0.19 and 0.04 ± 0.94, respectively. Maternal heritability decreased with age, because maternal effects in mammals are substantial in young animals, but diminish with age (Robison, 1981). This heritability's are in the range of those presented by K. Mohammadi, A. Aghaei et al. (2010a) for birth weight in Arabi, S. Savar-Sofla et al. (2011) in Moghani for birth weight, F. Ghafouri-Kesbi and H. Baneh (2012) in Makooei for weaning weight. Estimates of the present study were lower than those of M.R. Bahreini Behzadi et al. (2007) in Kermani for birth, weaning and yearling weights, K. Mohammadi, A. Aghaei et al. (2010a) in Arabi for weaning weight, S. Savar-Sofla et al. (2011) in Moghani for weaning weight, H.G. El-Awady et al. (2011) in Egyptian Rahmani for birth weight.

Conclusions

Heritability of growth traits ranged from moderate (0.26 to 0.29 for Model 3) to high (0.50 to 0.74 for Model 2 and 0.63 to 0.77 for Model 1) based on different models. The results of the present study showed that the addition of maternal and herd effects to the model resulted in a decrease in the estimates for direct heritability for all growth traits of the Latvian dark-head lambs. The results showed a strong herd influence possible due to different situation in Latvia because of quite small herds and rams are used only in one herd. Therefore, maternal and herd effects are significant sources of variation of growth traits and ignoring these effects in the model would cause overestimation of direct heritability and inaccurate genetic evaluation of lambs.

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ANALYSIS OF CONFORMATION OF FORELEGS AND HIND LEGS OF LATVIAN WARMBLOOD CARRIAGE TYPE MARES

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Abstract
The aim of the study was to analyze an occurence of forelimb and hind limb conformation traits in the population of the Latvian warmblood carriage type broodmares accepted as appropriate for the breed's genetic resources and an occurence of this traits depending on the origin. The conformation traits of limbs were analyzed in the population of the Latvian warmblood carriage types broodmares accepted as appropriate for the genetic resources from 2004 to 2012 and registered in the Stud Book, the group consisted of 301 mare of which 104 mares had a description of the conformation in Stud Book or database. Based on common female ancestors the broodmares were divided in families, recognized as important for improving the breed, and other related groups. The quality of limbs in the population of broodmares included in genetic resources was compared to the quality of limbs in the population of their female ancestors. Good limb conformation was characteristic to 28.8% of broodmares. The most common conformation faults were toeing-in of forelegs and base-narrow position of forelimbs and hind limbs. The occurence of conformation faults as sloping pasterns of forelegs and hind legs and sickle-hocks was rarer in nowaday population than in population of female ancestors. The occurence of toeing-in of forelegs increased from 9.7% to19.2%, a significant difference between contemporary population and ancestors was found ($p < 0.05$). A significant difference in limb quality between groups with different origins was not found.
Key words: genetic resources, broodmares, limbs, female families, conformation faults.

Introduction

The Latvian warmblood horse breed is divided in a sport type and carriage type. The development of sport type is based on breeding of horses suitable for show jumping and dressage. There are stallions of related breeds widely used to reach a breeding aim. Preservation of carriage type started in 2004 based on determination of preserving genetic resources of livestock, the breeding programme of carriage type horses was worked out. A steady temperament, an easiness of handling and a strong body conformation, suitability for tourism and driving are representative features of carriage type horses (Rozitis et al., 2008). The horses accepted as appropriate for the breed's genetic resources must conform to several criteria, an origin of horse is the most important one.

Performance is the basic horse productivity. Quality, durability and efficiency of performance are closely related to conformation of limbs. Conformation is a physical appearance of an animal due to the arrangement of muscles, bones and other tissue. Conformation is determined as the most important, second or third major selection criteria in breeding programmes of almost all breeding organisations of warmblood horses (Koenen et al., 2004). Conformation influences reliability of horse limbs and quality of gaits and lameness frequently occur due to a less than ideal joint and limb angulation (Dyson, 2000; Laizans, 2012). A special attention is paid to quality of limbs in valuation of horse conformation. A methodology of horse valuation at Breeding programme of the Latvian warmblood horses schedules seven conformation criteria, three of them are connected with the quality of limb conformation – forelegs, hind legs and correctness of movement. Each criteria should be valuated in ten point scale. There was no detailed description of conformation included in evaluation methodology for more than ten years without a possibility to determine the quality of conformation of each animal and the whole population. The evaluation of limbs in ten point scale hids many parameters as faults and advisable conformation traits, and it is also subjective due to various experts (Orbidane and Jonkus, 2013). The evaluation form, worked out in 2010, defines a recording of every conformation trait. Similar description of conformation can be obtained using linear evaluation score (Gordon et al., 2012).

A period of the past two decades in Latvia was characterized by horse breeding in private property in contrast to collective farms where united breeding aims for all of them were carried out. Consequently, a selection of broodmares was carried out based on owners' preference and knowledge without external control and export of the best broodmares led to production of large number of horses with low conformation quality. Currently, Latvian Horse Breeding Association with the help of a special prize and support payments, promotes breeders to include the best mares to stud herd.

The aim of the study was to analyze an occurence of forelimb and hind limb conformation traits in the population of the Latvian warmblood carriage type broodmares accepted as appropriate for the breed's genetic resources and an occurence of these traits depending on the origin.

Materials and Methods

An occurence of conformation traits of forelimbs and hind limbs was analyzed in a population of the Latvian warmblood horse breed broodmares accepted as appropriate for the breed's genetic resources from 2004 to 2012 and registered in the Stud Book and their female ancestors.

Information about accepted broodmares was received from databases of Agricultural Data Centre and Latvian Horse Breeding Association. There was pedigree information of each mare determined up to the last known female ancestor bred in Latvia. The information about pedigree of broodmares and their conformation traits were found out from Stud Book and public horse database of Latvian Horse Breeding Association, available at: www.lwhorse.lv.

The Latvian breed broodmares are evaluated in accordance with the Breeding programme from 2010 to 2015 of the Latvian warmblood horses or previous horse evaluation instructions.

Conformation traits of forelimbs and hind limbs of all mares were found out in the pedigree of broodmares accepted as appropriate for the breed's genetic resources. Mares without conformation description in their pedigree were not included in the study. In cases when limb conformation traits were not mentioned in common conformation's description or only positive conformation traits were mentioned, a record 'broodmare without conformation faults' was registered. An expression of trait was not recorded, except development level (good, moderate, low) of flexor tendons and ties, knees, hock joints, cannons and pasterns.

The occurence of conformation traits (undesirable or desirable) of forelimbs and hind limbs was analyzed to:

1) population of the Latvian warmblood horse breed broodmares accepted as appropriate for the breed's genetic resources from 2004 to 2012, registered in the Stud Book and had recorded description of conformation traits (n = 104);
2) population of broodmares' female ancestors (n = 585);
3) common population that consists of broodmares accepted as appropriate for the breed's genetic resources, registered in the Stud Book and all their female ancestors with recorded description of conformation traits (n = 689);
4) broodmare families, recognized as important for improving the breed (8 families);
5) other related groups with common origin from mother side (8 groups).

The comparison between current population (n = 104) and population of broodmares' female ancestors (n = 585) and between groups with different origins from mother side was carried out.

The statistical analysis was performed using IBM SPSS Statistics 20. The data were analyzed using nonparametric nominal data descriptive statistic method Crosstabs. The significance of the differences between the samples was assessed using Chi-square ($p < 0.05$).

Results and Discussion

There are 301 Latvian warmblood horse breed broodmare accepted as appropriate for the breed's genetic resources from 2004 to 2012 and registered in the Stud Book; however, only 104 mares from 301 had recorded description of conformation traits in population of the Latvian warmblood horse breed broodmares accepted as appropriate for the breed's genetic resources.

Good limb conformation without faults was detected to 28.8% of broodmares. Correct position of forelegs was found out to 13.5% of mares, correct postition of hind legs – 15.4% of mares. The most typical conformation fault of limbs was toeing-in of forelegs that occurred in 19.2% of broodmares while toeing-in of hind legs was recorded to only 2.8% of mares. An occurence of limb conformation faults are shown in Table 1. As it is seen, the base-narrow position of forelimbs and hind limbs was a characteristic undesirable trait of population. Several conformation faults of limbs as sickle-hocks, sloping pastern and toeing-out of hind legs, low or moderate development of knees, hocks and cannons occurred in population very frequently.

Comparison of conformation traits of broodmares accepted for the breed's genetic resources and their female ancestors shows that conformation faults were registered for 71.2% of broodmares and 67.4% of ancestors, a significant difference between groups was not found. The occurence of many traits was similar in both groups. Smaller number of mares had recorded devolopment level of flexor tendons and ties, good, moderate or low, while description of knees, hock joints and cannons in population of broodmares accepted for the breed's genetic resources was mentioned more often than in the population of their ancestors. The occurence of base-narrow position of forelimbs was more frequent at present time population; nevertheless, the base-narrow position of hind limbs was rarer characteristic in comparision with ancestors. The occurence of conformation faults as a sloping pastern of forelegs and hind legs, and sickle-hocks was rarer than in the population of female ancestors. The occurence of toeing-in of forelegs was more frequent (19.2% in current population and 9.7% in female ancestors), a significant difference between groups was found ($p < 0.05$). There was an incresed

occurence of mares with toeing-out of hind limbs in population.

Grouping of broodmares into historical female families (Rozitis, 1989) and determination of occurence of main characteric limb conformation traits was carried out. In general, 44 of mares accepted for the breed's genetic resources represented 8 broodmare families historically recognized as important for improving the breed (Table 2).

Other mares had varied origin. Current population of mares accepted for the breed's genetic resources consisted of progeny both of imported broodmares that were included in the first volumes of the Stud Book nevertheless formed their own female families, both of crossbreed mares with unknown native origin, in next generations improved by qualitative stallions. A part of broodmares had common female ancestors. Broodmares were divided in related groups, based on common, a first known female ancestor. The analysis was carried out for 8 related groups with a number of broodmares accepted for the breed's genetic resources not smaller than 4 and enough female animals with recorded description of conformation traits. Analysed groups had at least two branches that were developing from progenitress.

The female family of dam Luna Lb 238 (Балтакменс, 1988) was most represented, it continued by a branch of mare Laimrota Lb 659 with 7 broodmares. Laimrota was accepted as an important dam in breeding of carriage type. A number of broodmares in genetic resources – 7, female animals with recorded description of conformation traits – 17.

Related group of dam Aida L 2346, born in 1971, from Ugmis Lb 575 (Siego Old 66 sire line) and crossbreed mare Ausma LK 21580 with half-known pedigree, was relatively small in genetic resources

Table 1

The occurence of forelimb and hind limb conformation faults in population of the Latvian warmblood carriage type broodmares

Conformation fault	Occurence, %		
	population of broodmares in genetic resources n = 104	population of female ancestors n = 585	P-value*
Base-narrow position of forelimbs	9.6	6.5	0.071
Base-narrow position of hind limbs	14.4	16.2	0.382
Base-wide position of forelimbs	1.0	0.3	0.388
Base-wide position of hind limbs	0	0.2	0.849
Cow-hocked hind limbs	1.9	2.2	0.600
Bow-legged hind limbs	4.8	2.9	0.228
Long pasterns of forelimbs	0	1.4	0.268
Long pasterns of hind limbs	0	1.2	0.316
Short pasterns of forelimbs	3.8	0.7	0.021
Short pasterns of hind limbs	0	0.5	0.612
Sloping pasterns of forelimbs	1.9	6.0	0.062
Sloping pasterns of hind limbs	4.8	7.4	0.239
Upright pasterns of forelimbs	3.8	2.1	0.211
Upright pasterns of hind limbs	1.9	2.1	0.644
Synovitis of hock joints	2.9	3.4	0.533
Sickle-hocks	5.8	10.3	0.101
Moderate or low development of knees	5.8	0.9	0.003
Moderate or low development of hock joints	6.7	1.4	0.003
Moderate or low development of cannons	5.8	1.7	0.023
Moderate development of flexor tendons and ties	2.9	9.7	0.011
Toeing-in of forelimbs	19.2	9.7	0.006
Toeing-in of hind limbs	2.9	2.6	0.527
Toeing-out of forelimbs	2.9	3.8	0.463
Toeing-out of hind limbs	5.8	2.1	0.041

* $p < 0.05$

(n = 5). The group continued with three daughters of Aida, one of them included in genetic resources, while from others – their daughers. There was no good quality of limbs detected in this related group, most common conformation trait was toeing-in of forelimbs.

Related group of dam Faza Lb 1280 from Burtnieki stud farm was represented by 7 broodmares. Faza was a daughter of imported dam Anila (from Asterios) and Hanoverian breed stallion Fausts from Flingart sire line.

Other related group of Burtnieki stud farm started from Vizma L 2625. Vizma was a daugther of sire Grasis L 812 (Juveels Old 49 line) and Vigra L 1683 from Gaitis L 780 (Gotenfirsts Lsb 22 sire line). Vizma was born in 1976 and left three daughters in the stud farms breeding herd. The occurence of conformation traits showed that mares from this group had good conformation of limbs, a correct limb stand and no characteristic faults. Sloping pasterns only of hind limbs were mentioned.

Related group of crossbreed dam Eksa Lk 21275, a daughter of stallion Grundulis Lb 371 (Gotenfirsts Lsb 220 sire line), also excelled with good limb quality. Eksa produced an offspring of a high quality, referable to a sport type. Her son Daigirs L966 was the best show jumping horse in Latvia in 1984. Two branches from daughters Dina Lk 21140 and Ingoleta Lk 22147 continued the group, of which Dina's offspring had better quality of limbs.

Aida without a Stud Book number from sire Palejs Old 84 developed a related group with 7 mares in genetic resources and 14 mares with recorded description of conformation traits.

Related group of dam Sacere L 1969 developed in the stud farm of collective farm 'Viesturi'. Sacere, a daughter of a champion of the breed Sargs Lb 341 and broodmare Ciga Lbk 20800, increased the quality of stud farm's herd considerably.

An offspring of crossbreed dam Cilla Lbk 6160 was represented in genetic resources more widely (n = 10), female animals with recorded description of conformation traits – 12. Cilla's daughter Fata Lb 806 developed her own stud farm's family. Most known dam from this family was Unce L 1844 (daugther of Flagmanis L 703, founder of sport type sire line), a champion of the breed in 1980.

Female animals without conformation faults were 32.1% in common population. The family of Astra excelled with a good limb conformation, respectively

Table 2

Charasteristic limb conformation traits of female families in Latvian warmblood horse breed

Female family	Mares without conformation faults, %	Charasteristic limb conformation traits
Jula Angp 368	33	Base-narrow position of hind limbs, toeing-in of forelimbs, sickle-hocks.
Zenda Old 12 – Fata Old 16	33	Correct position, toeing-in of forelimbs, moderate development of flexor tendons and ties.
Briva Old 105 – Norma Lsbk 749	50	Base-narrow position of hind limbs, good development of flexor tendons and ties.
Laima Lsb 26	23	Correct position of forelimbs, base-narrow position of hind limbs, sloping pasterns of forelimbs and hind limbs, sickle-hocks, toeing-in of hind limbs, moderate development of flexor tendons and ties, flat feet.
Laumute Lsb 30	39	Toeing-in of forelimbs and hind limbs, base-narrow position of hind limbs, sickle-hocks.
Skaidrite Lsb 68	25	Correct position of forelimbs and hind limbs, base-narrow position of hind limbs, sloping pasterns, moderate development of flexor tendons and ties.
Astra Lb 532	54	Base-narrow position of hind limbs, good development of limbs.
Arta Lb 634	25	Correct position of hind limbs, good development of flexor tendons and ties, toeing-out or toeing-in of forelimbs.

more than half of mares was without pronounced faults of conformation of forelegs and hind legs (Table 2). There was frequent (more than 80%) occurence of faults in related groups of Aida from Palejs, Aida L 2346, Faza and Sacere. The occurence of faults in the family of Luna was even 88%.

Families of Eksa (44%), Vizma (30%) and Skaidrite (25%) were characterised by correct position of forelimbs. The occurence of correct position of hind limbs was 16%. This trait was often recorded to mares in families of Eksa (44%), Skaidrite and Arta (25%).

Good development of flexor tendons and ties was recorded to 6.8% of mares. This trait was characteristic to the related group of Cilla (23%).

Base-narrow position of hind limbs occurred in 16% of population and was most common limb conformation fault. This limb position was often recorded to evaluation of mares of several families - Skaidrite (33%), Briva-Norma (25%), Astra (23%) and Laima (20%). Base-wide position of forelimbs and hind limbs, also cow-hocked and bow-legged hind limbs are not typical for the population of Latvian warmblood carriage type broodmares.

Long pasterns of forelimbs was characteristic only for the related group of Cilla (15%), this trait was less frequent than too upright pasterns. Horse conformation studies found out a revelance between upright pasterns and sustaining synovitis (McIlwraith et al., 2003). Only related group of Sacere had to be characterized by occurence of synovitis of hock joints (30% of mares).

Sloping pasterns of forelimbs and hind limbs were recorded in families of Laima (13% and 10%) and Skaidrite (17% and 25%), sloping pasterns of hind limbs - in related group of Vizma (20%).

The occurence of sickle-hocks in the population was 9.6%. This conformation fault of hind limbs was not recorded in several families - Skaidrite, Astra, Arta and Vizma. Sickle-hocks were typical traits in the families of Jula (17%), Laumute (14%) and Eksa (22%). Too straight hock joints were not detected in the population of carriage type mares, both in populations of genetic resources and female ancestors.

Moderate or low development level of knees, hock joints, pasterns and cannons were recorded for a small number of mares. Insufficient development of cannons was clarified as a typical fault of the related group of Faza (29%).

The occurence of faults of forelimb knees (back-at-the-knee and over-at-the-knee) was extremely rare. Two cases of buck-kneed forelimbs from five in common population were recorded in the family of Laumute (7%).

Toeing-in of forelimbs was one of the most widely occurred limb fault in the population (11%). The occurence of this trait was 29% in the group of Aida L 2346, 26.7% - in the group of Aida from Palejs, 21% in the family of Laumute, 22.2% - in families of Jula and Zenda-Fata, 20% - in the group of Sacere. Toeing-in was not recorded in the families of Briva-Norma, Skaidrite, Eksa and Vizma. Toeing-in of hind limbs was less typical for the population, most frequent occurence between families was determined to the family of Laumute.

Toeing-out had rarer occurence in the population. It was recorded to mares in the families of Arta (17%) and Laima (7%) more frequently.

An analysis of development level of feet and hoofs showed that club feet almost did not exist in the population. Flat feet were characteristic traits of mares in the family of Laima (10%). Other faults of feet and hoofs were very rare or non existent.

A rare occurence of flawed and crisp hoofs was detected. In general, only 1.3% of mares had crisp hoofs, though this trait was recorded to mares in the family of Luna (17%).

A relatively large number of mares had moderate development of flexor tendons and ties (8.7%), low development – only 1.0% of mares. A comparison between groups showed moderate development of flexor tendons and ties in the families of Skaidrite (25%), Zenda-Fata (22%), Aida L 2346 (29%) and Luna (18%). A significant difference in limb quality between Latvian warmblood horse breed carriage type broodmares' groups with different origins was not found.

In1988, Baltakmens reported that improvement of Latvian breed horse limbs was on its way. The horses evaluated in 1980-s had such conformation faults as weakness of ties, toeing-in or toeing-out, cow-hocked and bow-legged hind limbs and sickle-hocks less expressed than horses evaluated in 1948. Incorrect toeing was characteristic for 21.8% of mares in 1948 and 15.0% in 1985, sloping pasterns of forelegs and hind legs – 13.4% and 19.1% - on 1948 and 5.2% and 8.5% in 1985. The occurence of flat feet decreased from 9.1% to 2.0%, cow-hocked hind limbs – from 26.2% to 1.1%, bow-legged hind limbs – from 15.0% to 3.7%, sickle-hocks – from 54.3% to 14.2% in 1985 (Балтакменс, 1988). The analyzed group from all female ancestors collected evaluation data from those periods. In comparison, the broodmares from genetic resources showed higher occurence of incorrect toeing than mares in 1985. The analysis of conformation faults in 1960-s (Stikans, 1970) detected that toeing-out was most frequent fault, but toeing-in occurred rarer (35.7% and 20.2%). Baltakmens R. (1988) pointed out that toeing-in was not considered as a conformation fault for carriage type horses, also sickle-hocks combined with good development of flexor tendons and ties might be considered as an advisable conformation trait for show jumping and

carring heavy loads. Altought several conformation faults were less recorded, we detected occurence of sloping pasterns and sickle-hocks in the population of broodmares from genetic resources noticeably rarer than it was registered in 1985.

Conclusions

1. A few limb conformation faults were observed. The most typical conformation faults of limbs in the population of the Latvian warmblood carriage type broodmares were toeing-in of forelimbs and base-narrow position of forelimbs and hind limbs. Limb faults were not observed to 28.8% of broodmares, part of population had correct limb position. Such abnormalities as base-wide limb position, incorrect hoofs and feet, back-at-the-knee and over-at-the-knee, 'tied in' cannons below knee, too straight hock joints were observed very rarely. Comparison of present day broodmares included in genetic resources with their female ancestors indicated that the occurence of several faults such as sloping pasterns of forelimbs, hind limbs and sickle-hocks were significantly rarer in the population of genetic resources, while the occurence of toeing-in of forelegs increased from 9.7% to19.2%.
2. A significant difference in the limb quality was not found among the groups with different origins. The number of broodmares in each group was too small to detect the most typical limb conformation traits in present day families of carriage type mares.

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SENSORY EVALUATION OF NEW BEAN SPREADS FOR VEGETARIANS

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Abstract
Vegetarianism is a growing trend in Latvia but there is a lack of spread-like products for vegetarians. There are about 10 plant protein spreads commercially available in Latvia that differ very much in nutritional value and ingredients. Common beans (*Phaseolus vulgaris* L.) which are popular among Latvian consumers and rich in important macro- and micronutrients could be a great source of protein for vegetarians in spread-like products, however, are not represented in foreign or Latvian food company products yet. The aim of this research is to develop new vegetarian spreads using commercially available beans in Latvia and to subject the newly developed bean spreads to sensory evaluation. Four bean spreads were developed using white beans: classic, with basil, with curry, and with sun-dried tomatoes. Samples of bean spreads were packed in 200 g polypropylene (PP) containers and after 12 h storage in a refrigerator (3 ± 1 °C) subjected to sensory evaluation. Sensory evaluation was carried out in 3 different groups of panellists using hedonic scale and line scale. The hedonic evaluation showed that bean spread with sun-dried tomatoes has the highest overall preference compared to other bean spreads ($p<0.05$). Significant differences among four bean spread samples in the intensity of their sensory properties – acidity, bean flavour, saltiness, and colour – were found ($p<0.05$). Based on sensory evaluation data further research should be continued with classic bean spread and bean spread with sun-dried tomatoes.
Key words: vegetarianism, beans, bean spreads, sensory evaluation.

Introduction

Vegetarianism is the practice of abstaining from the consumption of meat – red meat, poultry, seafood and the flesh of any other animal; it may also include abstention from by-products of animal slaughter, such as animal-derived rennet, gelatine, and animal fat. Vegetarianism can be adopted for different reasons: health-related, religious, ethical, political, environmental, cultural, aesthetic or economic (Craig, 2010; Marsh et al., 2012). The number of people turning to vegetarianism is increasing every year. According to the latest estimates about 3 to 5% of Latvian population identify themselves as vegetarians (Mazlovskis, 2012). A properly planned vegetarian diet is healthful, nutritionally adequate, and provides health benefits in the prevention and treatment for such diseases as diabetes, cancer and coronary heart disease (McEvoy et al., 2012; Rizzo et al., 2011).

European Food Declaration of the New Common Food and Agriculture Policy points out the need to promote healthy eating patterns, moving towards plant-based diets and a reduced consumption of meat, energy-dense and highly processed foods, and saturated fats, while respecting the regional cultural dietary habits and traditions. Over recent years the impact of meat in our diets has been studied. The main problem appears to be that the modern meat-eaters diet includes a greater proportion of meat than that of our ancestors. This increase can cause health problems for a variety of reasons. Vegetarian diets offer lower levels of saturated fat, cholesterol and animal protein, and higher levels of carbohydrates, fibre, magnesium, potassium, foliate, and antioxidants such as vitamins C and E and phytochemicals, compared to an omnivorous diet (Craig, 2010; Sabate and Blix, 2001).

Legumes (*Leguminosae*) are the most important source of protein for vegetarians (Messina et al., 2004). Common beans (*Phaseolus vulgaris* L.) are the most significant legume in human nutrition, accounting for more than 90% of the world's total bean production. Common beans are a rich and fairly inexpensive source of protein, carbohydrates, dietary fibre, minerals and vitamins, especially iron, potassium, selenium, thiamine, pyridoxine, and folic acid (Fageria et al., 2010; Gepts, 2008). When compared to other sources of protein e.g. meat (Van Heerden et al., 2004) beans are reasonably cheap (Osorio-Diaz et al., 2003), and easy to store with a longer shelf life than most fruits, vegetables and animal products (Sathe et al., 2003). Legumes play a key role in the acceptability of monotonous diets in many parts of the world. Beans have always been a part of the traditional Latvian diet.

One of the main objectives for vegetarians is to provide the body with optimal amounts of protein. For diversification of vegetarian diet there is a variety of plant-derived protein products available in the world: tofu, Tofurky (a vegetarian turkey replacement), soy-based vegetarian meat substitutes, peanut butter, bean and pea flour (as thickening agents for soups, stews and sauces), seitan (wheat gluten), tempeh and hummus. Meat alternative ingredients are nutritious with some offering specific health benefits. As well as increasing consumer choice, such products therefore have the potential to contribute to overall public health

(Van Roost, 2003). The main plant protein spread-like product is hummus which is very popular in Israel, Egypt, the Middle East and Mediterranean countries (Zubaida, 2001).

There are about 10 plant protein spreads commercially available in Latvia that differ very much in nutritional value and ingredients. Common beans which are popular among Latvian consumers and could be a great source of protein for vegetarians in spread-like products are not represented in foreign or Latvian food company products.

One main objective of sensory evaluation is the measurement of sensory attributes and the quantification of the influence of these attributes on consumer acceptance. Sensory attributes are directly linked to the concept of quality and thereby ultimately contribute to the success or failure of a product (Carbonell et al., 2009). Sensory attributes that influence acceptance of cooked beans and bean products are general visual appearance, texture and flavour (Ghasemlou et al., 2013; Sanzi et al., 1999).

The suitability of common beans for vegetarian food products has not been studied in Latvia, therefore, the aim of this research is to develop new vegetarian spreads using commercially available beans in Latvia and to subject the newly developed bean spreads to sensory evaluation.

Materials and Methods

Experiments were carried out at the Latvia University of Agriculture at Faculty of Food Technology and Paul Stradins Clinical University laboratories.

Preparation of bean spreads

For classic bean spread production the following materials were used: white beans (Ltd. *Voldemārs*, Kazakhstan, harvested in 2012), *Extra virgin* canola oil (Ltd. *Iecavnieks*, Latvia), 5% citric acid solution (Ltd. *Valezs*, Lithuania), drinking water, and salt (Ltd. *Voldemārs*, Latvia). Additional additives were used for other bean spread production – frozen fresh basil (local market), curry powder (Ltd. *Valezs*, Lithuania), and sun-dried tomatoes (Ltd. *Gemoss*, Turkey).

Bean spreads were prepared according to the vegetarian spread preparation technology in RL patent *Vegetarian bean spread production method* application (Kirse A., Karklina D. RL patent application No. P–13–59 with priority date 03.05.2013.). Dry white beans were soaked in water at 18 ± 2 °C for 15 h, then rinsed and boiled until tender (about 110 ± 5 minutes). Cooked beans were then grinded in a food processor and the homogeneous bean paste cooled to 60 ± 5 °C. Other ingredients were added to the bean paste; oil and salt were added at the end of mixing in the food processor. Vegetarian bean spreads were packed in 200 ± 5 g polypropylene cups and stored at 3 ± 1 °C for 12 h prior to sensory evaluation.

Vegetarian bean spreads were made using common white beans (75.0 - 89.0%), water (5.0 - 7.0%), unsaturated canola oil (4.0 - 7.0%), 5% citric acid solution (2.0 - 2.5%), basil (0.8 - 1.5%), curry powder (0.5 - 1.1%), sun-dried tomatoes (5.5 - 8.5%), and salt (0.03 - 0.08%).

Four different kinds of bean spreads were developed: classic bean spread, bean spread with basil, bean spread with curry, and bean spread with sun-dried tomatoes.

Sensory evaluation

Sensory evaluation of bean spreads for vegetarians was carried out in 3 different groups of panellists (n = 110), i.e., vegetarians (n = 50), semi-vegetarians (n = 30), and omnivores (n = 30). The average age in each group was 35 with gender distribution 80% female and 20% male. Because no significant differences between sensory evaluation results in groups were found ($p<0.05$), all results are given as the overall average.

Each panellist was served 4 samples of bean spreads in a randomized serving sequence: classic bean spread (sample A), bean spread with basil (sample B), bean spread with curry (sample C), and bean spread with sun-dried tomatoes (sample D). Classic bean spread was used as a conditioned control sample. Sensory evaluation was carried out using the nine point hedonic scale and line scale. The nine point hedonic scale was used in order to determine the degree of overall preference of the given samples (9 – extremely like and 1 – extremely dislike). The line scale was used for the intensity evaluation of sensory properties (acidity, bean flavour, saltiness, creamy texture, and colour) (ISO 4121:2003).

The obtained data processing was performed using mathematical and statistical methods with IBM SPSS Statistics 21.0 and Microsoft Excel 14 for Windows; differences among results are considered significant if p-value $<\alpha_{0.05}$. For the interpretation of the results it is assumed that $\alpha=0.05$ with 95% confidence (Næs et al., 2011). The data was analysed using analysis of variance (ANOVA) and Tukey's test.

Results and Discussion

The results of the variance analysis of new vegetarian bean spread hedonic evaluation are given in Table 1.

Table 1

Results of analysis of variance of bean spreads using hedonic scale (p<0.05)

Source of Variation	Sum of squares, SS	Degree of freedom, df	Mean squares, MS	Variance ratio, F	F critical value, F crit
Between Groups	286.44	3.00	95.48	47.72	2.63
Within Groups	872.45	436.00	2.00		
Total	1,158.89	439.00			

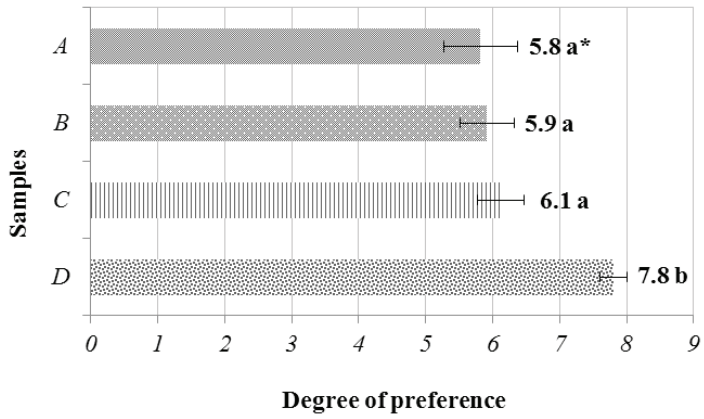

Figure 1. Results of hedonic evaluation of beans spreads.
A – classic bean spread, B – bean spread with basil, C – bean spread with curry,
D – bean spread with sun-dried tomatoes.
* – values, marked with the same letters, are not significantly different (p<0.05).

The results of the variance analysis demonstrate that $F_{cal} = 47.72 > F_{crit} = 2.63$ (n_1=3, n_2=327, α=0.05), that indicates significant differences among four samples of bean spreads in the degree of preference. Tukey's test shows which samples panellists prefer more and the ranking according to the degree of preference. The results of hedonic evaluation of bean spreads are given in Figure 1.

The overall preference of bean spread samples range from 5.8 – „like slightly" to 7.8 – „like very much". According to Tukey's test, sample D (7.8) has the highest degree of preference compared to samples A, B, and C. This assessment of bean spreads suggests that the bean spread with sun-dried tomatoes has a very pleasant taste.

Line scale was used to evaluate the most important sensory characteristics - acidity, bean flavour, saltiness, creamy texture and colour intensity. The obtained results of the variance analysis of intensity of bean spread sensory properties show that there are significant differences among acidity ($F_{cal} = 20.81 > F_{crit} = 2.63$), bean flavour ($F_{cal} = 14.34 > F_{crit} = 2.63$), saltiness ($F_{cal} = 12.16 > F_{crit} = 2.63$), and colour ($F_{cal} = 88.71 > F_{crit} = 2.63$) of four bean spread samples. No significant differences among creamy texture ($F_{cal} = 0.22 < F_{crit} = 2.63$) of four bean spread samples were found (p<0.05). Tukey's test was used to understand the differences between samples' acidity, bean flavour, saltiness and colour and their ranking according to the intensity of sensory properties.

The diagram given in Figure 2 illustrates the differences between the sensory properties of bean spreads.

According to the obtained results, sample D has the most acidic taste while sample A has the lowest acidity intensity. The pronounced acidic taste in sample D can be accounted for the added sun-dried tomatoes.

The most intense bean flavour was found in sample B, followed by sample A with a less intense bean flavour. It is implied that the added basil in sample B makes the bean flavour stand out.

Panellists rated samples A and D as bean spread samples with more intense saltiness than sample B, followed by sample C. No significant difference was established between saltiness intensity of samples A and D. A stronger salt taste in sample D can be explained with the sun-dried tomato additive which has extra salty flavour. In sample A saltiness could be the only pronounced flavour other than bean flavour because no other additives were added, also basil and curry in samples B and C, respectively, reduces the

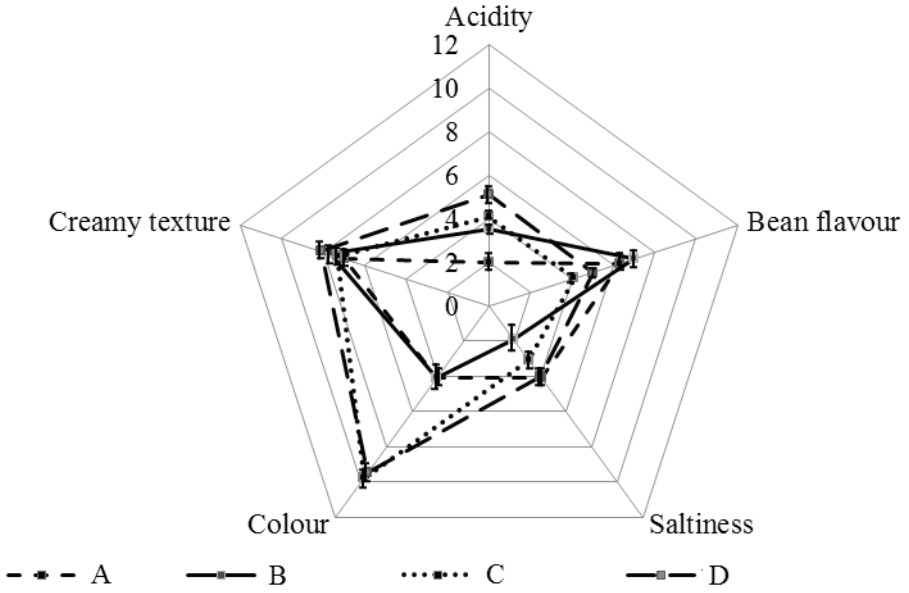

Figure 2. The assessment results of the intensity of bean spread sensory properties.
A – classic bean spread, B – bean spread with basil, C – bean spread with curry,
D – bean spread with sun-dried tomatoes.

saltiness of bean spreads because of possibly toned down sensory activity of one's palate accounted by additional solid flavours.

Consumer appetite for food is stimulated or dampened by its colour. This is because the colour of food indicates the flavour of food (Downham et al., 2000). Samples B and D were evaluated as having the most intense colour, whereas bean spread samples A and C had less intense colour. No significant differences were found between samples within these two bean spread sample pairs. Colour intensity of bean spread samples can be referred to curry powder giving sample B its intense yellow colour and sun-dried tomatoes giving sample D a soft red colour.

Acidity, saltiness and colour are possibly the main sensory attributes that influence acceptance of new bean spreads. Classic bean spread has a somewhat bland taste but it has enough saltiness so it could be satisfactory for consumers who like adding different spices to their food themselves. Bean spread with sun-dried tomatoes has a very pleasant taste and colour and is highly appreciated both by vegetarians and omnivores.

Conclusions

1. Overall preference of new bean spreads for vegetarians range from "like slightly" to "like very much" (5.8-7.8). Panellists prefer the bean spread with sun-dried tomatoes ("like very much" – 7.8) most ($p<0.05$).
2. There are significant differences among four bean spread samples in the intensity of their sensory properties: acidity, bean flavour, saltiness, and colour. No significant differences among creamy texture of four bean spread samples were found ($p\leq0.05$).
3. Based on sensory evaluation data further research should be continued with classic bean spread and bean spread sun-dried tomatoes.

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DIFFERENT TEMPERATURE TREATMENT EFFECTS ON THE CHANGES OF THE FUNCTIONAL PROPERTIES OF BEANS (*PHASEOLUS*)

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Abstract
The experiment was carried out in the Latvia University of Agriculture. The objectives of this research were to study changes of bean (*Phaseolus*) protein fractions occurring under the thermal treatment conditions and determine the critical temperature for Maillard reactions in beans. In these reactions lignified protein is made from amino acids and sugars, and it decreases the nutrition value of the food. If lignified protein exceeds 50% of crude protein content in food, then food is considered unsuitable for daily diet. In this experiment beans were milled and then heated at 50 ± 3 °C, 75 ± 3 °C, 100 ± 3 °C, 125 ± 3 °C and 150 ± 3 °C temperature in the drying oven for 20 h. As a control sample non heated beans were used, - they all were kept in a plastic jar at room temperature (20 ± 1 °C). Dry matter, crude protein, starch, sugar and lignified protein content were determined in heated beans and control samples. No significant changes of crude protein content were observed due to thermal treatment. It was from 24.3 ± 0.3 g 100 g^{-1} of dry matter. Similarly, no significant changes were observed in starch content, as it stayed averagely 47.1 ± 0.2 g 100 g^{-1} of dry matter. Medium high correlation was observed (0.64) between lignified protein content and sugar content for in different temperature treated beans. Lignified protein showed exponential growth if the samples were heated at a temperature of 100 ± 3 °C and higher, giving exponential change.
Key words: lignified protein, crude protein, sugars, Maillard reactions.

Introduction

Food supplies the human body with materials that are used in the creation of the body cells and energy generation. The food delivers substances which are needed for energy. The main structural material of the human body is protein. It is needed for body growth and new cell creation. Some proteins in the human body promote energy production processes, others help to fight against germs that cause diseases. Many products of animal origin are rich in protein, such as, milk, cottage cheese, meat, fish, eggs, as well as many plant products: peas (*Pisum sp.*), beans, cereals, bread (Shuyo and Waye, 1996; Vadivel et al., 2010).

All plants and products of animal origin contain protein, but not all of it is biologically usable for organism. It needs to contain all essential amino acids for each organism group or at least replaceable amino acids. Biologically more valuable protein is in the food of animal and microbial origin; the protein content in these products is 40-70 g 100 g^{-1}. But in the food of plant origin, the highest protein content is in canola (*Brassica*), legumes (*Fabaceae*), such as soy (*Glycine sp.*), pea (*Pisum sp.*) and bean seeds. The protein content in these products is 20-45 g 100 g^{-1} (Maillard et al., 1979; Tessier, 2010; Shuyo and Waye, 1996).

Legumes are widely used as a source of protein and carbohydrates in the human diet in many countries. Although legume seeds contain a moderately high amount of protein, mineral elements and even some of the vitamins, their use in food and feed is limited due to several antinutritional factors (Morrow, 1991), such as tannins (Reddy et al., 1985), phytic acid (Urbano et al., 2000), trypsin inhibitors (Gupta, 1987; Singh, 1988) and flatulence-causing oligosaccharides (Singh, 1988; Udensi et al., 2007). Tannins inhibit the digestibility and digestion system enzymes, thus lowering digestibility of most nutrient elements, especially protein and carbohydrates (Reddy et al., 1985) while phytic acid reduces the bioavailability of some essential minerals (Duhan et al., 1989; Poel, 1990). Besides, phytic acid is considered an antinutrient - mainly due to its ability to bind essential dietary minerals including proteins and starch, and thus reduce their bioavailability for human digestion (Phillippy, 2003). Moreover, the α-galactosides raffinose, stachyose and verbascose in legume seeds are one of the main reasons why legumes are not used in animal and human diet as much as they could be (Wang et al., 2003). It has been reported that different cooking methods improve the nutritional quality of food legumes to various extents (Chau et al., 1997; Mubarak, 2005). Improvement in protein quality of legumes after the partial removal of polyphenols by simple boiling has been documented (Singh, 1988). Z. U. Rehman et al. (2001) observed an improvement in the protein digestibility of black grams following the removal of tannins by pressure-cooking. A. Kataria et al. (1989) reported that pressure-cooking was more effective than ordinary cooking in reducing the amount of antinutrients in black grams (*Vigna mungo* (L.) Hepper) and mung beans (*Vigna radiate* (L.) R. Wilczek). The findings of S. S. Kadam et al. (1987) revealed that boiling and autoclaving in water improved the protein quality of winged beans (*Psophocarpus tetragonolobus* (L.) D.C.) because of the reduction in the level of antinutrients. C. M. F. Mbofung et al. (1999) showed that the digestibility

of starch from cowpeas was distinctly improved as a result of steam cooking, whereas, in an earlier study, Tuan and Phillips (1991) found that simple boiling improved the protein and starch digestibility of cowpeas to some extent. Still in other research different treatments are reported to drastically reduce the antinutritional content of the seeds with heating, like boiling, microwave cooking and autoclaving. And as legume seeds are proposed as ingredients in human diet, any of these conducted treatments are advised to be applied prior to their consumption to ensure their safety and quality in the food and feed (Khattab and Arntfield, 2009).

Strong heating or prolonged storage may damage the quality of protein. Then Maillard or the so called browning reactions may occur. In these reactions carbonyl group of sugars reacts with free amino group of amino acids, peptides and proteins. This process occurs with a variety of compounds, but each product can only provide the specific compounds, that ensures that each product has its own specific flavor (Belitz et al., 2009).

As a result, a complex of amino-sugar is formed, that cannot be used in metabolism of living organisms. Such products can be harmful to human health, because high temperature can accelerate the formation of phenylalanine. Phenylalanine and its metabolites can inhibit protein synthesis in the liver (Maillard et al., 1979; Tessier, 2010). Simple sugars, sugar acids, ascorbic acid and other carbonyl compounds may be subject to browning reactions at high temperatures, even in the absence of a free amino group. When the reaction is carried out in acid or alkaline solutions, it is called a caramelisation. In addition, many of the resulting reaction products are similar to those that are obtained in Maillard reactions. As nonenzymatic browning is one of the most complex reactions in food chemistry, and a number of components that can participate in these reactions gives large variable of resulting compounds, making it a multi-component mixture (Belitz et al., 2009), Maillard reactions are still hard to investigate. Maillard reactions give food the brown colour and smell. For the baking process, it is a desirable phenomenon, but for others, like storage and sterilization, these reactions are bad, and not welcome in both color and taste. Also because of the decrease of the nutritional value of product and increase of potentially toxic compounds (Linden, 1996; Nollet, 2004). These reactions result in the formation of lignified proteins and reduce the usable protein part that can be used by the body (Belitz et al., 2009).

According to Soest method, proteins are divided in usable protein and lignified or bound (insoluble) protein. It is also called acid detergent insoluble nitrogen (ADIN) and is determinate in acid detergent fibre (ADF) fraction. Insoluble protein is not available for microorganisms or higher animal digestive enzymes (Soest et al., 1979).

Insolubility of lignified protein formations depends on several factors such as temperature, humidity etc. The optimal conditions are 70% humidity and 60 °C or higher temperature. This process mainly brakes down hemicelluloses. So if the product is exposed to overheating, its nutrition value can be damaged. Lignified protein usually is less than 10% of the total crude protein content in the product, but if it is 50% or more, food practically loses its value. Thus if lignified protein content is high, it should be taken into account when calculating the nutrition value and daily diet (Maillard et al., 1979; Soest et al., 1979).

Food legumes are usually cooked either by simple boiling or in a pressure cooker. The literature is replete with reports that the boiling method improves the nutritionally quality of food legumes because of the reduction in antinutrients. However, there is scarce information in the literature about the improvement in the nutritional quality of food legumes when cooking under different cooking conditions. The cooking of food legumes is related to the heating temperature and time, initial moisture and the amount of water added during the cooking process. Therefore, the present work was undertaken to study changes of bean (*Phaseolus*) protein fractions occurring under thermal treatment conditions and to determine the critical temperature for Maillard reactions in beans.

Materials and Methods

The experiments were carried out in Latvia University of Agriculture at Agronomical Analysis Scientific Laboratory. White beans (*Phaseolus*) with high protein (more than 20 g $100g^{-1}$) content were used in experiments. The samples were prepared according to method ISO 6498:1998. Beans were milled with sieve 1 mm, and then sample was separated in 6 samples. As the control were used not heated beans, kept at room temperature (20 ± 3 °C). Petri plates were dried for two hours at 55 ± 3 °C, then cooled and weighted. Then the sample was placed on Petri plates, weighted and placed in the drying oven with ventilation for 20 h until the sample is dry (dry matter ≥85%) at 50 ± 3 °C, 75 ± 3 °C, 100 ± 3 °C, 125 ± 3 °C and 150 ± 3 °C temperature. Then cooled till the room temperature and weighed. As in the course of previous study, 20 ± 3 °C, 30 ± 3 °C, 40 ± 3 °C temperatures showed no sufficient changes in protein fractions, but the temperature more than 150 ± 3 °C was too high. All analyses were taken in four replicates each.

The dry matter content

The container with a lid was dried at 103 ± 3 °C temperature for half an hour, then cooled to the room temperature in a desiccator. The weight was measured

with an accuracy of 1 mg. 5 g of the sample weighed to the nearest 1 mg, placed in the container and put in the drying oven at 103 ± 3 °C for 4.0 ± 0.1 h. After four hours, the container lid was put on, the sample was removed from the oven and cooled in the desiccator to the room temperature. The sample was weighed with the container and absolute dry matter was calculated (ISO 6496, 1999). Absolute dry matter was used in the calculations of other parameters.

Crude protein (CP) determination by Kjeldahl method

Well milled sample was weighed as approximately 0.5 g (to the nearest 0.0001 g). The sample was transferred in a temperature resistant glass flask, then copper catalyst was added as well - 20 mL of concentrated H_2SO_4. The flask was placed in a preheated stove (420 ± 5 °C), gas vacuum suction cap was fixed on and the wet mineralization continued for 1 hour until the solution in the flask is bright and clear. The flask and digested sample is removed from the stove and allowed to cool for 15-20 min. The cooled sample was placed in a distillation unit, 50 mL of water, 80 mL of 40% NaOH were added. Ammonium was distilled in 65 mL of 3% H_3BO_3 solution. The steam distillation was carried out for 220 s, then boric acids solution was titrated with 0.2 *M* HCl (its concentration was checked with 0.1 *M* NaOH solution) till pH 4.70. As blank titration 1g of sucrose in place of sample was used, prepared in the same way as samples, and with the coefficient of 6.25 (LVS EN ISO 5983-2, 2009) It is calculated as follows: − protein contains an average of 16% nitrogen, so 100:16 = 6.25. Since individual nutrients nitrogen content ranges from 15.0 to 18.4 %, there are cases where other coefficients should be taken (Gill, 1989), but the most common is coefficient 6.25 which was used in this case, too.

Lignified protein determination

This protein fraction is determinate in the acid-washed fibre (ADF) fraction, so ADF must be determined first. Borosilicate crucible is dried for 4 hours at 103 ± 3 °C, mass is weighed to the nearest 0.1 mg. The weight of the sample is 1000 ± 2 mg. Crucible is placed in the analyzer. 100 mL of the ADF solution at room temperature and 2-4 drops of 1-octanol were added and the heater turned on. Water cooling is switched on after 5-10 min. The sample boils for 5-10 min, then boils for 60 ± 5 min. When the sample has finished boiling, the hardware glass walls are washed with less than 5 mL of ADF solution. The heater is turned off and the solution is filtered off. The sample is washed 3 times with 30 mL of hot (90-100 °C) distilled water allowing it to stay on sample for 3-5 min. After washing, crucible with the sample is transferred to the cold extraction unit and washed with 25 mL of acetone and filtered. Then crucible with the sample is put in the drying oven at 103 ± 3 °C for more than 5 hours or 130 ± 3 °C for more than 2 hours. Extraction of fats using acetone needs to be done beforehand, if the fat content of samples is more than 10% (LVS EN ISO 13906, 2008).

After determining the ADF fraction, the dried sample is transferred to a temperature resistant glass flask and proceeded the same as crude protein. Sample weight, which is used for calculation, is one that was taken before ADF determination (Undersander et al., 1993).

Polarimetric determination of starch

Method is based on a fact that disaccharides are able to turn the optical plane. To determinate starch, 2.50 ± 0.05 g of sample is transferred into a 100 mL measuring flask. Then 25 mL of 0.309 *M* HCl are added and the flask is shaken. Then another 25 mL of 0.309 *M* HCl are added. After that the flask is put in a hot water bath for 15 ± 5 min, and shaken in the process. After 15 min, 30 mL of cold water are added and the flask is cooled rapidly under running water at a temperature of 20 ± 2 °C. After cooling the flask, 5 mL of Carrez I solution are added and for 1 min shaken, then 5 ml of Carrez II solution are added and again shaken for 1 min. The flask is filled with water till the mark, shaken and filtered. If the filtrate is not clear, procedure is repeated by adding 10 mL each of Carrez solution. The polarimeter is set to 589.3 nm and measuring 200 mm cell optical rotation angle of the plane is measured (LVS EN ISO 10520:2001).

The sugar content determination by modified Bertran method

The sample preparation is made according to Anthrone method, but analyses are made with Bertrand's method. The sample is weighed to the nearest 0.001 g, placed in the volumetric flask. Approximately 30 mL water is added and the sample is put in a steam bath for 1 hour. Then 5 mL of 6% $ZnSO_4$ solution are added and the sample is left for 3 min. After that 5 mL of 3% $K_4[Fe(CN)_6]$ solution is added and the flask filled till 50 mL (ГОСТ 26176, 1991)). The sample is filtered and 10 mL of filtrate transferred into a new flask. Then 25 mL of freshly prepared Fehling's solution is added and the solution is boiled for 10 min, then centrifuged and decanted carefully discarding the excess liquid from the red/brown precipitate. The precipitate is washed with hot water and centrifuged. After the second decantation, precipitate is dissolved into a small amount of $FeNH_4(SO_4)_2$ solution in sulphuric acid. The resulting light solution is titrated with 0.1 *M* $KMnO_4$ to a slightly pink colour (Вальтер et al., 1957; ГОСТ 26176, 1991). Calibration graph is made using pure glucoses solution.

The standard deviation is calculated by the Excel function STDEV and data are processed with the

function of Data Analysis using ANOVA analyses and descriptive statistics.

Results and Discussion

In the scientific literature it is mentioned that beans contain averagely 28.3 g 100 g^{-1} (from 19.8 to 36.9 g 100 g^{-1}) of protein, and 2.3 g 100 g^{-1} (from 0.98 to 3.5 g 100 g^{-1}) of fat (Dairy One, 2012).

As shown in Figure 1 crude protein content in beans for all treatments was averagely 24.3 ± 0.3 g 100g^{-1} and data mathematical analysis shows no significant changes in crude protein content as it was expected, because crude protein content should not change under thermal treatment. (Alonso et al., 2000)

While the Figure 2 shows that bean starch content in different processing temperatures averagely is 47.1 ± 0.2 g 100 g^{-1}, the temperature does not make significant changes in starch content. Thus it can be concluded that the total starch content is not affected by the temperature changes.

Lignified protein content in the bean samples heated at different temperatures is presented in Fig. 3. Lignified protein content changes under different temperature conditions and data mathematical analysis shows significant differences for the control sample and samples heated over 125 ± 3 °C. Increment of lignified protein in beans is suspected to show even from 100-125 °C.

Comparing with control sample (20 ± 1 °C), no significant changes were observed in the samples with thermal treatment at 50 ± 3 °C, 75 ± 3 °C and 100 ± 3 °C, while the sample treated at 125 ± 3 °C had differences from the control, and the sample treated at 150 ± 3 °C temperature had significant differences ($F>F_{crit.}$) compared with the control.

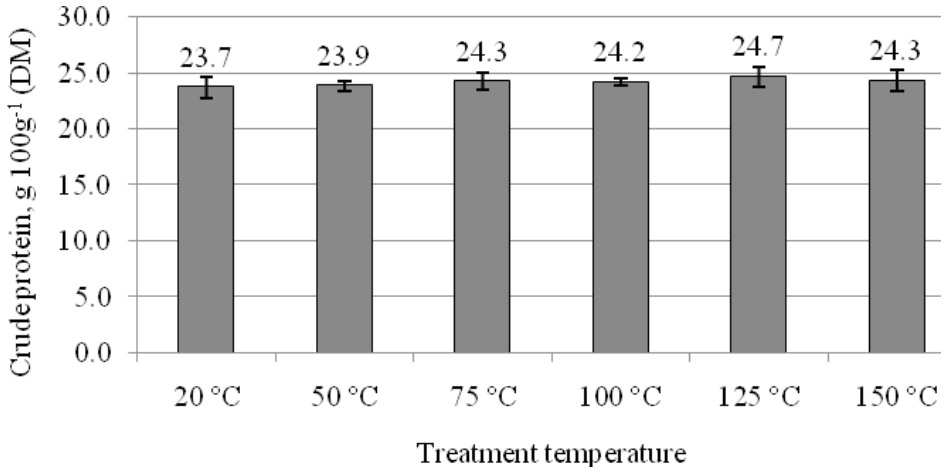

Figure 1. Total protein content in beans heated at different temperatures (20 °C control sample without heat treatment).

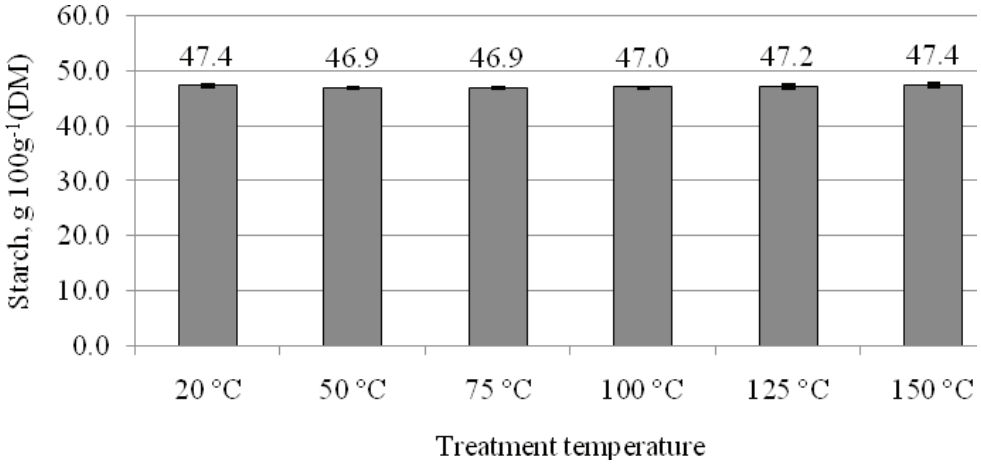

Figure 2. Starch content in beans heated at different temperatures (20 °C control sample without heat treatment).

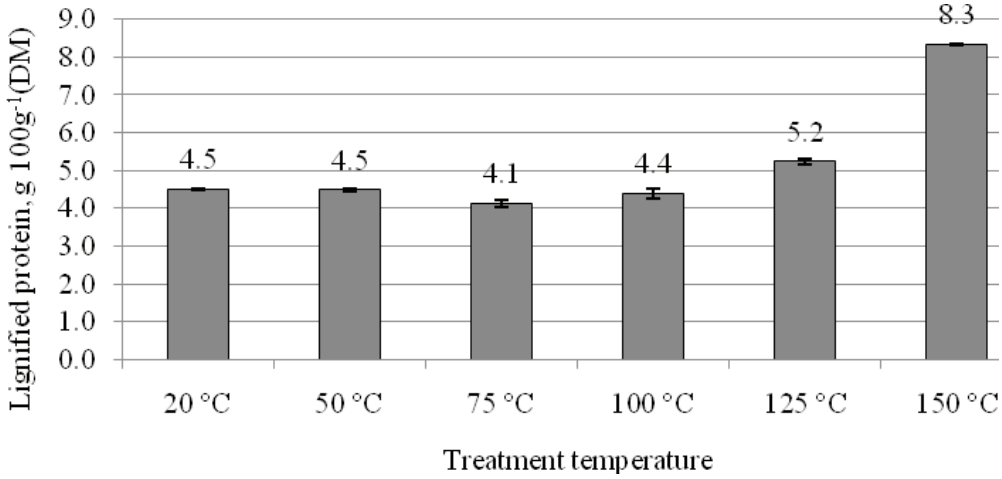

Figure 3. Lignified protein in beans heated at different temperatures
(20 °C control sample without heat treatment).

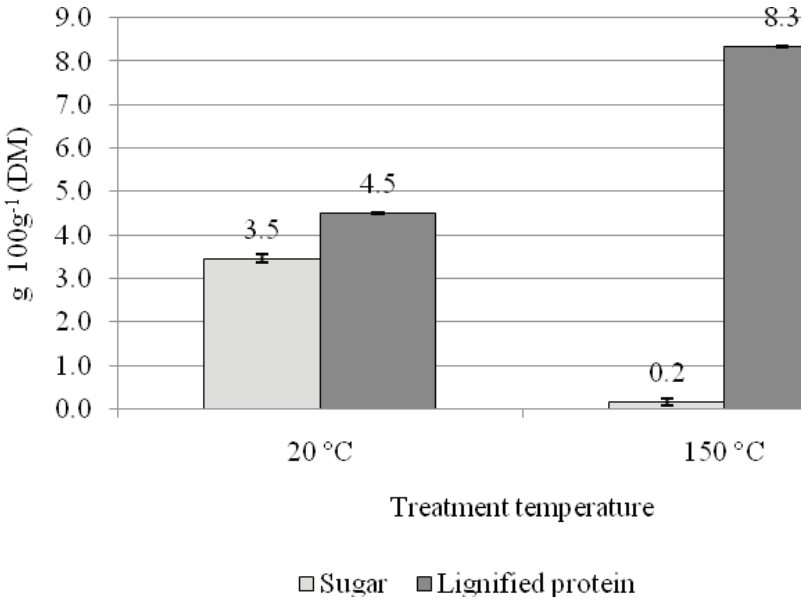

Figure 4. Lignified protein and sugar content changes at different temperatures
(20 °C control sample without heat treatment).

Lignified protein increased from 4.13 ± 0.03 g 100 g^{-1} to 8.34 ± 0.02 g 100 g^{-1}. The figure also shows that the lignified protein increase is not linear, it is the fastest between 125 ± 3 °C and 150 ± 3 °C.

Figure 4 shows the amount of reducing sugars and lignified protein. It shows that if at 20 ± 1 °C reducing sugar is traceable, then in the sample heated at 150 ± 3 °C there is almost no trace of sugars, but lignified protein content has grown substantially.

It is possible, that Maillard reactions have occurred. Increasing the heating temperature till 150 °C, 94% of sugar content is lost, while the related protein increases more than 84%.

As there is a tendency to decrease the quantity of sugar and increase the amount of lignified protein, it can be suggested that sugar loss is due to Maillard reactions, as correlation coefficient between the two variables is 0.64. It corresponds to H. D. Belitz et al. (2009) research.

Literature data (Curtis and Powers, 2010) suggests that the lignified protein is dependent not only on the amount of sugar, but also on the free amino group in the product. As an example, in rye (*Secale cereal* L.) grain asparagine concentration is higher than in beans and rape seeds (Curtis and Powers, 2010).

Conclusions

1. Lignified protein changes in beans compared with the control showed no significant changes (α=0.05) if heated till 100 ± 3 °C, but above 125 ± 3 °C – lignified protein increases exponentially. In the temperature range from 50 ± 3 °C to 150 ± 3 °C bean starch content changes are irrelevant to the control (20 ± 3 °C).
2. By increasing the processing temperature, lignified protein increases and reduced sugar content in the sample decreases, with the correlation coefficient 0.64.

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EFFECT OF HYDROGEN PEROXIDE ON THE QUALITY PARAMETERS OF SHREDDED CARROTS

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Abstract
The main purpose of the present experiments was to investigate the effect of various hydrogen peroxide (H_2O_2) concentrations and for various lengths of treatment on the total carotenoid, β–carotene content, colour intensity and microbiological safety on the fresh shredded carrots. Shredded carrots were dipped in 0.5, 1.0 and 1.5% H_2O_2 water solution for 30 ± 1s, 60 ± 1s and 90 ± 1s. Negative effect of H_2O_2 on β–carotene content and colour parameters of analyzed shredded carrots samples was not detected. In carrots treated with H_2O_2 ($p = 0.008$) for 60 – 90s the total content of carotenoids significantly decreased during treatment compared to untreated carrot samples. There was significant difference ($p<0.05$) observed between treated and non–treated shredded carrot samples on the total bacteria count. It was possible to reduce significantly ($p<0.05$) the content of yeasts and mould up to 99.99% by shredded carrots treatment with 1.5% hydrogen peroxide water solution for 30 ± 1s. In the non–processed carrots E.coli was detected; however, it was possible to destroy *E.coli* by treating carrots with 0.5% H_2O_2 water solution for 30 ± 1s. Considering all experimentally obtained results, we have concluded that fresh shredded carrots could be treated in water with the addition of hydrogen peroxide 1.5% for 30 ± 1s to maintain quality.
Key words: hydrogen peroxide, carotenoid, β–carotene, colour, microbiology.

Introduction

Carrot (*Daucus carota* L.) is among the top-ten most economically important vegetable crops in the world, in terms of both area of production and market value. Carrot improvement today includes several academic, private and government research programs around the world that work in concert with local, regional, and global industries (Prohens and Nuez, 2008). Fresh–cut carrots can be found in the market place as: whole peeled (baby), sticks, or sliced, shredded, grated and diced. There is a shelf life limitation for minimally processed carrots from 4 to 5 days due to high respiration rate, development of off–flavour, acidification, and loss of firmness, discolouration, and microbial spoilage. Shredded carrots are an increasingly popular product, but their sales are restricted due to rapid deterioration during storage (Barry-Ryan et al., 2000). Strained carrots sold in retail markets exhibit a diverse range of colour and taste characteristics. From a single processor, this variability may be influenced by differences in raw product, growing conditions, processing parameters and the degree of physiological stress. However, much of this variability is avoided by selecting carrot cultivars for desirable colour, taste and aroma characteristics (Talcott et al., 2001). Carrots have the highest β–carotene, a precursor of vitamin A, content among human foods (Hsieh and Ko, 2008). Nutritionally the most important elements in carrots are phenolic compounds, carotenoids, soluble dry matter, β–carotene, sugars and others. Carotenoids are fat soluble compounds that are associated with the lipid fractions. This class of natural pigments occurs widely in nature. Furthermore, some of them are involved in the cell communication and xanthophylls have shown to be effective as free radical scavengers (Rodríguez–Bernaldo and Costa, 2006). Carotenoids, the main pigments that are responsible for the colour of carrots, are of importance to food and nutrition due to their pro–vitamin A and antioxidant activity. ß–carotene constitutes a large portion (60 – 80%) of the carotenoids in carrots, followed by α–carotene (10 – 40%), lutein (1 – 5%) and the other minor carotenoids (0.1 – 1%) (Sun and Temelli, 2006).

The global processing and storage design to achieve high–quality minimally processed foods requires a combination of different strategies and technologies that would help reduce degradative processes in fresh–cut vegetables. In the design of new processes to obtain precut fruits and vegetables with improved nutritional and health–promoting characteristics, processing and storage technology is selected on the basis of how they improved the nutritional constituents and antioxidant characteristics of the plant products (Martín–Belloso et al., 2011). The cutting process leads to cellular damage, which coupled with increased adhesion and leakage of intracellular material, can lead to increased growth rate of spoilage microorganisms or pathogens. Minimally processed fruit and vegetables are often washed in water or water containing chemicals. The wash water can help distribute bacteria into the damaged sites on the processed fruit or vegetables (Watson et al., 2007). It is important therefore, that processors use the best practice available to supply fruit and vegetables with the minimum risk to consumers through digestion of pathogens and with as long a shelf life as possible to aid distribution channels, minimise waste and increase

profits for processors. One of the new approaches is the use of "generally recognized as safe" (GRAS) compounds due to minimal concerns about their environmental impact and low residues in the treated commodity. The US Food and Drug Administration (FDA) have published lists of GRAS compounds that can be used in many food processing applications where they have been declared safe by expert panels. Regarding the FDA list as a reference, among the chemicals used in this study, chlorine dioxide (ClO_2), hydrogen peroxide, citric acid ($C_6H_8O_7$), and ethanol (EtOH) are listed as GRAS (Generally Recognized As Safe) substances (Loredo et al., 2013; Vardar et al., 2012). The Food and Drug Administration (FDA, 2011), under the ruling 21 C.F.R. 173.315, has approved the use of hydrogen peroxide as plant protection agent in the processing of fresh fruits and vegetables (Rodrigues et al., 2012). Hydrogen peroxide (H_2O_2) is also a well studied oxidant agent (Rodrigues et al., 2012; Sahin et al., 2012; Yildiz et al., 2009), directly toxic to pathogens. Hydrogen peroxide (oxygenated water) is characterized by containing a pair of oxygen atoms (–O–O), which are highly oxidative with the release of O_2 in aqueous solutions, and this create antimicrobial activity, mainly for Gram-positive and Gram–negative bacteria, due to its capacity to generate other cytotoxic oxidizing species, such as hydroxyl radicals (Alexandre et al., 2012; Delgado et al., 2012; Demirkol et al., 2008; Loredo et al., 2013; Malik et al., 2013; Rodrigues et al., 2012; Ruelas et al., 2007; Tornuk et al., 2011). Hydrogen peroxide is a strong oxidizing agent proposed as an alternative for decontamination of fruits and vegetables due to its low toxicity and safe decomposition products (Alexandre et al., 2012; Loredo et al., 2013; Ruelas et al., 2007). Hydrogen peroxide is a stable, partially reduced form of oxygen, and its rapid turnover is characteristically mediated by enzyme action. H_2O_2 plays a dual role in plants. H_2O_2 provides a host of benefits by cleansing water from harmful substances such as spores, dead organic material and disease-causing organisms while preventing new infections from occurring. H_2O_2 is of great use in hydroponics and soilless gardening and is sometimes used for root initiation in cuttings (Khandaker et al., 2012).

Further studies on the effectiveness of hydrogen peroxide treatment on the carotenoid, β–carotene content and colour intensity of fresh shredded carrots are needed. The main purpose of the present experiments was to investigate H_2O_2 effect on the carotenoid, β–carotene content, colour intensity and microbiological safety on the fresh shredded carrots.

Materials and Methods

Experiments were carried out at the Department of Food Technology of the Latvia University of Agriculture. The object of the research was carrots *(Daucus carota* L.*)* 'Nante' cultivar carrot hybrid 'Nante/Forto' grown in Latvia and harvested in Zemgale region in the first part of October 2012 and immediately used for experiments. Meteorological data were obtained from "Latvian Environment, Geology and Meteorology Centre". Meteorological conditions of 2012 were characterised by relatively high temperatures in the first two months of the summer of 2012 in Latvia. In June and July the average monthly air temperature was + 18.2 °C. In summer of 2012 the average rainfall was 312 mm, respectively, close to optimal precipitation. The autumn of 2012 in Latvia was warm and relatively dry - it was warmer and drier than normal. The autumn temperature was 3 degrees above normal. The quantity of autumn precipitation was 61 mm (87% of normal).

Serotinous 'Nante' carrot hybrid Nante/Forto was analyzed. Shredded carrots were treated with 0.5, 1.0 and 1.5% H_2O_2 water solution for 30 ± 1s, 60 ± 1s and 90 ± 1s. The scheme of experiments is shown in Figure 1.

Preparation of hydrogen peroxide solutions - to prevent degrading of the hydrogen peroxide solutions were prepared by mixing food grade concentrated hydrogen peroxide 30 g 100 g^{-1} (Peróxidos do Brasil Ltda, Curitiba, Brazil) with sterile deionised water; solution was prepared one minute before the treatment process (Augspole et al., 2013; Delgado et al., 2012).

The total carotenoids were analyzed with spectrophotometric method (used the UV/VIS spectrophotometer Jenway 6300) at 440 nm. A sample of 1g of homogenized shredded carrots was placed in 100 ml conic flask and 30 ml of 96 g 100 g^{-1} ethanol was added. The sample was stirred on a magnetic stirrer for 15 min, then 25 mL of petrol ether were added and stirring was continued for one more hour. After 1 hour when both layers were completely divided, the top yellow layer was used for further detection of carotenoids at 440 nm. Carotene equivalent (KE) was found, using a graduation curve with $K_2Cr_2O_7$. The content of carotenoids (mg $100g^{-1}$) was calculated by equation (Kampuse et al., 2012; Biswas et al., 2011).

$$X = \frac{12.5 \times 100 \times KE}{36 \times a} \times 100 \qquad (1)$$

Where 12.5 and 36 – coefficients for relationship between $K_2Cr_2O_7$ and carotenoids;
KE – carotene equivalent by graduation curve;
a – sample weight, g.

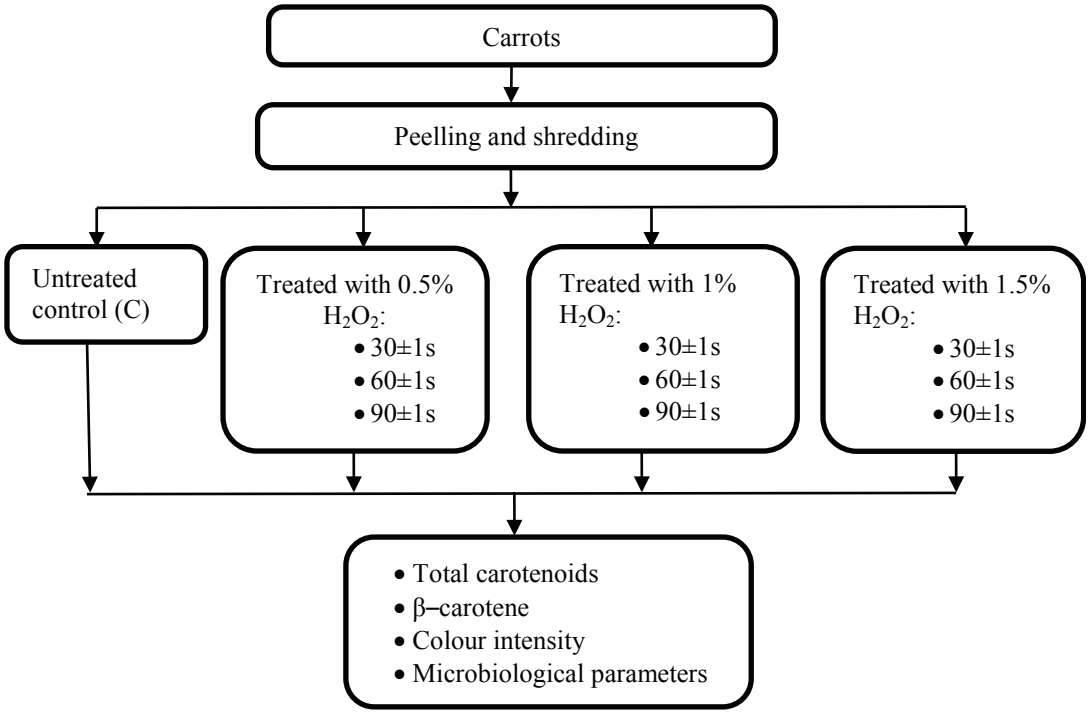

Figure 1. The scheme of experiments.

ß–carotene content was analyzed with spectrophotometric method. For extraction, a representative portion of this sample (1 g) was accurately weighed in a glass test tube. Then 5 ml of chilled acetone was added to it, and the tube was held for 15 min with occasional shaking at 4 ± 1 °C, vortex at highspeed for 10 min, and finally centrifuged at 1370×g for 10 min. Supernatant was collected into a separate test tube, and the compound was re–extracted with 5 ml of acetone followed by centrifugation once again as before. Both supernatants were pooled together and then passed through the Whatman filter paper No. 42. The absorbance of the extract was determined at 449 nm wave length in a UV–Vis spectrophotometer (Rakcejeva et al., 2012; Biswas et al., 2011). A working standard containing 32 µg mL^{-1} was prepared from the 1 mg mL^{-1} stock solutions kept at 4 °C. From this working standard different dilutions were made to spike the samples. Blank samples of 1.0 g were spiked with working standards to obtain the final concentrations 16.000, 8.000, 4.000, 2.000, 1.000, 0.500, 0.250, 0.125, 0.062, 0.031 and 0.015 µg g^{-1} of β–carotene and extracted as described previously. Calibration curves were plotted by taking Optical Density value to the respective in concentrations by back extrapolation methods. These curves were used to quantify the β–carotene content in the samples analyzed (Rakcejeva et al., 2012).

Colour of the carrot samples was evaluated by measuring CIE L*, a*, and b* parameters by means of "ColorTec–PCM/PSM" (ColorTec Associates, Clinton, USA). L*, a*, and b* indicate whiteness/darkness, redness/greenness, and blueness/yellowness values, respectively. The maximum value for L* is 100, which would be a perfect reflecting diffuser. The minimum for L* would be zero, which would be black. The values of a* and b* axes have no specific numerical limits. Positive a* is red and negative a* is green. Positive b* is yellow and negative b* is blue (Rakcejeva et al., 2012).

Samples for microbiological testing were prepared by dilution method in conformity with standard LVS EN ISO 6887-1:1999 and 6887-4:2044. TPC (total plate count) – determined in conformity with standard LVS EN ISO 4833:2003A; yeast and mould plate count – determined in conformity with standard LVS ISO 21527-2:2008. Plate counts evaluated as decimal logarithm of colony forming units (CFU) per gram of a product (log cfu g^{-1}).

Statistical analysis. The results were processed by mathematical and statistical methods. Statistics on completely randomized design were determined using the General Linear Model (GLM) procedure SPSS, version 16.00. Two–way analyses of variance ($p \leq 0.05$) were used to determine significance of differences between different samples.

Results and Discussion

Food processing has both positive and negative effects on the levels of carotenoids in food, but overall it is more evident that processing may be beneficial through disruption of matrix (cell walls) which facilitates their release and solubilisation as free or esterified/glycosylated forms in appropriate solvents, or after long heating times, leading to chemical changes (Patras et al., 2009). The results of this study indicate the needs for further insights into the carotenoid role of H_2O_2 treatment.

Colour is an important quality parameter for fresh and processed carrots (Patras et al., 2009). The colour intensity of carrots is considered a reliable indicator of higher nutritive value (Goncalves et al., 2010). The colour of shredded carrots is especially important when carrots for fresh–cut industrial use are prepared. Interesting results were obtained in relation to product colour (Table 1). It was found that colour values were retained unchanged when samples were treated with H_2O_2 solutions. The colour of carrot roots results from pigment accumulation in the shredded carrots tissue. The samples of the investigated carrots differed in L*, a* and b* colour parameters. The highest L* value, related to the lightness, was found for carrot samples of 1% H_2O_2 90 ± 1s (56.53 ± 1.04) and 1% H_2O_2 60 ± 1s (56.18 ± 1.08) (Table 1).

In the case of a* and b* parameters, related to the redness and yellowness, respectively, the highest values were obtained for 1.5% 60 ± 1s (20.41 ± 0.84) and 0.5% 90 ± 1s (46.57 ± 0.98), respectively (Table 1). The L*, a* and b* colour parameters of the carrot samples were not significantly influenced by H_2O_2 treatment compared to control samples. All colour parameters were fairly consistent. There were no significant differences found in the L* ($p = 0.058$) colour parameter, a* colour parameter ($p = 0.610$) and b* colour parameter ($p = 0.247$). Therefore it is possible to conclude, that several concentrations and treatment times of H_2O_2 do not significantly influence colour changes of fresh shredded carrots. The results obtained in the present research conform to results cited in scientific literature, namely, Watson et al. (2007) reported that no colour changes were found of carrots treated with H_2O_2 in different concentration and treating times.

Carotenoids in fresh–cut products are highly susceptible to oxidative deterioration due to the enhanced susceptibility under acute abiotic stress. In general, H_2O_2 negative impact wasn't reported on total carotenoid, β–carotene content of the shredded carrots. In the present study it was detected, that the total content of carotenoids significantly decreased in carrots treated with H_2O_2 ($p = 0.008$) comparing to untreated carrot samples. In our experiment we applied hydrogen peroxide treatment, which is the recommended form for carrot processing. It was found that the effect of different doses of hydrogen peroxide had insignificant effect on total carotenoid content accumulation in shredded carrots (Table 1). In the present research it was determined, that more advisable H_2O_2 concentration for maximal carotenoid content preservation in fresh shredded carrots 1.5% is recommendable with treating time of 30s. However, the 1.5% H_2O_2 concentration and 60 – 90s long treating time were not suitable for preservation of carotenoids. In scientific literatureit has been reported, that food processing has both positive and negative effects on the levels of carotenoids in food, but overall it is more evident that processing may be beneficial through disruption of matrix (cell walls) which facilitates their release and solubilisation

Table 1

Results of colour intensity, total carotenoids and β–carotene content for shredded carrots

Concentration of H_2O_2, %	Treatment time, s	Colour intensity			Total carotenoids, mg $100g^{-1}$	β–carotene, mg $1g^{-1}$ DW
		L*	a*	b*		
0.0	-	56.81±0.61	19.98±1.06	46.55±1.43	185.14±0.16	164.44±0.00
0.5	30±1	54.88±1.57	18.18±1.03	46.17±1.14	172.89±0.11	164.18±0.19
	60±1	52.69±1.18	17.39±1.03	41.23±1.09	167.86±0.41	155.18±0.15
	90±1	52.61±1.58	18.28±0.52	46.57±0.98	132.70±0.10	156.90±0.25
1.0	30±1	54.47±1.23	18.09±0.75	42.99±0.80	153.58±0.16	151.25±0.27
	60±1	56.18±1.08	18.42±0.76	45.35±1.28	157.05±0.20	155.36±0.17
	90±1	56.53±1.04	18.61±0.23	46.17±0.94	153.10±0.12	162.72±0.15
1.5	30±1	53.47±0.84	19.60±0.65	41.72±0.67	166.14±0.06	163.20±0.21
	60±1	55.43±0.75	20.41±0.84	35.96±0.54	132.52±0.15	156.12±0.10
	90±1	54.00±1.06	18.02±0.61	43.65±0.79	146.82±0.10	150.23±0.10

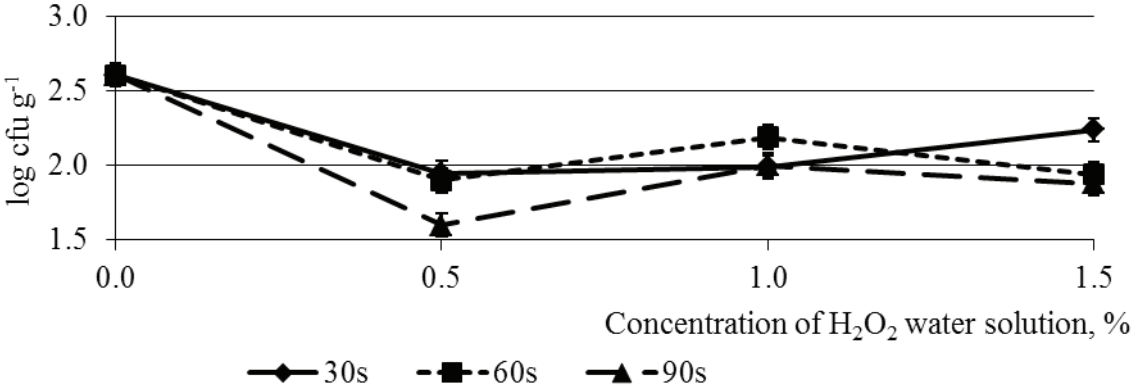

Figure 2. The dynamics of hydrogen peroxide treatment on the total bacteria count.

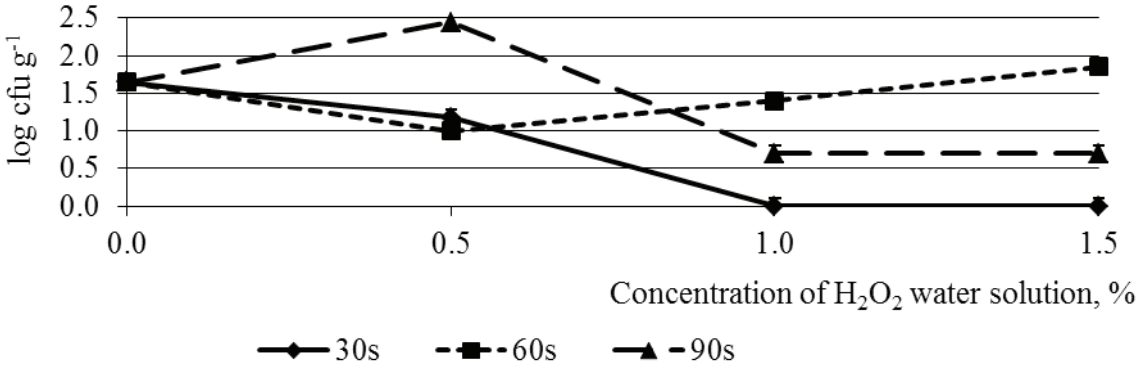

Figure 3. The dynamics of hydrogen peroxide treatment on yeasts and moulds count.

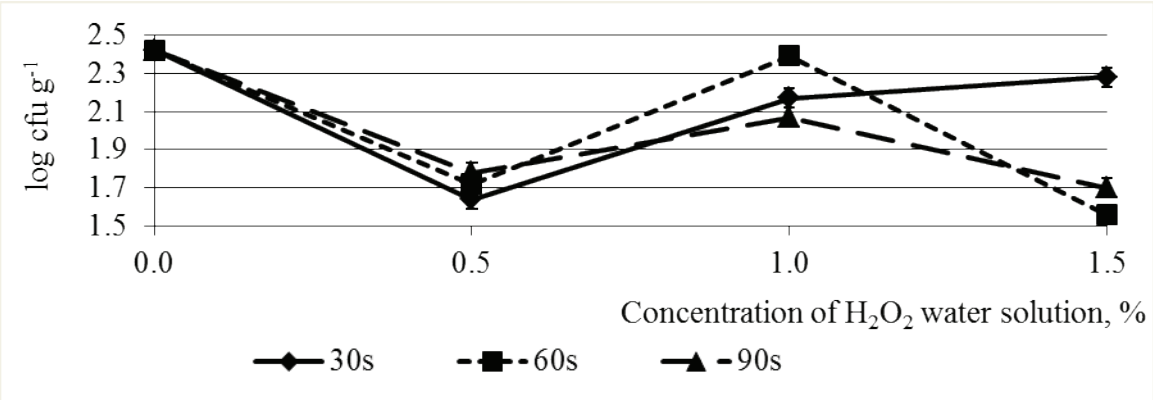

Figure 4. The dynamics of hydrogen peroxide treatment on the *E.coli*.

as free or esterified/glycosylated forms in appropriate solvents, or after long heating times, leading to chemical changes (Patras et al., 2009). However, the negative effect of H_2O_2 on β–carotene content was not observed in this study, too. There was no significant difference ($p = 0.240$) between analysed carrot samples (Table 1). The results of this study indicate the need for further insight into the total carotenoid and β–carotene role of H_2O_2 treatment.

Microbiological safety is the main quality parameter of food. Therefore for the determination of shredded carrots, the microbiological parameters were evaluated as main quality indicator. In the present research possible hydrogen peroxide disinfection functions were proved. There was significant differences ($p<0.05$) between the total mesophilic aerobic bacteria count of treated and non–treated shredded carrot samples. The present study showed, that hydrogen peroxide solutions of 0.5% and treatment time of 90 ± 1s are more effective (Figure 2).

However, it was possible significantly reduce ($p<0.05$) the content of yeasts and mould up to 99.99% by shredded carrots treatment with 1.5% hydrogen peroxide water solution by 30 ± 1s (Figure 3).

Minimally processed carrots were microbiologically spoiled by bacteria rather than yeasts and mould. It wasreported that microbial growth increased due to the increase in surface by peeling and cutting, high pH, and the moisture content of minimally processed carrots (Augspole et al., 2013).

In the non–processed carrots *E.coli* was detected, however, it was possible to destroy *E.coli* by carrot treatment with 0.5% hydrogen peroxide water solution and treatment time of 30 ± 1s. Therefore excellent microbiological effect of hydrogen peroxide was proved (Figure 4).

Conclusions

1. The negative effect of H_2O_2 on total carotenoids, β–carotene content and colour parameters of analyzed shredded carrot samples is not detected.
2. The results of physicochemical property measurements of total carotenoids, β-carotene content and colour value show that samples treated with hydrogen peroxide could be suitable for the market.
3. There is a significant difference ($p<0.05$) between the total mesophilic aerobic bacteria count of treated and non–treated shredded carrot samples. It was possible significantly reduce ($p<0.05$) the content of yeasts and moulds for up to 99.99% by shredded carrots treatment with 1.5% hydrogen peroxide water solution for 30 ± 1s. It is possible to destroy *E.coli* by treating carrots with a minimal concentration of hydrogen peroxide water solution and treatment for 30s.
4. Fresh shredded carrots treated in water with the addition of hydrogen peroxide 1.5% for 30 ± 1s can provide the maintenance of the quality.

Acknowledgements

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TOTAL POLYPHENOLS, FLAVONOIDS AND ANTIRADICAL ACTIVITY OF VEGETABLES DRIED IN CONVENTIVE AND MICROWAVE- VACUUM DRIERS

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Abstract
Fruits and vegetables are a major source of antioxidants. The aim of current research was to study and compare the antiradical activity, the total polyphenol content (TPC) and the total flavonoid content (TFC) in dried carrots (*Daucuscarrota*), pumpkins (*Cucurbita maxima)*, leeks (*Allium ampeloprasumvarporrum)* and black radish (*Raphamussativus)* using a traditional conventive drier and a microwave-vacuum drier. For each vegetable steaming as pretreatment was used. Vegetables were harvested in Latvia in 2012, gathered when ripe and then dried. Analyses were made in Latvia University of Agriculture, Faculty of Food Technology laboratories. The total polyphenol content was determined by the Folin- Ciocalteu method and the total flavonoid content - using spectrophotometric method. The antiradical activity was analyzed by the 2,2-diphenyl-1-picrilhydrazyl (DPPH) assay. The results of experiments demonstrate that the total amount of polyphenols ranged from 98.97 to 623.70 mg gallic acid equivalent (GAE) 100 g^{-1} in dry weight and the total amount of flavonoids ranged from 40.32 to 100.23 mg catechin equivalent (CE) 100 g^{-1} in dry weight. The value of DPPH antiradical activity for vegetable samples ranged from 6.10 to 45.14 percentes.
Key words: dried **v**egetables, polyphenols, flavonoids, antiradical activity.

Introduction

Vegetables are considered a traditional healty diet. They are a rich source of antioxidant content, such as phytochemicals and vitamins, as well as other minerals, and has a beneficial effect on health (Sulaiman et al., 2011). Fruits and vegetables are important sources of folacin, vitamin C, carotenoids, flavonoids (anthocyanins, flavanones, flavones, flavonols), glucosinolate, indoles, isothiocyanates, lignan, phenolic acids, plant sterols, pectin, rutin, salicylates and limonene, as well as potassium and other elements, such as magnesium, iron, manganese and copper (Pennington and Fisher, 2010)

The health benefits and nutritional characteristics of vegetables depend on the diversity of agricultural kind and growing conditions, and of the type of processing techniques used. Minimally processed vegetables (for example, drying) are a rapidly expanding segment of the fresh food industry due to the convenience and increased demand by the consumers (Siddiq et al., 2013)

Significant amounts of phenolic compounds frequently occur in foods such as fruits and vegetables and are routinely consumed in our diets. They attribute to the sensory qualities (colour, flavour, taste) of fruits, vegetables and their products (Kim et al., 2003). The number of natural polyphenols has been estimated to be over one million, because they generally occur as glycosides, and the sugar species and binding forms show a great diversity. Pre-treatment by hydrolysis produces a loss of content due to the decomposition and polymerisation of polyphenols. For example, under optical conditions, hydrolysis led to an underestimation of up to 50% of the true polyphenol level in food (Sakakibara et al., 2003)

Phenolic compounds comprise a wide variety of molecules that have a polyphenol structure, but also molecules with one phenol ring (phenolic acids and phenolic alcohols). Polyphenols are divided into several classes according to the number of phenol rings that they contain and to the structural elements that bind these rings to one other. The main groups of polyphenols are flavonoids, phenolic acids, tannins (hydrolysable and condensed), stilbenes and lignans (Ignat et al., 2011).

Enviromental factors have a major effect on polyphenol content. These factors may be soil type, sun exposure, rainfall or agronomic culture (in greenhouses or fields, biological culture, hydroponic culture, fruit yield per tree, etc). Exposure to light has a considerable effect on a large part of flavonoids. The degree of ripeness considerably affects the concentrations and proportions of various polyphenols. Storage may also affect the content of polyphenols that are easily oxidized. Oxidation reactions result in the formation of more or less polymerized substances, which lead to changes in food quality, particularly in color and organoleptic characteristics. Methods of culinary preparation also have a marker effect on the polyphenol content of foods (Manach et al., 2004)

Flavonoids are one of major phenolic compounds in vegetables and are important components of the human diet. Daily intake of flavonoids can range between 50 to 800 mg per day, depending on the consumption of vegetables and fruits (Seyoum et al., 2006). Flavonoids are widely distributed in the leaves, seeds, bark and flowers of plants. In plants, these compounds afford protection against ultraviolet radiation, pathogens and herbivores (Heim et al., 2002). They have anti-inflammatory, antidiabetic,

antifungal, antiallergic, antiviral, antioxidant and anticancer properties.

Flavonoids are categorized into following classes: flavones, flavonols, flavanones, flavonols, anthocyanidins, isoflavones and chalones. Dietary flavonoids that possess anticancer properties are also known to have antioxidant activities because of their ability to scavenge free radicals (Bennet et al., 2012).

Flavonoids are powerful antioxidants against free radicals, because they act as „radical scavengers". This activity is attributed to their hydrogen-donating ability and the activity degree depends on the structure. It is based on the ability to inhibit the hydroxyl radical and it shows the importance of double substitution also the hydroxyl groups are esterified with methyl groups (Tripoli et al., 2007).

Antioxidants are defined as compounds that can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Oxidative stress is an imbalanced state where excessive quantities of reactive oxigen and/or nitrogen species overcome endogenous antioxidant capacity, leading to oxidation of a varieties of biomacromolecules, such as enzymes, proteins, DNA and lipids. Oxidative stress is important in the development of chronic degenerative diseases including coronary heart disease, cancer and aging. Phenolic compounds have been considered powerful antioxidants and proved to be more potent antioxidants than vitamin C and E and carotenoids (Dai and Mumper, 2010).

Simple polyphenols, glycosides of flavones and flavanols are widely distributed in root vegetables and in leef vegetables. Some of these polyphenols are: chlorogenic, caffeic, ferulic and gallic acids, apigenin, luteolin, quercetin, kaempferol and myricetin glycosides (Sakakibara et al., 2003). According to J.A.T. Pennington (2010) pumpkin (*Cucurbita maxima*) and carrot (*Daucuscarrota*) are grouped as deep orange/ yellow fruits, roots and tubers; leek (*Allium ampeloprasumvarporrum*) as Allium family bulbs. In each of these groups the flavonoid content changes. According to J.A.T. Pennington (2010), leek contains 0.04 ± 0.05 mg 100 g^{-1} flavan-3-ols, 0.01 ± 0.01 mg 100 g^{-1} flavones, 16.63 ± 10.03 mg 100 g^{-1} and their antiradical activity are 1097 ± 136 µmol Trolox equivalent (all parameters estimated per 100 g fresh weight). Carrots and pumpkins contain 1.55 ± 3.41 mg anthocyanidins, 2.37 ± 3.88 mg flanav- 3-ols, 0.23 ± 0.50 mg flavones, 0.49 ± 0.61 mg flavonols and their antiradical activity can be 972 ± 547 µmol Trolox equivalent per 100 g fresh weight (Pennington et al., 2010).

Vegetables may be drier using different methods. One of the oldest methods for the preservation of food is drying, which means removing water from the product in order to provide microbiological safety. The most popular drying method includes convection. In this method the drying agent supplies heat to the material and at the same time removes moisture (in the form of water vapour) from the material. The method has the disadvantage of entailing a time- consuming process. In contact with oxygen that is present in the air, the product becomes exposed to high temperatures for a long time: such exposure reduces the content of some valuable components which readily undergo oxidation at elevated temperature. Other disadvantage of the convective method is the concomitant substantial shrinkage (Nawirska et al., 2009)

Microwave-vacuum drying is a modern, efficient method of food preservation. During microwave-vacuum drying the energy of microwave is absorbed by water located in the whole volume of the material being dried. This makes a large vapour pressure in the centre of the material, allowing rapid transfer of moisture to the surrounding vacuum and preventing structural collapse. As a result, the rate of drying is considerably higher than in the traditional methods of dehydration. The decisive factor enchancing drying rate is the wattage of microwaves. Using this method, the rapid process of dehydration creates a pourus texture food sample and stimulates obtaining a crispy and delicate texture, this way reducing the product's density as well as shrinkage (Rakcejeva et al., 2011)

The aim of the present work is to study and compare the changes of the total polyphenol and flavonoid content, and antiradical activity in dried carrot, pumpkin, leek and black radish with and without steaming pre-treatment using traditional conventive and microwave-vacuum drying methods.

Materials and Methods

Sample preparation and the drying process

Vegetables were grown and harvested in a farm in Zemgale region, Latvia, in 2012 during the vegetable season. Analyses were made in Latvia University of Agriculture, Faculty of Food Technology laboratories. Then they were washed, nonedible parts removed, peeled and cut into equal small pieces. For each drying process about 1.00 kg of sample were used. One part of vegetables was dried in a traditional conventive dryer (Memmert, Model 100- 800) at 45 °C for 48 h. Before microwave-vacuum drying all vegetables were steamed using a steam cooker (Tefal, Vitamin+, Model VC4003) and after that the samples were rapidly cooled in cool water (Turkmen et al., 2005). Carrots and pumpkins were steamed for 1.5 and 3.0 min, leek and black radish (*Raphamus sativus*) for 6, 8 and 10 min (Fig.1.). Steaming time was chosen based on vegetable hardness condition (Turkmen et al., 2005). The samples were dried using a microwave-vacuum dryer (OOO Ingredient, Russia) at 35 ºC.

The necessary amount of microwave energy was calculated using empirical formulas when the initial moisture of vegetables was known and the final was estimated (9 ± 1%) (Rakcejeva et al., 2011).

Extraction of polyphenols

Extracts were made according to S.F. Sulaiman et al. (2011) with modifications. A dried sample (3 g) was grounded using electronic grinder and then extracted with 30 mL pure acetone (to remove chlorophill) for 1 h at 18 °C. The resulting solution was filtrated; residue was soaked in 30 mL ethanol-water (1:1) mixture and extracted for 30 min at 18 °C. Solvent was filtrated and extracts were stored at 4 ± 1 °C until further analysis for 72 h. The extraction process was carried out in triplicate for each sample.

Total polyphenol content

Total polyphenols were determined according to the Folin-Ciocalteu reagent method (Pande and Akoh, 2010) with some modifications. To 500µL of extracted sample were added 2.5 ml of 0.2 N Folin-Ciocalteu reagent and 2.0 mL sodium carbonate solution (7.5 g per 100 mL). The resuling solution was mixed and allowed to stand for 30 minutes at 18 ± 1 °C in a dark place. Absorbtion was read at 760 nm using JENWAY 630 Spectrophotometer. Quantification was based on the standart curve generated with 0.120 mg mL^{-1} of gallic acid (y=10.161x + 0.0805; R^2 = 0.9986). Results were expressed as miligram gallic acid equivalent per 100 gram dry weight (mg GAE 100 g^{-1} DW).

Total flavonoid content

The total flavonoid content was determined by F.D. Olivera et al. (2008) with modificatons. To 500 µL of extracted sample were added 2.0 mL destiled water and 150 µL $NaNO_2$ (5 g per 100 mL) and left to incubate for 5 min. After that 150 µL $AlCl_3$ (10 g per 100 mL) was added and incubated for 6 min. Then 1 mL 1 *M* NaOH and 1.2 mL destiled water was added. Solution was mixed and incubated at 18 ± 1 °C in dark for 20 min. Absorbance was measured at 510 nm using JENWAY 630 Spectrophotometer. A standart curve was constructed based on a range of catechin concentrations (from 400 µgmL^{-1} to 10 µg mL^{-1}) (y=0.0030x – 0.0362; R^2=0.9980). The results were expressed as miligram catechin equivalent per 100 gram in dry weight (mg CE100 g^{-1} DW)

Antiradical activity

The antiradical activity of vegetable extracts was determined on the radical scavenging ability in reacting with stable 2,2-diphenil-1-picrylhydrazyl (DPPH) free radical according to A.F. Afifyet et al. (2012) with modifications. 4 mg of DPPH was dissolved in 100 ml pure ethanol and 3.5 mL of this solution was added to 0.5 mL vegetable extract. This mixture was shaken and kept in dark at 18 °C for 30 min. The absorbance was measured at 517 nm using JENWAY 630 Spectrophotometer. The radical percentage was calculated using the following equation:

$$\% \text{ Antiradical activity} = ((A_{blank} - A_{sample}) / A_{blank}) \cdot 100$$

Statistical analysis

All analyses were triplicated and the results of total polyphenol, flavonoid content and antiradical activity are presented as a mean value ± standart deviation (SD) and analyzed using Microsoft Office 2007 software. Statistically significant differences between the drying methods and steaming times were calculated at the level of confidence α=0.05.

Results and Discussion

The total polyphenolic content (TPC) in selected vegetables ranged from 98.97 (leek steamed for 10.0 min and dried in a microwave-vacuum dryer) to 623.70 (black radish dried in a conventive dryer) mg GAE 100 g^{-1} DW (Fig.1.).

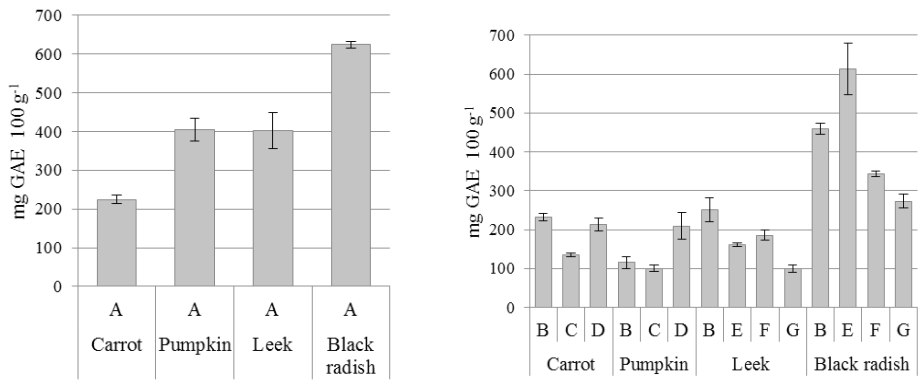

Figure 1. Total polyphenol content in dried vegetables.

(A- conventive drying; B- microwave-vacuum drying; C- steaming 1.5 min and dried in a microwave-vacuum dryer; D- steaming 3.0 min and dried in a microwave-vacuum dryer; E- steaming 6.0 min and dried in a microwave-vacuum dryer; F- steaming 8.0 min and dried in a microwave-vacuum dryer; G- steaming 10.0 min and dried in a microwave-vacuum dryer).

Significant differences ($p<0.05$) were found in conventive dried pumkin and microwave-vacuum dried pumpkin. No differences in the total polyphenol content ($p>0.05$) between microwave-vacuum dried pumpkin and steamed and then microwave-vacuum dried pumpkin were found. There were significant differences between all dried and steamed leek samples (10.0 min), but no significant differences between 6.0 and 8.0 min steamed leek samples. The highest polyphenol content in black radish can be obtained with 6.0 min steaming. Carrot has significant diffecences between microwave- vacuum drying and steaming for 1.5 min. Our obtained results compared with data in literature (Ninfali et al., 2005; Bernaert et al., 2012) are similar. According to N. Bernaert et al. (2012), the total polyphenolic content in leeks ranged from 5.31 to 15.14 mg GAE 100 g^{-1} DW. According to P. Ninfali et al. (2005), TPC in leeks ranged from 41.6 to 88.2 mg GAE 100 g^{-1} fresh weight; in carrot TPC was determined 14.6 mg GAE 100 g^{-1} fresh weight; in radish TPC ranged from 30.0 to 61.4 mg GAE 100 g^{-1} fresh weight. Comparing two different drying methods, the highest polyphenol content was obtained using traditional conventive drying, -it can be explained by the fact that polyphenols are exposed to heat for many hours and it is easier to extract polyphenols from vegetables. When the steaming has no positive effect on increasing polyphenol content in leek, it may be explained by the fact that the chosen steaming times reduce polyphenols in this vegetable. The highest results are reached when steaming pumpkin for 3.0 min and black radish for 6.0 min; the explanation may be that polyphenols start to hydrolysed and it is easier to extract more from these vegetables.

Polyphenols are mostly taken in our diet using fruits, vegetables and spices. They may reduce the risk of cardiovascular diseases and have antioxidative, anticancerogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory activities (Kim et al., 2003).

The total flavonoid content in selected vegetables ranged from 40.32 (pumpkin dried in a microwave-vacuum dryer) to 100.23 (leek dried in a conventive dryer) mg CE 100 g^{-1} DW (Fig. 2.).

The obtained results compared with data in literature (Ninfali et al., 2005) are similar. According to P. Ninfali et al. (2005), the flavonoid content (FC) in carrot was determined 12.80 mg CE 100 g^{-1} fresh weight; in leek FC ranged from 10.10 to 28.00 mg CE 100 g^{-1} fresh weight; in black radish FC ranged from 10.80 to 10.90 mg CE 100 g^{-1} fresh weight. Comparing both drying methods, the flavonoid content is higher in carrot, pumpkin and black radish using microwave-vacuum drying. Using steaming process, the flavonoid content rises - the highest content in carrot and pumpkin was obtained by using 3.0 min steaming. Significant differences were found between steaming carrots for 1.5 min; 3.0 min and microwave-vacuum drying. As for leeks, the flavonoid content is highest, whileusing conventive drying and steaming has no significant differences. Steaming black radish for 6.0 min increases the flavonoid content. Marked diferences in the concentration exist depending on both exposure to sunlight and heat. A large part of flavonoids exist as dimers, oligomers and contain C-glycosides. During the industrial processing, heat results in the hydrolysis of glycosides, and releases flavonoid monomers (Manach et al., 2004). Heat increases chemical extraction in samples and may be induced by the steaming, microwave-vacuum drying process, too (Olivera et al., 2008).

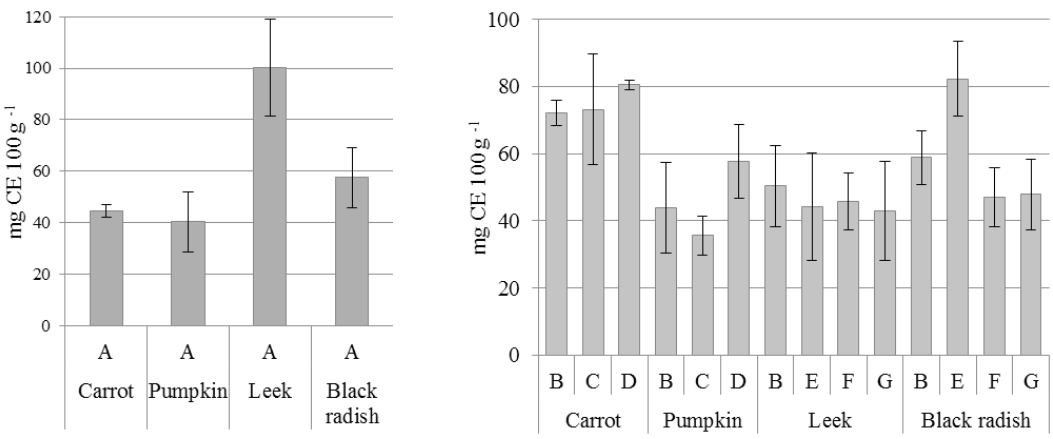

Figure 2. Total flavonoid content in dried vegetables.

(A- conventive drying; B- microwave-vacuum drying; C- steaming 1.5 min and dried in a microwave-vacuum dryer; D- steaming 3.0 min and dried in a microwave-vacuum dryer; E- steaming 6.0 min and dried in a microwave-vacuum dryer; F- steaming 8.0 min and dried in a microwave-vacuum dryer; G- steaming 10.0 min and dried in a microwave-vacuum dryer).

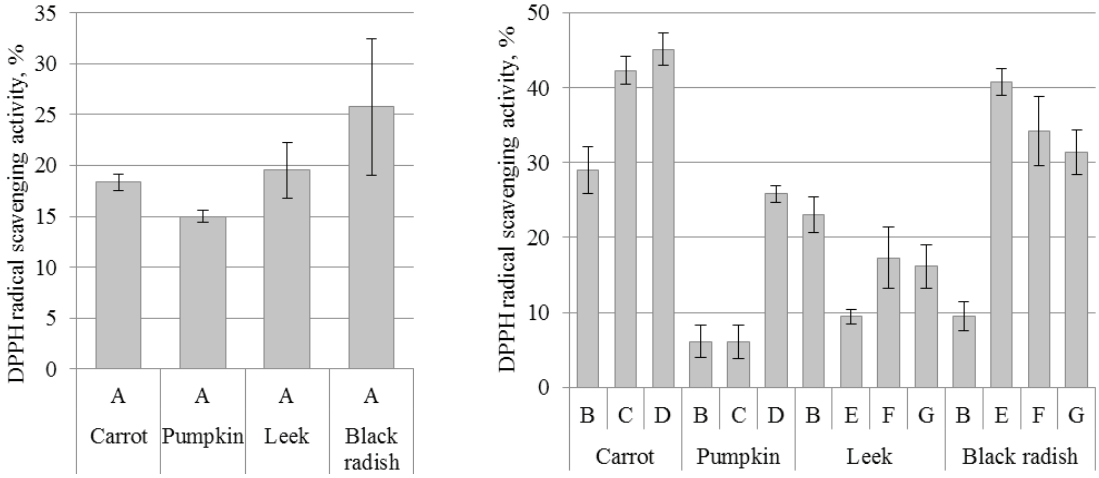

Figure 3. DPPH antiradical activity in dried vegetables.

(A- conventive drying; B- microwave- vacuum drying; C- steaming 1.5 min and dried in a microwave-vacuum dryer; D- steaming 3.0 min and dried in a microwave-vacuum dryer; E- steaming 6.0 min and dried in a microwave-vacuum dryer; F- steaming 8.0 min and dried in a microwave-vacuum dryer; G- steaming 10.0 min and dried in a microwave-vacuum dryer).

Stable organic radical DPPH has been widely used for the determination of antiradical activity in vegetables and other products. For evaluation of antiradical activity of selected vegetables, all extracts were measured and compared with their DPPH radical scavenging activities. Results are expressed as percentage and are shown in Fig. 3. The values of DPPH scavenging activity for carrots, pumpkins, leek and black radish ranged from 6.10 (pumpkin dried in a microwave-vacuum dryer and pumpkin steamed for 1.5 min and dried in a microwave-vacuum dryer) to 45.14% (carrot steamed for 3.0 min and dried in microwave-vacuum dryer).

The results show no significant differences between conventive drying and microwave-vacuum drying on carrot, pumpkin and leek. Radical scavenging activity in black radish was two times higher using conventive drying than using microwave-vacuum drying. Pre-treatment of all vegetable samples has a significant influence on radical scavenging activity. There is a positive correlation between changes in the total flavonoid content and the radical scavenging activity of all analyzed vegetable samples. According to F.D. Olivera (2008) boiling (5 min), microwave cooking and steaming over boiling water induced significant increases in the total antiradical activity on green vegetables.

From the obtained results it was concluded that long term pre-treatment does not always give a positive effect. Because of that in further analysis pre-treatment time for leek and black radish will be reduced.

Conclusions

1. The total polyphenol content in selected dried vegetables ranges from 98.97 (leek steamed for 10.0 min and dried in a microwave-vacuum dryer) to 623.70 (black radish dried in a conventive dryer) mg GAE 100 g^{-1} DW. The highest polyphenol content from analyzed vegetables is in black radishes. The highest content is reached using conventive drying for all analyzed vegetables compared with microwave- vacuum drying. The best effect is reached using steaming for 6 min on black radishes.
2. The total flavonoid content in dried vegetables ranged from 40.32 (pumpkin dried in a microwave-vacuum dryer) to 100.23 (leek dried in a conventive dryer) mg CE 100 g^{-1} DW. The highest flavonoid content is in leeks and carrots. The most suitable drying for reaching a higher favonoid content is microwave-vacuum drying. Vegetable steaming has a positive effect on flavonoid content - in all cases TFC is raised compared to microwave-vacuum drying without steaming.
3. The antiradical activity in dried vegetables ranged from 6.10 (pumpkin dried in a microwave-vacuum dryer and pumpkin steamed for 1.5 min and dried in a microwave-vacuum dryer) to 45.14% (carrot steamed for 3.0 min and dried in a microwave-vacuum dryer).The highest activity was reached in all samples with steaming pre-treatment.

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COMPARISON OF DIFFERENT SOLVENTS FOR ISOLATION OF PHENOLIC COMPOUNDS FROM HORSERADISH (*ARMORACIA RUSTICANA* L.) LEAVES

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Abstract
Horseradish (*Armoracia rusticana* L.) is a perennial herb belonging to the *Brassicaceae* family and contains biologically active substances. The aim of the current research was to determine the most suitable extraction method and solvent for obtaining horseradish leaf extracts with high antiradical activity. For experiments fresh leaves of horseradish were extracted with seven different solvents: n-hexane, diethyl ether, 2-propanol, acetone, ethanol (95%), ethanol / water / acetic acid (80/20/1 v/v/v) and ethanol / water (80/20 by volume) using two extraction methods (conventional and Soxhlet). For all extracts, total phenolic, flavonoid content and DPPH$^{\bullet}$**,** ABTS radical scavenging activity, and reducing power were determined using a spectrophotometric method. As the best solvent ethanol can be chosen. Total phenolic content and total flavonoid content was higher in Soxhlet extracts. Comparing to other plants, the proportion of flavonoids in the amount of total phenolics is average, and it increases by increasing the polarity of used solvent. It can be concluded that by6 using Soxhlet extraction method it is possible to obtain extracts that are effective antioxidants. A very strong and a strong correlation has been identified between levels of phenolic compounds and antioxidant capacities of the extracts.
Key words: antioxidants, phenolics, horseradish leaves, solvent, extraction.

Introduction

Vegetables and spices contain a wide variety of biologically active phytochemicals (Caragay, 1992; Cai et al., 2004; Wojdyo et al., 2007). Plant-derived antioxidants may function as reducing agents, scavengers of free radicals and metal ion chelators, among others (Rice-Evans et al., 1996). Plants provide abundant natural antioxidants, which are vitally important for human health (Naczk and Shahidi, 2006). Experments about natural antioxidants are important in the food industry because they can serve as an alternative to synthetic antioxidants (Michiels et al., 2012). Natural antioxidants from many spices and vegetables can be used in food to help prevent oxidation processes and neutralize free radicals (Raghavan Uhl, 2000). Studies show different antioxidant activity for each plant type, stimulated by the antioxidant components, such as α-tocopherol, β-carotene, vitamin C and phenolic compounds (Ismail et al., 2004).

Polyphenols are large, important and diverse class of antioxidants, beneficial to both plants and humans. Extensive studies on functions and the role of polyphenols in humans began in the last century and are continued today (Rappoport, 2003). Plant phenolic compounds is one of the most important primary antioxidants, so it is important to investigate their occurrence in different plant species. Phenolic compounds commonly found in spices are biologically active substances having antiseptic, vitamin activity expression, and other properties (Daayf and Lattanzio, 2008). Phenolics are ubiquitous secondary metabolites in plants with diverse chemical nature (Kahkonen et al., 1999). Phenolic composition of plants is affected by different factors – variety, genotype, climate, harvest time, storage, processing (Marrelli et al., 2012). Flavonoids, a subject of comprehensive studies in recent years, belong to polyphenols group. Flavonoids exist widely in most parts of the plant and have been attributed with multiple biological activities such as anticarcinogenic, anti-inflammatory, antibacterial, antiviral and immune-stimulating effects (Rahimi et al., 2010). Recently, the antioxidant activity of flavonoids has given rise to much attention. Flavonoids are naturally occurring plant-based free radical scavengers, and many flavonoids have been reported to possess better antioxidant properties than α-tocopherol when assessed by the in vitro oxygen radical absorbency capacity method (Cao et al., 1997). The flavonoids scavenge free radicals by acting as a hydrogen atom donor to the free radical during the oxidation-reduction reaction (Younes and Siegers, 1981). Flavonoids are widely distributed in plant fulfilling many functions. Flavonoids and other plant phenolics are especially common in leaves, flowering tissues and woody parts such as stems and bark (Kahkonen et al., 1999).

Many researchers reported influence of different extraction solvents and techniques on the content of natural antioxidants in extracts, that are strongly dependent on plant matrix used (Michiels et al., 2012). Solvents, such as methanol, ethanol, acetone, propanol and ethyl acetate have been commonly used for the extraction of phenolics from fresh product (Durling et al., 2007; Alothman et al., 2009). Very important parameter is solvent polarity – the higher the polarity, the better the solubility of phenolic compounds (Naczk and Shahidi, 2006).

Based on these statements, it can be concluded that it is very important to develop the most suitablemethod for extraction of phenolics compounds from plants.

Horseradish is a perennial plant indigenous to Eastern and Northern Europe and the Mediterranean, with a particularly pungent flavour, rich in glucosinolates and usually consumed as a pickled vegetable. It is also cultivated in central Europe, but not very broadly. Horseradish has about 0.2 to 1.0 g 100 g^{-1} of essential oil, mainly sinigrin, sinigrin-derived allylisothiocyanate, diallylsulfide, phenylpropyl and phenethylthiocyanate. Myrosinase enzyme acts on sinigrin to give allylisothiocyanate, which gives horseradish its burning taste. Horseradish has a high vitamin C content (302 mg 100 g^{-1}) (Raghavan Uhl, 2000). Its leaves are considered to prevent food spoiling processes. Although glucosinolates, with their antioxidant properties, play an important role in the human diet, they have not been systematically investigated (Majewska et al., 2004). A. Majewska et al. (2004) reported that leaf and root extracts derived from four Polish types of horseradish did not exhibit strong antioxidant properties, but the different environmental conditions of plant growth affected these properties significantly.

The aim of the current research was to determine the most suitable extraction method and solvent for obtaining horseradish leaf extracts with high antiradical activity.

Materials and Methods

Materials

Fresh horseradish leaves (*Armoracia rusticana* L.) were collected from Pure Horticultural Research Centre collection field (latitude–57° 03' N, longitude–22° 91' E), Latvia in August 2012. For analysis the average sample of 300 grams was taken. Fresh leaves were washed, peeled and homogenized (for 5 minutes). All samples of leaves were homogenized together in order to obtain a representative sample.

Chemicals

Gallic acid, Folin-Ciocalteu phenol reagent, and 2,2-diphenyl-1-picrylhydraziyl ($DPPH^{\bullet}$) were purchased from Sigma-Aldrich (Switzerland). All other chemicals and solvents (Na_2CO_3, ethanol) used in the research were obtained from Acros Organic (USA). For extraction seven different solvents were used: n-hexane (HE), diethyl ether (DI), 2-propanol (PR), acetone (AC), ethanol (95%) (ET), ethanol / water / acetic acid (80/20/1 v/v/v) (EWA), and ethanol / water (80/20 v/v) (EW).

Extraction procedure

For extraction of phenolic compounds the conventional extraction (CONVE) and Soxhlet extraction (SOXE) were used.The extraction process was performed in triplicate.

CONVE - five grams of the homogenized sample were extracted with 50 mL of an appropriate solventin a conical flask with a magnetic stirrer (magnet 4.0 × 0.5 cm) at 700 rpm for 1 h at room temperature (20±1ºC). The root extracts were then filtered (paper No. 89).

SOXE - three grams of the sample were placed in the filter cartridge (paper No. 89) in a classical Soxhlet apparatus and extracted with 170 ml of an appropriate solvent for 2 h. Extracts were cooled to room temperature.

Analytical methods

For all extracts total phenolic and flavonoid content and $DPPH^{\bullet}$, ABTS radical scavenging activity and reducing power were determined using a spectrophotometer JENWAY 6300 (Baroworld Scientifid Ltd., UK). Total phenolic content and $DPPH^{\cdot}$ were determined for all extracts, but total flavonoid content, ABTS and reducing power for extracts obtained by PR, AC, ET, EWA and EW. For all tests of antioxidant activity the control sample contained all the reaction reagents except the extract, but as positive control maximal allowed concentration 100 mg kg^{-1} of butylated hydroxytoluene (BHT) was used (MKN Nr.146). All determination was performed in triplicate.

Determination of total phenolic content (TPC)

The TPC of the leaf extracts was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999) with some modifications. To 0.5 ml of extract 2.5 ml of Folin–Ciocalteu reagent (diluted 10 times with water) was added and, after 3 minutes 2 ml of sodium carbonate (Na_2CO_3) (75 g L^{-1}) was added. The sample was mixed. The control sample contained all the reaction reagents except the extract. After 2 h of incubation at room temperature, the absorbance was measured at 765 nm. Total phenols were expressed as gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of the horseradish leaves.

Determination of total flavonoid content (TFC)

The TFC was measured by a colorimetric method (Kim et al., 2003) with minor modification. The extraction solution used was the same as TPC. To 0.5 mL of extract 2 mL of double distilled H_2O was added, and mixed with 0.15 mL of 5% sodium nitrite ($NaNO_2$) (50 g L^{-1}). After 5 min, 0.15 mL of 10% aluminium chloride ($AlCl_3$*$6H_2O$) solution was added. The mixture was allowed to stand for another 5 min, and then 1 mL of the 1M sodium hydroxide (NaOH) was added. The reaction solution was mixed well. After 15 min of incubation at room temperature, the absorbance was measured at 415 nm. Total

flavonoids were expressed as catehin equivalents (CE) 100 g^{-1} DW of the horseradish leaves.

For calculation of TFC proportion in content of phenol copounds, also TPC was expressed as CE 100 g^{-1} DW, and calculation was performed TFC/TPC.

Determination of DPPH$^{\bullet}$ radical scavenging activity

Antioxidant activity of the plant extracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydraziyl (DPPH$^{\bullet}$) radical as outlined by Yu et al. (2003). The antioxidant reaction was initiated by transferring 0.5 mL of plant extract into a sample cavity containing 3.5 mL of freshly prepared DPPH$^{\bullet}$ methanol solution (0.004 g DPPH$^{\bullet}$ to 100 mL methanol). After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity (Zhao et al., 2008). The radical scavenging capacity (RSC) was expressed as Trolox mM equivalents (TE) 100 g^{-1} DW of the horseradish leaves.

Determination of ABTS$^{\bullet}$

The RSC of extract was also measured by ABTS$^{\bullet+}$ radical cation assay (Re et al., 1999). A stock solution of ABTS (2 mM) was prepared by dissolving in 50 mL of phosphate buffered saline (PBS) obtained by dissolving 8.18 g sodium chloride (NaCl), 0.27 g potassium dihydrogen phosphate (KH_2PO_4), 1.42 g hydrogenated sodium phosphate (Na_2HPO_4) and 0.15 g potassium chloride (KCl) in 1 L of ultra pure water. If the pH was lower than 7.4, it was adjusted with sodium hydroxide (NaOH). Ultra pure water was used to prepare 70 mM solution of potassium persulfate ($K_2S_2O_8$). ABTS$^{\bullet+}$ radical cation was produced by reacting 50 mL of ABTS stock solution with 0.2 mL of $K_2S_2O_8$ solution and allowing the mixture to stand in the dark at room temperature for 15-16 h before use. The radical was stable in this form for more than 2 days when stored in the dark at room temperature. For the assessment of extracts, the ABTS$^{\bullet+}$ solution was diluted with PBS to obtain the absorbance of 0.800 ± 0.030 at 734 nm. Five mL of ABTS$^{\bullet+}$ solution were mixed with 0.05 ml of extract. The absorbance was read at ambient temperature after 10 min. PBS solution was used as a blank sample.

The RSC was expressed as Trolox mM equivalents (TE) 100 g^{-1} DW of the horseradish leaves.

The higher the Trolox equivalent antioxidant capacity (TEAC) value of a sample, the stronger the antioxidant activity.

Determination Reducing power

The reducing power can be determined by the method of Athukorala et al. (2006). One mL of extract is mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of potassium ferricyanide ($K_3[Fe(CN)_6]$) (30 mM) and incubated at 50 °C for 20 min. Thereafter, 2.5 mL of trichloroacetic acid (CCl_3COOH) (600 mM) is added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 mL) is mixed with 2.5 mL of distilled water and 0.5 mL of iron chloride ($FeCl_3$) (6 mM) and absorbance is measured at 700 nm. Reducing power was expressed as ascorbic acid equivalent (AAE) 100 g^{-1} DW of the horseradish leaves.

Additionally for all horseradish leaves the **moisture content** was determined according to standard ISO 6496:1999 and all results are expressed according to dry basis.

Statistical analysis

Experimental results were means of three parallel measurements and were analyzed by Microsoft Excel 2010 and SPSS 17.00. Analysis of variance (ANOVA) and Tukey test were used to determine differences among samples. The linear correlation analysis was performed in order to determine the relationship between TPC, TF, antioxidant activity such as DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and reducing power. Differences were considered as significant at $p < 0.05$.

Results and Discussion

Total phenolics and flavonoids content

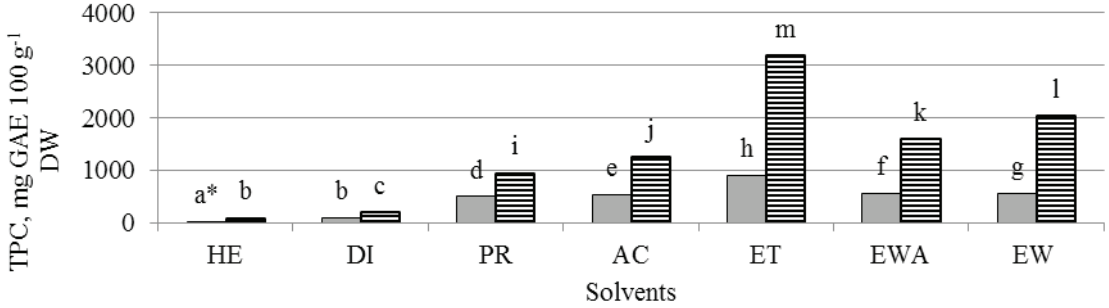
TPC determined in horseradish leaf extracts made by CONVE and SOXE depending on used solvent ranged from 18.85 to 904.66 mg GAE 100g^{-1} DW and from 94.30 to 3192.96 mg GAE 100g^{-1} DW, respectively (Fig. 1).

Previous experiments about TPC in horseradish roots extracts obtained by CONVE and SOXE ranged from 334.29 mg GAE 100 g^{-1} DW to 985.87 mg GAE 100 g^{-1} DW depending on solvents used (Tomsone et al., 2012), and it is almost three times lower amount compared to results obtained in the current research about horseradish leaves. Also other studies showed that qualitative and quantitative content of phytochemicals in plant parts differ significantly (Marrelli et al., 2012).

TFC determined in extracts of horseradish leaves depending on different solvents used ranged from 770.45 to 2306.65 mg CE 100 g^{-1} DW (CONVE) and from 2121.69 to 8929.22 mg CE 100 g^{-1} DW (SOXE) (Fig. 2.). Due to cross-reactions between the solvents (HE and DI) and the reagents, it was impossible to read the absorbance, so TFC, ABTS$^{\bullet+}$ and Reducing Power were not determined.

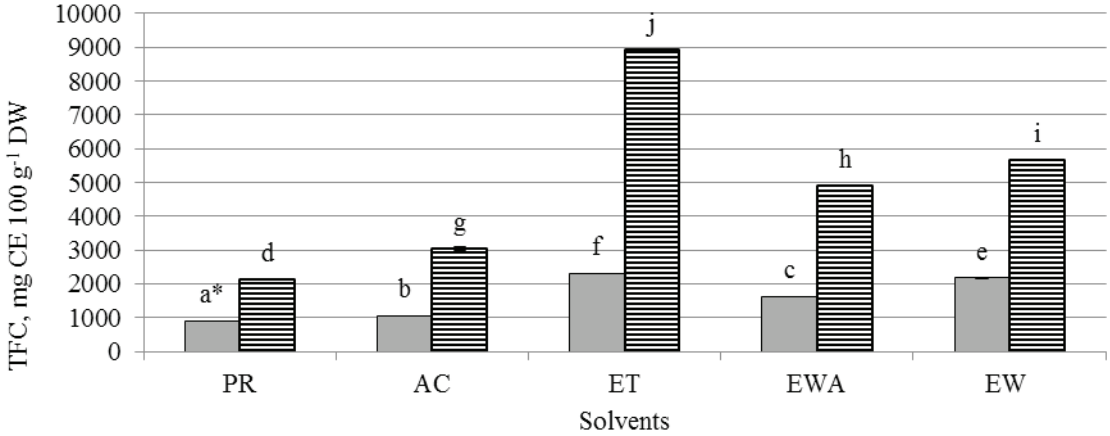
Results of multivariate dispersion analysis showed that both used solvent and extraction method are significant factors affecting TPC and TFC ($p < 0.05$). Mainly the results of TPC and TFC obtained using a SOXE are higher compared to the CONVE.

TFC proportion in the content of total phenols ranged from 0.21 to 0.44, and this proportion tends to



* Mean values within the same row followed by different letters significantly differ according to the LSD test ($p<0.05$).

Figure 1. TPC in horseradish leaves depending on solvent:
CONVE–conventional extraction, SOXE–Soxhlet extraction.



* Mean values within the same row followed by different letters significantly differ according to the LSD test ($p<0.05$).

Figure 2. TFC in horseradish leaves depending on solvent:
CONVE–conventional extraction, SOXE–Soxhlet extraction.

increase by increasing polarity of extraction solvent. Consequently it could be concluded that solvents with higher polarity are better isolated flavonoid type compounds. In literature it was found that for fruit the proportion between TFC/TPC ranged from 0.15 to 0.56, but for vegetables 0.07 – 0.78 (Marinova et al., 2005). The results of our experiments are within the range of results obtained by other scientists.

The recovery of polyphenols from plant materials is influenced by the solubility in the solvent used for the extraction process (Nićiforović et al., 2010). In the current research seven solvents with different polarity were used, and they can be arranged as follows (starting from more unpolar solvents, according to indexes of polarity): HE < DI < PR < AC < ET < EWA < EW. From the selected solvents the lowest polarity is for HE, but the highest for EWA and EW.

Solvent polarity plays a key role in increasing phenolic solubility (Naczk and Shahidi, 2006). Obtained results showed that phenolic compounds and antioxidant activity generally increased by increasingthe polarity of solvents, andthe tendency is more pronounced in the SOXE. Results of Tukey's test showed that using both extraction methods solvents significantly influence changes of all analysed parameters. Polarity of phenolic compounds differs, therefore it is hard to develop a standard extraction procedure suitable for the extraction of all plant phenols.

The results of analyses showed that the highest TPC of horseradish leaves was extracted using 95% ethanol (by both extraction methods). Similar results were found in experiments about leaves of *Moringaoleifera* L., where TFC increased by increasing concentration of ethanol in extraction (Vongsak et al., 2012). Also in experiments about *Potentilla fulgens* roots (Jaitak et al., 2010) and canola meal (Hassas-Roudsari et al., 2009) showed that higher TFC were obtained with more polar solvents. Extraction is strongly influenced by plant matrix because in the study about *Artemisiaargyi* L. leaves TPC in methanolic extract

was higher comparing to aqueous extract, although *Pyrrosialingua* L. leaves showed oposite effect (Cai et al., 2004).

Antioxidant activity

The antioxidant activity has been attributed to various mechanisms, for example, prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997). The most commonly used antioxidant methods are those with $ABTS^{\bullet+}$ and $DPPH^{\bullet}$. Both of them are characterized by excellent reproducibility under certain assay conditions, but they also show significant differences in their response to antioxidants. The $DPPH^{\bullet}$ free radical does not require any special preparation, while the $ABTS^{\bullet+}$ radical cation must be generated by enzymes or chemical reactions (Wojdyo et al., 2007). In our research for the measurements of the reductive ability, it has been investigated from the Fe^{3+}–Fe^{2+} transformation in the presence of extract samples using the method followed by Athukorala (2006).

There are variations of antioxidant activity of horseradish leaf extracts depending on extraction method and solvent used (Table 1).

Comparing to antioxidant activity of maximally allowed concentration of BHT, in horseradish leaf extracts obtained by SOXE results are higher, but by CONVE – are lower. These results confirmed that application of horseradish leaf extracts in food stuffs can replace synthetic antioxidants and it is necessary to continue experiments in real food matrixes.

Literature data showed that antioxidant activity differs depending on used solvent and food matrix. Antioxidant activity of horseradish leaves differed significantly depending on solvents used and the highest activity was determined in SOXE extract with ET. Using both extraction methods it is possible to observe increase in antioxidant activity by an increased polarity of solvent. In the studies about horseradish roots (*Armoracia rusticana* L.) (Tomsone et al., 2012), *Potentilla fulgens* roots (Jaitak et al., 2010), canola meal (Hassas-Roudsari et al., 2009) it was also found that the antioxidant activity increases by increasing solvent polarity. In the study about *Artemisia argyi* L. leaves it was found, that the same as for TPC, antioxidant activity of aqueous extract was higher than in methanolic extract, and oposite tendency in *Pyrrosia lingua* L. leaves was determined (Cai et al., 2004). Fresh dill (*Anethum graveolens* L.) flower ET extracts also showed higher TEAC activity comparing to HE (Shyu et al., 2009), and this observation is in accordance with our results.

Relationship between total antioxidant capacity and phenolic content

Phenolic compounds have radical scavenging activity. The Pearson`s coefficients between the phenolic compounds levels and antioxidant capacities, are presented in Table 2. The antiradical capacity of an extract is often related to its polyphenolic constituents. In our study all correlations are positive. For all

Table 1

Analysis of antioxidant capacity of horseradish leaves depending on extraction solvent and method

		$DPPH^{\bullet}$ mM TE 100 g^{-1} DW	$ABTS^{\bullet+}$ mM TE 100 g^{-1} DW	Reducing power mg AAE 100 g^{-1} DW
CONVE	HE	5.35±0.03[a*]	n.d.	n.d.
	DI	5.52±0.02[a]	n.d.	n.d.
	PR	6.76±0.05[b]	14.31±0.01[a]	1130.42±2.05[a]
	AC	7.29±0.02[c]	15.36±0.02[b]	1225.98±4.66[b]
	ET	10.11±0.04[e]	19.61±0.01[e]	2729.94±4.81[e]
	EWA	8.80±0.02[d]	16.98±0.08[c]	1741.63±4.12[c]
	EW	8.92±0.03[d]	17.21±0.01[d]	2043.34±4.80[d]
SOXE	HE	35.58±0.10[g]	n.d.	n.d.
	DI	36.59±0.29[h]	n.d.	n.d.
	PR	43.75±0.07[i]	84.87±0.07[g]	4815.94±5.76[g]
	AC	47.01±0.44[j]	85.50±0.31[h]	5216.38±9.48[h]
	ET	68.78±0.22[m]	116.79±0.06[k]	9616.67±3.84[k]
	EWA	51.71±0.36[k]	100.44±0.06[i]	5512.39±4.12[i]
	EW	55.24±0.31[l]	102.02±0.04[j]	5801.34±9.79[j]
BHT	ET	27.10±0.05[f]	66.82±0.04[f]	3660.32±4.65[f]

* Mean values within the same columnfollowed by different letters significantly differ according to the LSD test ($p<0.05$). n.d. – not determined.

extracts a very strong correlation has been identified between all levels of phenolic compounds and the antioxidant capacities of the extracts.

Table 2

Pearson`s coefficients between total antioxidant capacity, total phenolic and total flavonoid content for horseradish leaves

	TPC	TF	DPPH	ABTS
TPC	1			
TF	0.987**	1		
DPPH	0.779**	0.860**	1	
ABTS	0.847**	0.825**	0.996**	1
Reducing power	0.955**	0.937**	0.958**	0.934**

**Correlation is significant at the 0.01 level (2-tailed).

Similar results with very strong correlation between TPC and antioxidant activity were reported for different lychee (*L. chinenesis* Sonn.) flower solvent extracts (Liu et al., 2009).

Conclusions

The analysis of the phenolic compounds and antioxidant activity of horseradish extracts showed differences depending on extraction method and solvent used. As the best solvents ethanol, but extraction method - SOXE can be chosen to obtain horseradish leaf extracts with high antioxidant activity. Solvents with higher polarity isolated flavonoids from horseradish leaves better and their proportion in TPC was higher. Horseradish leaf extracts have strong antioxidant activity, comparable to synthetic antioxidants and in it is necessary to continue experiments with their efficiency in food.

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EVALUATION OF BUTTER OIL OXIDATIVE STABILITY AND NUTRITIONAL VALUE AFFECTED BY COW FEEDING

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Abstract
Nutritional value and shelf life of milk and dairy products depend on the composition and stability of their constituents. The **aim** of the present study was to evaluate the effects of carrots as cow feed carotenoid source on butter oil fatty acid (FA) composition and oxidative stability. Milk was obtained from one trial group (TG; n=5) and one control cow group (CG; n=5) in a conventional dairy farm in Latvia. TG cows received carrots 7 kg per cow per day; the length of the supplementation period was 39 days. The stability of butter oil exposed to sunlight (3h) and held for 14 days in the temperature of 60 °C was analyzed by peroxide value method.
The changes of the FA content and ratios in TG milk fat were more positive with respect to the fat nutritional value as observed in CG – stronger increase in the content of polyunsaturated FA ($p<0.05$), and in the ratio between stearic and palmitic acids ($p<0.05$); also a tendency was seen of increasing content of short and monounsaturated FA, as well as decreasing the ratio between ω6 and ω3 FA groups.
Oxidative stability of the carrot supplemented cow group's milk butter oil samples that were collected after 25- day trial period, exposed to sunlight (3h) and stored at a temperature of 60 °C was significantly ($p<0.05$) higher compared to CG samples. After the 39-day long trial period stability difference was not significant, despite the tendency that average polyunsaturated FA content in TG samples was higher compared to the control.
Key words: butter oil, fatty acid, peroxide value, carotenoids, cow feed, carrots.

Introduction

Nutritional value and shelf life of milk and dairy products depend on the composition and stability of their constituents. Milk lipids confer distinctive nutritional, textural and organoleptic properties to dairy products, such as cream, butter, whole milk powder and cheese (MacGibbon and Taylor, 2006). However, the health benefits of milk fat is the cause for the debate among scientists that continues for years. Unbalanced fat composition with predominance of saturated fat is related to increased rates of heart disease (German and Dillard, 2006). Therefore trials are made to achieve a more healthful milk fat composition by altering the cow's diet (Bobe et al., 2007). Natural antioxidants – carotenoids are known well in health and food protection amongst which b-carotene is particularly involved in prevention of photo-oxidation, hindering unfavourable quality changes (Namitha and Negi, 2010). However, the potential of carotenoids is employed insufficiently in dairy. Carrots are one of the richest sources of carotenoids containing mainly a- and b-carotenes (Kotecha et al., 1998). The **aim** of the present study was to evaluate the effects of carrots as cow feed carotenoid source on butter oil fatty acid (FA) composition and oxidative stability.

Materials and Methods

Experimental design. Milk was obtained from one trial group (TG; n=5) and one control cow group (CG; n=5) in a conventional dairy farm in Latvia. The average stage of lactation (5.3 months), the average lactation number (i.e. 2.8) and cow breed (Latvian Brown, Danish Red, and crossed) were as similar as possible in all groups. Feed supplementation was implemented at the end of the indoor period (April and May). The basic feed (equal in both groups) was haylage, mixed feed concentrate and hay. TG received 7 kg of carrots, additionally providing 145 mg of total carotenes per cow per day.

Milk sample collection and storage. Individual cow milk samples were obtained from the morning milking before the feed supplementation (D0) and on days 25 (D25) and 39 (D39) from the start of the feed supplementation. One bulk milk sample per each group was obtained pooling 5 L of individual cow's milk and transported to the laboratory.

Butter oil extraction and storage. Milk was warmed up to 40-45 °C subsequently separating cream with a milk separator to approximately 30% of fat content. Cream was ripened at 4-6 °C, for 20 ± 1 h, than churned till the formation of butter. The buttermilk was removed and butter was rinsed with cold distilled water. Then butter was warmed up to 40-50 °C and centrifuged 14360×g, for 10 minutes at 40 °C to separate the pure butter oil, that was used for fatty acid analysis or split into smaller (20 g) sub-samples for peroxide value analyses, e.g. fat was poured into the appropriate number of transparent plastic Petri dishes and subjected to direct sunlight at 20 ± 1 °C for 3 h to hasten the fat ageing. Then, the samples were placed into thermostatic oven at 60 ± 1 °C for 14 days.

Peroxide value (PV) of the milk fat was determined in accordance with the iodometric titration method

(Охрименко и др., 2005). The length of the induction period was established by setting the point of intersection of lines of linear functions corresponding to the induction period and active phase of peroxide and hydroperoxide development.

Analyses of fatty acid (FA) composition were performed according to the method of Semporé and Bézard (1996) with some modifications. Extracted butter oil was transesterified to methyl esters in a sodium methylate solution, e.g., 7-15 mg of the oil was mixed with 1 ml of hexane (with 50 ppm of butylated hydroxytoluene) and 10 µL of Na methylate solution (12.5 g 100 mL^{-1}) (self-made, containing 5.32 g of sodium in methanol 100 mL^{-1} solution (wv^{-1})), shaken 1 min, left for 10 min in 20 ± 3 °C and centrifuged for 5 min at 4 °C and 14360×g . The upper layer containing FA methyl esters was used for further analysis by gas-liquid chromatography using the ACME model 6100, GLC (Young Lin Instrument Co.) gas chromatograph fitted with the flame ionization detector, and a 30 m long, 0.25 mm i.d. Alltech AT-FAME analytical column. The carrier gas (He) flow rate was 2 ml min^{-1}. The injector and detector temperatures were 225 °C and 250 °C, respectively. The oven temperature was programmed from 50 °C (4 min) till 170 °C at a rate of 8 °C min^{-1} (held 15 min), till 240 °C at a rate of 6 °C min^{-1}. Peaks were identified using standard mixture Supelco FAME Mix C4-C24, Sigma Aldrich. Results were evaluated with an integrator program (Autochro-2000, Young Lin Instrument Co.) The sum of FA groups' content was calculated according to the following formulas (1-6):

- **Saturated FA (SFA)** = sum of C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0 (1)
- **Short and medium FA** = sum of C4:0, C6:0, C8:0, C10:0 (2)
- **Monounsaturated FA (MUFA)** = sum of C14:1, C15:1, C16:1, C18:1w9c, C18:1w9t, C20:1w9 (3)
- **Polyunsaturated FA (PUFA)** = sum of C18:2cw6, C18:2t, C18:3w6, C18:3w3, C20:4w6, C20:5w3,C22:5w3, C22:6w3; (4)
- **w3 FA** = sum of C18:3w3, C20:5w3, C22:5w3, C22:6w3 (5)
- **w6 FA** = sum of C18:2cw6, C18:3w6, C20:4w6 (6)

Changes (%) in fatty acid content or ratios in milk fat before and during the supplementation were calculated using the following formula (7):

$$Changes = \frac{(\text{FA content}_{\text{AVER}_{(D25,\,D39)}} \times 100)}{\text{FA content}_{D0}} - 100 \ (\%) \quad (7)$$

Analyses were carried out in the Scientific Laboratory of Biochemistry and Microbiology of the Research Institute of Biotechnology and Veterinary Medicine 'Sigra' of the LLU.

Chemicals were of analytical or higher purity. Water was purified with Simplicity (Millipore SAS, France). Potassium iodide was from Stanchem, Poland, glacial acetic acid from Lach-Ner, Czech Republic, chloroform from Riedel-De-Haën, Germany, sodium thiosulphate from AVSISTA, Lithuania, sodium from Charlau Chemie, methanol and hexane (HPLC grade) from Chromasolv.

Statistical analyses were made using Microsoft Office program Excel and Microsoft Windows for SPSS (SPSS 17.0, SPSS Inc. Chicago, Illinois, USA). Differences between the groups were tested for significance ($p < 0.05$) by ANOVA.

Results and Discussion

The FA content and ratios before and after the cow feed supplementation are given in Table 1.

Table 1

Content of FA groups and ratios before and after the cow feed supplementation

Sampling	Cow groups	Content of fatty acid groups (% of total FA)				Fatty acid ratios	
		short and medium FA	SFA	MUFA	PUFA	ω6 / ω3	C18:0 / C16:0
Before supplementation (D0)	**TG (carrots)**	**9.22**	**74.80**	**18.81**	**1.54**	**2.21**	**0.11**
	±SD	0.52	1.86	0.89	0.15	0.77	0.02
	CG (control)	**10.38**	**73.62**	**20.30**	**1.70**	**1.82**	**0.13**
	±SD	1.50	2.14	2.17	0.21	0.29	0.01
After supplementation (average result of the D25 and D39 samples)	**TG (carrots)**	**9.37**	**71.05**	**21.11**	**2.08**[a]	**1.77**	**0.16**[a]
	±SD	0.15	1.99	1.69	0.14	0.09	0.01
	CG (control)	**9.79**	**71.56**	**21.25**	**1.92**	**1.71**	**0.17**[a]
	±SD	0.15	0.56	0.65	0.07	0.14	0.00

[a] – the diference between parameters before and after the supplementation is significant ($p<0.05$).

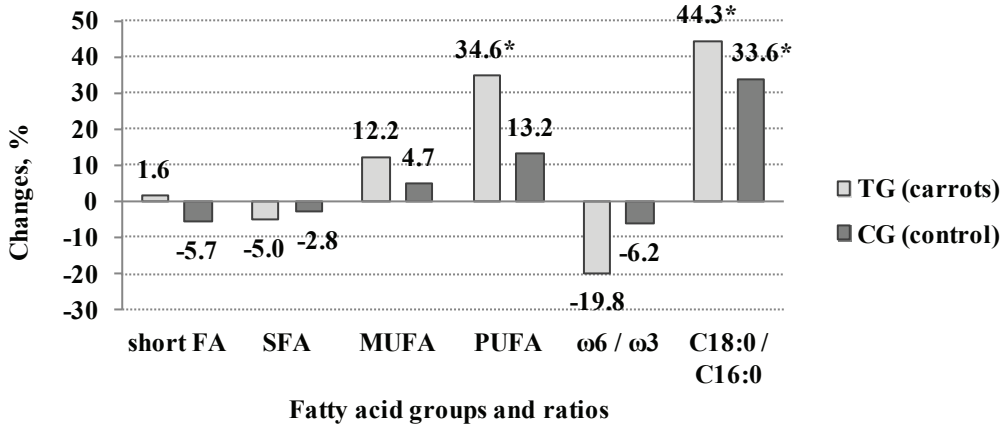

Figure 1. Changes in the fatty acid content and ratios in milk fat of cows fed differently
* – changes of parameters are significant ($p<0.05$).

The content of the short and medium FA, SFA, MUFA, and PUFA, as well as ratios between ω6 / ω3 and C18:0 / C16:0 did not differ significantly between the groups before the feed supplementation (D0).

After the feed supplementation, there was a significant increase observed in the milk fat PUFA content of the trial group by 34.6%, as well as in the ratio of stearic and palmitic acids (C18:0 / C16:0) of both groups (see Fig. 1). The changes of stearic and palmitic acid ratio were more pronounced in the trial group. Other parameters did not change significantly in either group, however, the tendency of the mean value changes was more positive in the trial group with regard to the increase of short and medium FA, MUFA content, and the decrease of SFA content and ratio between ω6 / ω3 FA.

In summary, the FA content and ratios changes in the trial group were more positive as observed in CG. Higher content of short and medium FA, MUFA, PUFA content in milk fat, as well as higher ratio between stearic and palmitic acids are considered to be more favorable for human health (Chillard et al., 2000; German and Dillard, 2006). The lower ratio of the ω6 / ω3 FA also is considered to be favourable for the prevention of number of diseases (Gebauera et al., 2005)

To our knowledge, little studies have been carried out on FA composition of milk as affected by the supplementation of cow forage with carrots. In the study of Nałęcz-Tarwacka et al. (2003), cow feed was supplemented with 5 kg of carrots per cow per day. The supplementation was started at the beginning of the cow dry period till the 20th day after calving. Their observations about the positive changes of FA composition were similar to our results. There was a significant increase ($p\leq0.01$) in the content of linoleic acid, conjugated linoleic acid (CLA), as well as in MUFA and PUFA contents in milk. Also the content of oleic acid increased significantly ($p\leq0.05$), but the content of lauric-, palmitic acids and the total SFA content decreased significantly ($p\leq0.01$).

The possible explanation of results can be related to the effects of carrot forage components as, for example, fiber or sugars, altering rumen biohydrogenation and milk FA synthesis processes in cows. The effect of carrot supplementation has to be investigated further. Many studies assure that milk FA composition is affected by feed components. For example, higher contents of CLA and C18:3n3 have been related to the use of grass-based forage rather than maize silage and to higher proportions of clover and other legume species than grasses (Larsen et al., 2010).

Oxidative stability of milk fat

The oxidative stability of milk fat was compared by PV changes affected by the initial storage of butter oil in the light (3h) and further storage at a temperature of 60 ± 1 °C, and by measuring the concentration of primary oxidation products (hydroperoxides and peroxides) in the fat. The oxidative stability of food system can be characterized by the length of the induction period when low oxidation intensity is followed by rapid increase in hydroperoxide concentration (O'Connor and O'Brien, 2006).

The changes of the peroxide value of samples collected after 25-day feed supplementation period and stored in the light and at a temperature of 60 ± 1 °C are represented in Fig. 2.

The oxidative stability of the fat depends on the FA composition, fat-soluble antioxidant content and other anti- and prooxidative factors (O'Connor and O'Brien, 2006). Its relation to the fat PUFA content was analyzed, however, the length of induction period also can be related to the antioxidant content of butter oil and other factors. The established induction

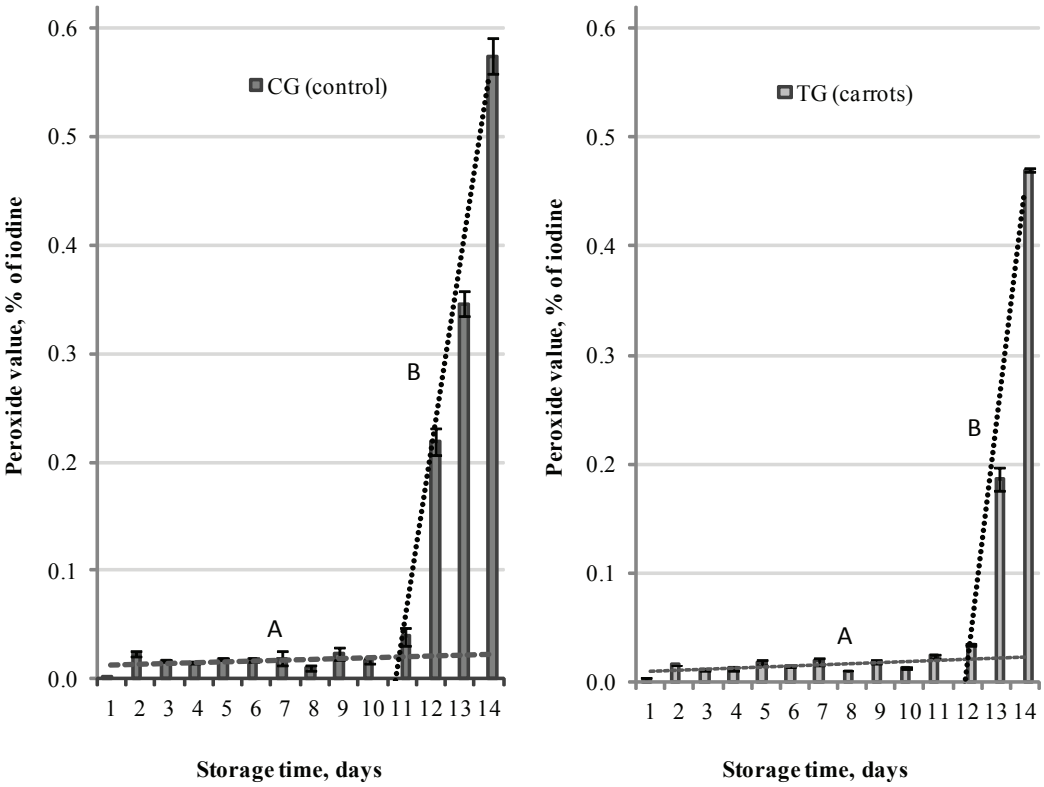

Figure 2. Changes of peroxide value of butter oil samples stored in light (3 h) and at 60 ± 1 °C
A– induction period, B – active phase of peroxide and hydroperoxide development.

periods of light-affected butter oil samples and their PUFA content are showed in Tab. 2.

Table 2

Induction periods established by PV method and PUFA contents of butter oil samples affected by light and stored at a temperature of 60 °C

Sampling days	PUFA content (mean ± SD), % of total FA		Induction period, days	
	TG	CG	TG	CG
D25	1.98±0.38	1.96±0.27	12.03	10.97
D39	2.17±0.24	1.87±0.23	12.00	11.85

After the 25-day trial period the induction period of TG samples was significantly ($p<0.05$) longer compared to CG. At the same time the PUFA content was similar in the butter oil of both groups.

Concerning D39 samples, the average PUFA content was higher in TG fat, consequently leading to a higher susceptibility to oxidation, however, the length of the induction period was insignificantly ($p>=0.05$) longer compared to CG. Therefore it can be assumed that cow feed enrichment with carotenoid supplements can leave a certain impact on health benefits of milk fat through its FA composition and stability improvement.

Conclusions

1. A significant ($p<0.05$) increase of the polyunsaturated fatty acid content in the trial group's milk fat by average 34.6% was observed after 25 and 39-day cow feed supplementation period with carrots compared to D0.
2. The changes in the fatty acid content and ratios in the trial group's milk fat were more positive with respect to the fat nutritional value as observed in the control group – stronger increase in the content of polyunsaturated fatty acids ($p<0.05$), and in the ratio between stearic and palmitic acids ($p<0.05$); also the tendency was seen of increasing content of short and monounsaturated fatty acids, as well as decreasing the ratio between ω6 and ω3 fatty acid groups.
3. Oxidative stability of the carrot supplemented cow group's milk butter oil samples that were collected after 25-day trial period, exposed to sunlight (3h) and stored at a temperature of 60 °C was significantly ($p<0.05$) higher compared to the control group samples. After 39-day long trial period the butter oil stability difference was not significant, despite the tendency of average

polyunsaturated fatty acid content in the trial group samples to be higher compared to the control.

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THE INFLUENCE OF SELENIUM AND COPPER ON MICROBIOLOGICAL INDICATORS OF RYE MALT

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Abstract
Malt is a natural food product produced by germinating cereal grains. Safety of cereal grains and cereal products is a very important area. Experiments were carried out at the Faculty of Food Technology of the Latvia University of Agriculture. The research object was rye malt. The purpose of the research was to investigate and compare the influence of copper and selenium on microbiological indicators of rye malt. Rye grains of 96% viability were soaked and germinated at temperature +6 ± 2 °C for 4 days, using copper(II) sulphate pentahydrate $CuS0_4*5H_2O$ solutions (Cu concentration 10 mg L^{-1}, 50 mg L^{-1}, 100 mg L^{-1}) and for 3 days using sodium selenate Na_2SeO_4 solutions (Se concentration 3 mg L^{-1}, 5 mg L^{-1}, 10 mg L^{-1}) then the soaked grains dried in the oven for 24 hours. The total plate count and yeast colony forming units were determined in rye malt samples. The obtained results showed that the increasing of copper and selenium concentration in solution significantly changes microflora of rye malt comparing with the control sample.
Key words: selenium, copper, rye malt, microbiological indicators.

Introduction

Cereals and cereal products are significant and important human food resources. Cereal grains are food staples in many, if not most, countries and cultures and are the raw materials of many of our foods and certain beverages (Bullerman and Bianchini, 2011; Downes and Ito, 2001). Rye (*Secale cereale*) is a cereal commonly grown in Central and Eastern Europe, especially Poland and Germany. Malt is a natural food product produced by germinating cereal grains (Edney and Izydorczyk, 2003). Rye malt is a natural sweetener and flavour with a rich taste that varies from mild and sweet to deep and robust. Malt gives a pleasing appetizing colour. Malt products promote the fermentative process, by nourishing the yeast so that sugar does not need to be added to the dough. Substantial quantities are also used as raw material in the distilling industry (Hübner et al., 2010).

Because of their extensive use as human foods, the safety of cereal grains and cereal products is a very important area. The sources of microbial contamination of cereals are many, but all are traceable to the environment in which grains are grown, handled, and processed. Microorganisms that contaminate cereal grains may come from air, dust, soil, water, insects, rodents, birds, animals, humans, storage and shipping containers, and handling and processing equipment. Many factors that are a part of the environment influence microbial contamination of cereals, including rainfall, drought, humidity, temperature, sunlight, frost, soil conditions, winds, insects, bird and rodent activity, harvesting equipment, use of chemicals in production versus organic production, storage and handling, and moisture control (Bullerman and Bianchini, 2011). Microorganisms are an important factor to consider when dealing with cereal grain and product because microbial spoilage has economic consequences of importance but most importantly because consumer safety is at risk with food borne illness (Downes and Ito, 2001). The microbial communities greatly influenced grain germination and malt properties (Laitila, 2007).

Copper is an essential micronutrient for plants that is a component of several electron transport enzymes and is involved in catalyzing the redox reactions in mitochondria and chloroplasts (Lombardi and Sebastiani, 2005). Virtually all organisms require copper as a catalytic cofactor for biological processes such as respiration, iron transport, oxidative stress protection, peptide hormone production, pigmentation, blood clotting and normal cell growth and development (Puig and Thiele, 2002). However, copper also induces toxicity at tissue concentrations slightly above its optimal levels. Excess leaf copper can induce alterations in the photosynthetic and respiratory processes, enzyme activity, DNA, and membrane integrity, all of which could lead to growth inhibition (Lombardi and Sebastiani, 2005).

Selenium (Se), an essential element for animals and humans, has also been found to be beneficial to plants. In some countries around the world, such as China and Egypt, Se deficiency in the diet is a common problem. To counteract this problem, Se compounds have been used to increase Se content in the edible parts of crops, through foliar sprays or base application of fertilizers. Se has also been shown to counteract various abiotic stresses induced in plants by cold, drought, high light, water, salinity and heavy metals (metalloids) (HMs), but the associated mechanisms are rather complicated and still remain to be fully elucidated (Feng et al., 2013; Bitterli et al., 2010). Although the essentiality of Se for higher plants has not been conclusively proved, there have been reports showing beneficial effects of Se on plant

growth. Trace amounts of Se can promote growth in a variety of Se accumulator and non-accumulator plants. Studies have also shown that Se can protect the plants from a variety of herbivores and pathogens, probably by volatile Se (dimethylselenide) emitted or Se elevated production of ethylene, jasmonic acid and other defense-related proteins (Wang and Wang, 2012; Terry et al., 2000). Duran (2013) notes that certain rhizosphere microorganisms, such as rhizobacteria and arbuscular mycorrhizal fungi can increase the selenium uptake in plants. It is reported in literature that cereals and cereal products contain a wide range of selenium concentration between 0.0010 and 0.550 mg kg^{-1} (FAO, WHO, 2001) mean concentrations of copper in rye 4.99 ± 0.65 mg kg^{-1} (Shtangeeva et al., 2011).

Although the physiological activity of copper and selenium is the subject of research of many authors, there are few data about the influence of these elements on the microflora. Because of the few existing studies, the research was to investigate and compare the influence of copper and selenium on microbiological indicators of rye malt.

Materials and Methods

Plant material

The research objects were rye grains (variety ´Kaupo´) from Ltd. ´Naukšēni´, harvested in 2011. Rye grain samples were divided into two parts. 3 kg of rye grain were soaked for 48 h in 10 L copper (II) sulphate pentahydrate $CuS0_45H_2O$ solutions and germinated for 48 h at temperature of +6 ± 2 °C. The concentration of copper was 10, 50, and 100 mg L^{-1}. The germination of grain with deionized water served as a control. After germination, all sprouts were dried for 24 h at a temperature of +73 - 108 °C; then they were grounded. Moisture of malt samples ranged from 8.9% to 10.2%.

Other 3 kg of rye grain were soaked and germinated at temperature of +6 ± 2 °C for 3 days, using sodium selenate Na_2SeO_4 solutions (Se concentration 3 mg L^{-1}, 5 mg L^{-1}, 10 mg L^{-1}). The concentration of selenium was 3, 5, and 10 mg L^{-1}. The germination of grain with deionized water served as a control. After germination, all sprouts were dried for 24 h at a temperature of +70 - 112 °C; then they were grounded. Moisture of malt samples ranged from 7.10% to 8.97%.

Moisture

Moisture content of rye malt was determined by ISO 6496:1999

Microbiological analysis

Malt samples for microbiological testing were prepared by dilution method in conformity with standard LVS EN ISO 6887-1:1999, 6887-4:2004. TPC (total plate count) – determined in conformity with standard LVS EN ISO 4833:2003A; yeast colony forming units – determined in conformity with standard LVS ISO 21527-2:2008. Plate counts evaluated as decimal logarithm of colony forming units (CFU) per gram of a product (log cfu g^{-1}). Experiments were carried out in threefold manner. The obtained measurement results were compared with the values given in the Sanitary and epidemiological rules and regulations "Hygienic safety and nutritional value of foods" SanPin 2.3.2.1078-01.

Statistical analysis

Data are expressed as mean ± standard deviation. For the mathematical data processing p-value at 0.05 was used to determine the significant differences.

Results and Discussion

The microflora of cereals and cereal products is varied and includes moulds, yeasts, bacteria (psychrotrophic, mesophilic, and thermophilic/ thermoduric), lactic acid bacteria, rope-forming bacteria (*Bacillus spp.*), bacterial pathogens, coliforms, and enterococci. Bacterial pathogens that contaminate cereal grains and cereal products and cause problems are *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Escherichia coli*, *Salmonella spp.*, and *Staphylococcus aureus* (Bullerman and Bianchini, 2011). Coliforms and enterococci also occur as indicators of unsanitary handling and processing conditions and possible fecal contamination (Downes and Ito, 2001). Ehrlich (1997) notes that all microbes, whether prokarotic or eukaryotic, employ metal species for structural functions and/or catalytic functions. The alkali metals Ca and Mg serve structural as well as catalytic functions. The metals V, Cr, Mn Fe, Co, Ni, Cu, Zn, Mo, and W, and the metalloid Se may participate in catalytic functions.

In this experiment we have followed the regulation of the Sanitary and epidemiological rules and regulations "Hygienic safety and nutritional value of foods" SanPin 2.3.2.1078-01, being in force from 15.04.2001 in Russia, as well as considered previously accomplished microbiological studies. In this research we have supposed, that CFU count could not be allowed more than 4.0 log cfu g^{-1}, and yeast count not more than 2.0 log cfu g^{-1} in grains (seeds), flour - cereals and bakery products, including grain malts.

Different copper concentrations influence on the rye malt microbiological indicators is shown in Figure 1.

Results show microbial growth inhibition effect of copper in different concentrations when added to rye malt. The total plate count in rye malt control sample (Figure 1) was 4.1 log cfu g^{-1}. At copper concentration in solution 10, 50 and 100 mg L^{-1} the total plate

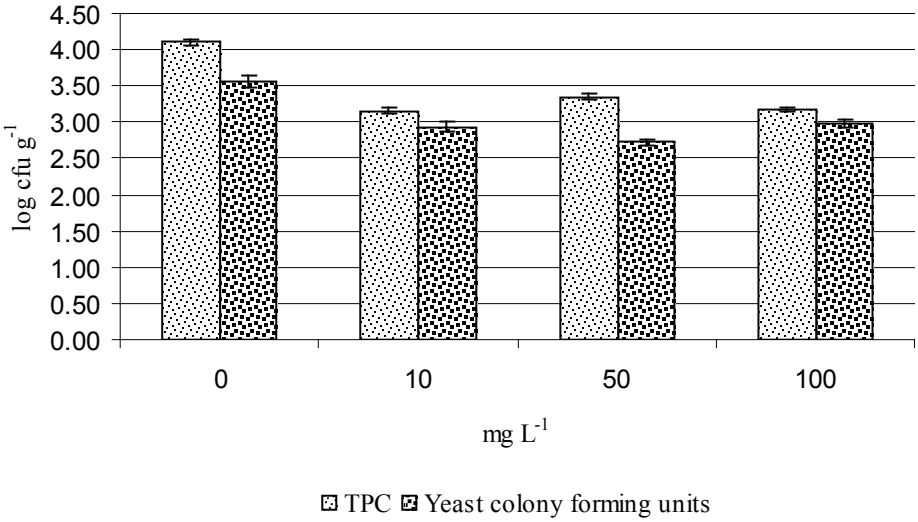

Figure 1. Different copper concentration influence on rye malt microbiological indicators.

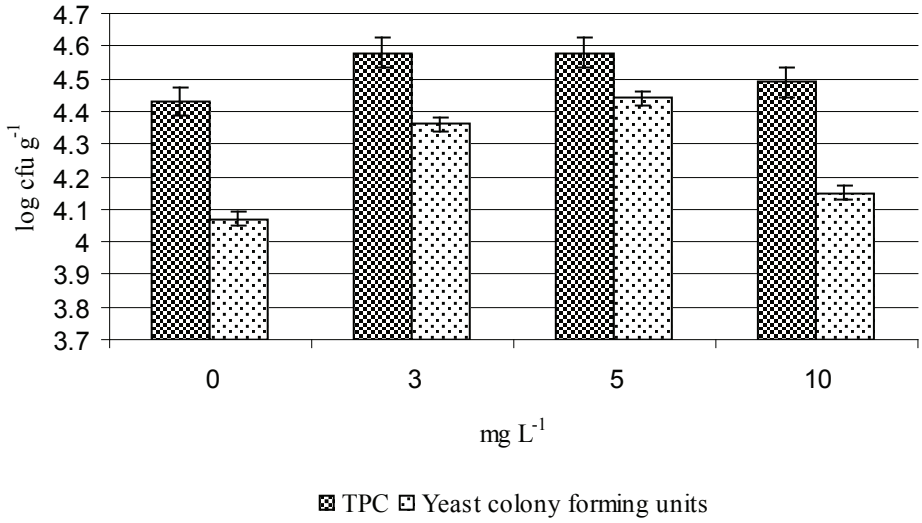

Figure 2. Different selenium concentration influence on rye microbiological indicators.

count of rye malt was 3.16, 3.25 and 3.18 log cfu g^{-1} which is considerably less compared with the control sample($p<0.05$). Different copper concentrations (10, 50 and 100 mg L^{-1}) in solution have an inhibitory effect also on yeast cells 2.94, 2.72 and 2.98 log cfu g^{-1} compared with the control sample (3.56 log cfu g^{-1}). Insufficient amount of information about copper influence on rye malt microflora can be found, but scientists have found high concentrations of copper to be toxic (Lombardi and Sebastiani, 2005).

Different selenium concentration influence on the rye malt microbiological indicators is shown in Figure 2.

Results show that no inhibitory effect on growth of microorganisms in rye malt was found by adding selenium in different concentrations. Furthermore increase of selenium concentration in solution promotes TPC and yeast plate count substantially ($p<0.05$). The total plate count in rye malt control sample (Figure 2) was 4.43 log cfu g^{-1}. At the selenium concentration in solution 3, 5 and 10 mg L^{-1} TPC was significantly higher compared to the control sample (4.58, 4.58 and 4.49 log cfu g^{-1}). At the selenium concentration in solution 3, 5 and 10 mg L^{-1} the yeast colony forming units of rye malt was 4.36, 4.44 and 4.15 log cfu g^{-1} which is considerably higher compared with the control sample ($p<0.05$). The yeast plate count in rye malt control sample was 4.07 log cfu g^{-1}. Similarly as with copper influence, few scientific articles are published about selenium influence on rye malt microbiological indicators. At low levels, selenium contributes to normal cell growth and function.

Conclusions

Increasing copper concentration in solution 10, 50 and 100 mg L^{-1}, the plate count of microorganisms and yeast colony forming units in rye malt decreases significantly ($p<0.05$). Increase of selenium concentration in solution 3, 5 and 10 mg L^{-1} promotes the total plate count and yeast colony forming units substantially.

Acknowledgements

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INVESTIGATION OF PHYSICALLY-CHEMICAL PARAMETERS OF CONVENTIONAL AND ORGANIC HULL-LESS BARLEY HARVESTED IN LATVIA

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Abstract
The main purpose of the research was to investigate the physically-chemical parameters of organic and conventional hull-less barley (*Hordeum vulgare*) harvested in Latvia. In the experiment the following hull-less barley harvested in 2012 from the State Priekuli Plant Breeding Institute (Latvia) was used: 'Irbe', PR4651, PR3808.21 and PR5099. The following quality parameters of grain – protein, starch, the bulk density and β-glucan content – were measured by standard methods – Infratec™ 1241 Grain Analyser (Denmark) corresponding to ISO 12099; thousand-grain weight (TGW) (ISO 520:2010); the falling number (ISO 3093:2009); moisture content (LVS 272:2000) were also determined. In the present experiments no significant differences in protein, moisture, starch, β-glucan content and TGW were detected, however, significant differences in the bulk density of all analyzed hull-less barley samples were established. The lower analyzed parameter value of 780 ± 1 g L^{-1} was obtained for the conventional and organic hull-less barley PR 5099. However, the higher bulk density value of 821 ± 1 g L^{-1} was obtained in the conventional hull-less barley variety 'Irbe'. No significant differences in the bulk density of conventional and organic hull-less barley 'Irbe', PR4651 and PR3808.21 were found. Significant differences in the falling number were detected in the analyzed hull-less barley samples harvested in the conventional and organic farming. The lower falling number value was obtained in the organic hull-less barley line PR 5009 – 81 ± 4 s, the higher in the conventional and organic hull-less barley line PR 3808.21 as 362 ± 5 s and 373 ± 2 s, respectively.
Key words: physically-chemical parameters, hull-less barley, organic and conventional farming.

Introduction

Cereal-based foods have been staples for humans for millennia. Cereal grains contain the macronutrients (protein, fat and carbohydrate) required by humans for growth and sustenance. They also supply important minerals, vitamins and other micronutrients essential for optimal health. However, it is becoming apparent that cereals in general have the potential for health enhancement beyond the simple provision of these nutrients and that their consumption can lower the risk of significant diet-related diseases quite substantially. This is an important attribute given the social and personal impact of these conditions (Topping, 2007).

Barley (*Hordeum vulgare* L.) is the fourth largest cereal crop produced in the world (Damiran and Yu, 2012). Cultivated barley (*Hordeum vulgare*) is the fourth largest cereal grain crop produced worldwide and the most under-utilized cereal grain in terms of human consumption. About 90% of barley grain is used in alcoholic beverage production and as a livestock feed. Barley is an excellent source of complex carbohydrates, which constitute about 80% of barley grain weight (Lia et al., 2001). Barley grains used as food for pearl barley, grits, flour (in small quantities), malt, barley coffee, alcohol and yeast. Although barley has been of little importance in the modern diet, when compared to other cereals, like wheat (*Triticum*), rye (*Secale*) and oats (*Avena sativa*), recent evidence about considerable amounts of nutritionally important β-glucan found in barley has focused a lot of attention on the matter of designing new foods containing barley. β-glucans are recognised as having an important positive health impact, centred on their benefits in case of coronary heart disease, cholesterol lowering and reducing the glycaemic response. Compared to wheat and rye grain, the highest content of natural antioxidants (copherol and tocotrienols) and of vitamin E was established in barley grain. Inclusion of barley flour in plain wheat bread formulation enhances the β-glucan content of bread, which may have a beneficial effect on human health (Škrbić et al., 2009; Lazaridou et al., 2006).

In food industry, hull-less barley is considered as more valuable and more economical compared to hulled barley. Protein content in hull-less barley is from 9 to 20% from total dry matter (Rakcejeva et al., 2007). The hull-less barley flour has a little darker colour, because compared to flour from soft wheat it has a higher ash value, and a higher protein and β-glucan content. Soluble dietary fiber, mainly β-glucan, provides a promoted viscosity. As a result, digestion, cholesterol and fat absorption are decreased. Compared with hulled barley, hull-less barley has the major diferences in the β-glucan content. Barley contains 70 mg 100 g^{-1} arabinoxylan and 25 mg 100 g^{-1} β-glucan, but hulless barly contains 75 mg 100 g^{-1} β-glucan and 20 mg 100 g^{-1} arabinoxylan. It can be expained by the fact that the hull- less barley flakes are not coalescing with a grain threshing and peeling process, reducing the amount of fiber (cellulose and arabinoxylans), while increasing β-glucan content and reducing the required energy consumption (Fastnaught, 2009).

In organic farming systems under temperate climatic conditions, cereals have lower yields compared with similar conventional systems. In organic cereal production, the management practices adopted to control weeds, pests and diseases and the optimization of nutrient availability to the crops to a large extent determine the yields obtained. As the best management practices for organic systems are still being tested for specific crop species and sites, there is a high potential to improve the organic cereal grain yields. This is in contrast with the intensive systems using high amounts of fertilizers and pesticides, where evidences of yield stagnation are now being reported (Doltra and Olesen, 2013).

The main purpose of the research was to investigate physico-chemical parameters of organic and conventional hull-less barley harvested in Latvia.

Materials and Methods

The study was conducted at the Agronomy Research Laboratory of Latvia University of Agriculture and JSC Jelgavas dzirnavas.

In the present experiment the following conventional and organic hull-less barley was harvested from the experimental and certified organic fields of the State Priekuli Plant Breeding Institute (Latvia) in 2012 – 'Irbe', PR4651, PR3808.21 and PR5099 – according to LVS 271:2000 standard method. The conventional hull-less barley used was: Podzols sod (Pv), sandy loam (ms), plant available P_2O_5 208 mg kg^{-1}, K_2O 215 mg kg^{-1} soil., pH 5.8 and 2.3% compast, embedded in the basic complex fertilizer NPK 5:20:30 150 kg ha^{-1} and ammonium nitrate 244 kg ha^{-1}. The conventional hull-less barley were sowing on 4 May. In addition on Decis Mega 0.125 l ha^{-1} and herbicide Sekators 0.1 l ha^{-1} and esthete 1.0 l ha^{-1}.

Organic hull-less barley used previous plants pea green manure. The organic hull-less barley used was: Podzols sod (Pv), sandy loam (ms), plant available P_2O_5 160 mg kg^{-1}, K_2O 93 mg kg^{-1} soil, pH 5.7 and 2.3% compost. Sowing took place on 28 May and harvested on 20 August.

- Protein, starch, bulk density and the content of β-glucan in the hull-less barley were measured by "Infratec™ 1241 Grain Analyser" (Denmark) according to ISO 12099.
- Thousand-grain weight (TGW) was measured in grams as the average weight of two different samples of 1000 grains from each line ISO 520:2010.
- The falling number of grains was analyzed using standard Hagberg-Perten method ISO 3093:2009.
- Grain moisture content was analyzed according to LVS 272:2000 standard method.

Data are expressed as mean ± standard deviation; for the mathematical data processing p-value at 0.05 (ANOVA) was calculated.

Results and Discussion

The reported values indicate that relatively small differences exist within and between varieties and that these are amplified by environmental factors (Shewry, 2007). In the present experiments protein content of analyzed grain samples range from the conventional 123 ± 1 g kg^{-1} to organic 141 ± 2 g kg^{-1} hull-less barley (Table 1). The higher protein content was found in organic hull-less barley compared to the conventional one. However, no significant differences in protein content (p=0.448) were established between the analyzed hull-less barley samples.

Water migration is a common problem in many food products in the baking industry, such as mixes of food products. Water diffuses from the wet component to the dry cereal-based one (Roca et al., 2007). Therefore, elevated moisture content in cereals could be a negative factor influencing quality parameters and shelf-life mainly, because it demands the harvest grain drying. In the present experiments the moisture content of the analyzed hull-less barley samples does not exceed 150 g kg^{-1} (Table 1). However, it is necessary to indicate, that the legislation of the Republic of Latvia (Requirements of the Cabinet of Ministers, No. 1455 from 15.12.2009) regulate that the maximum moisture content in barley cannot exceed 140 g kg^{-1}. Therefore, to provide the quality for the analyzed hull-less barley additional drying is necessary.

It is necessary to indicate, that barley endosperm is mainly composed of starch, and has many genotypes, waxy, normal and high amylose varieties, similar to other cereals (Tang et al., 2002). The amount of starch in the analyzed hull-less barley ranged from 615 ± 8 g kg^{-1} to 643 ± 11 g kg^{-1} (Table 1); significant differences were not detected (p=0.119).

Barley β-glucan is a water-soluble dietary fibre that can form highly viscous aqueous solutions at concentrations as low as 50 g kg^{-1} (Faraj et al., 2006). Research on barley β-glucan has demonstrated its multiple human health benefits. Thus, the industrial demand for this natural cereal based compound is fast growing. Functional food products containing β-glucan are now being commercially introduced to the market. Since starch is one of the major components of foods, understanding the mechanism of interaction of β-glucan with native starch and its hydrolytic products and its implication for rheological properties is highly important in order to achieve a product with a high sensory appeal (Faraj et al., 2006). No significant differences (p=0.224) were found in β-glucan content between the analyzed grain samples. However, it is

Table 1

Chemical composition of hull-less barley

Parameter	In conventional farming harvested grain				In organic farming harvested grain			
	Irbe	PR 4651	PR 3808.21	PR 5099	Irbe	PR 4651	PR 3808.21	PR 5099
Protein, g kg^{-1}	125±1	125±2	138±1	123±1	134±3	126±2	141±2	132±1
Moisture, g kg^{-1}	143±8	157±9	150±9	150±7	151±9	152±8	149±5	148±5
Starch, g kg^{-1}	626±12	635±11	615±8	643±11	621±10	641±10	619±9	640±4
b-glucan, g kg^{-1}	48±1	63±1	56±03	65±4	53±1	63±1	54±4	68±2
1000 grain weight, g	43.5±1.2	43.0±1.9	40.6±1.8	43.6±1.4	41.9±1.7	43.3±1.1	41.5±1.4	41.4±1.8

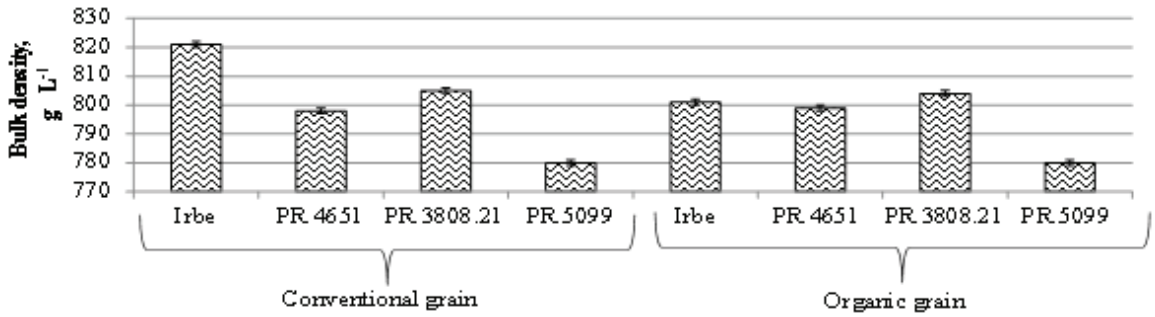

Figure 1. Bulk density of the analyzed hull-less barley.

necessary to indicate, that higher β-glucan content was detected in the organic hull-less barley (Table 1).

Thousand-grain weight (TGW), one of the three main agronomic (or numerical) components of grain yield in involved in the emergence of agriculture and crop domestication. Automatic selection due to planting and harvesting seeds of cereals may have increased seedling vigour through an increase in seed size. The TGW of the analyzed grain samples range from 40.6 ± 1.8 to 43.6 ± 1.4 g (Table 1), which is not substantially different (p=0.558).

Bulk density is a direct measure of the closeness of packing of particles in a defined volume; it depends on the local conditions when the measurement is made, and unlike a density or the skeletal density of a specified material, does not have a unique value (Davies et al., 2005). In the literature (Korunic et al., 1998) it is mentioned that bulk density of barley could be minimum of 750 g L^{-1} (Figure 1).

In the present research significant differences (p=0.009) in bulk density of all analyzed hull-less barley samples were established. Lower analyzed parameter content of 780 ± 1 g L^{-1} (Fig. 1) was obtained for conventional and organic hull-less barley line PR 5099. However, the higher bulk density value of 821 ± 1 g L^{-1} (Fig. 1), was obtained in the conventional hull-less barley variety 'Irbe'. Still, no significant differences (p=0.139) were found in the bulk density of conventional and organic hull-less barley 'Irbe', PR4651 and PR3808.21 (Fig. 1). Differences in the bulk density of the analyzed hull-less barley samples can mainly be explained with specific properties of the analysed line and variety.

The falling number traditionally is used widely in grain classification, quality control and marketing. Grain with a low falling number due to high α-amylase activity causes substantial economic losses to growers, significant processing and storage problems and is generally reflected in poorer quality end-products. Indeed with the advent of highly automated food production plants, particularly bakeries, variation in α-amylase in the starting material is now even more undesirable. Low falling number is generally associated with pre-harvest sprouting; however, it is now clear that there are a number of additional causes of low falling number (Mares and Mrva, 2008). There is no information in the legislation of the Republic of Latvia for falling number value in hull-less barley. The only information about wheat which can be found: the falling number of wheat grain could be 220–350 s, but

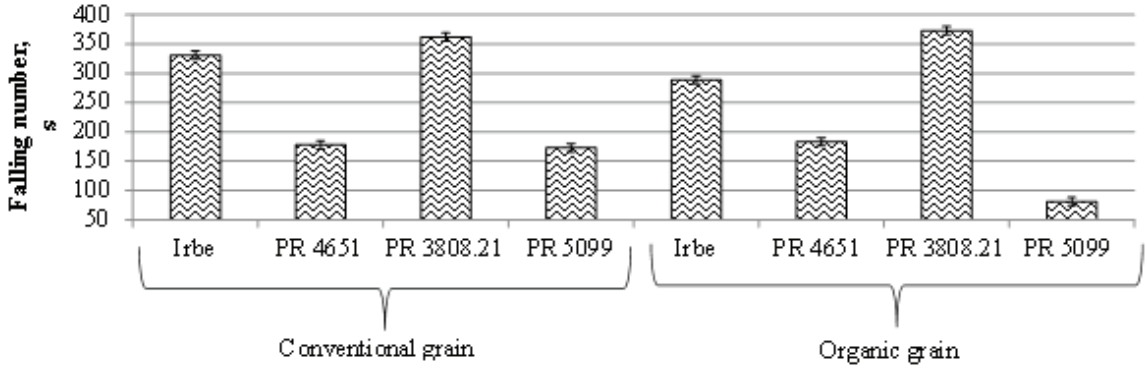

Figure 2. The falling number of the analyzed hull-less barley.

for rye it is stated that the falling number of rye grain could be 120-250 s (Requirements of the Cabinet of Ministers, No. 1455 from 15.12.2009). In the present experiment the falling number values of the analyzed hull-less barley were detected in range from 81 s to 373 s (Fig. 2).

Significant differences (p=0.116) in the falling number were found in the analyzed hull-less barley samples harvested in the conventional and organic farming. The lower falling number value was obtained in the organic hull-less barley grain line PR 5009 – 81 ± 4 s, the higher in the conventional and organic hull-less barley line PR 3808.21 as 362 ± 5 s and 373 ± 2 s respectively (Fig. 2). Low falling number value indicates possible elevated α-amylase activity; as a result quality properties of the analyzed grain worsen. The falling number can be influenced by crop conditions and weather. Weathering of the grain begins to germinate and the falling number significantly declines (German, 2006). It is necessary to indicate, that the falling number of organic hull-less barley 'Irbe' and PR 3808.21 was very close to the falling number of wheat grain 220–350 s mentioned in the literature (Requirements of the Cabinet of Ministers, No. 1455 from 15.12.2009), which mainly proves the excellent quality parameters of the grain.

Conclusions

1. In the present experiments no significant differences in protein, moisture, starch, β-glucan content and 1000 grain weight were found between the analyzed conventional and organic hull-less barley 'Irbe', PR4651, PR3808.21 and PR5099.
2. In the present research significant differences in the bulk density of all analyzed hull-less barley samples were established. Lower analyzed parameter content of 780 ± 1 g L^{-1} was obtained for the conventional and organic hull-less barley line PR 5099. However, the higher bulk density value of 821 ± 1 g L^{-1} was obtained for the conventional hull-less barley variety 'Irbe'. No significant differences were found in the bulk density of conventional and organic hull-less barley 'Irbe', PR4651 and PR3808.21.
3. Significant differences in the falling number were found in the analyzed hull-less barley samples harvested in both the conventional and organic farming. The lower falling number value was obtained in the organic hull-less barley line PR 5009 – 81 ± 4 s, higher in the conventional and biological hull-less barley line PR 3808.21 as 362 ± 5 s and 373 ± 2 s respectively.

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NUTRITIONAL VALUE AND SENSORY PROPERTIES OF YOGHURT ENRICHED WITH BARLEY GRAINS AND MALT EXTRACT

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Abstract
Growing interest of consumers in healthy eating has provided the development of new range of food. Therefore the task of research was to determine the nutritional value, calculate energy value and investigate the sensory properties of yoghurt samples enriched with flakes from biologically activated hull-less barley grains and malt extract.
Results showed that by adding flakes from biologically activated hull-less barley grain and malt extract it was possible to improve the nutritional value of yoghurt, i.e., increased protein, carbohydrate and decreased fat content. The energy value of yoghurt samples enriched with flakes from biologically activated hull-less barley grain and malt extract ranged between 65.96 and 75.72 kcal 100 g^{-1}, which is significantly lower comparing with mean energy value of commercial yoghurts. The changes of sensory properties were affected by the amount of added malt extract in yoghurt samples. The optimal amount of added malt extract for sensory evaluation in yoghurt samples was determined as 2%. The content of carbohydrate in yoghurt sample enriched with 5% of biologically activated hull-less barley grain and 2% of malt extract was two times lower than commercial yoghurts therefore its energy value was significantly lower. Yoghurt enriched with flakes from biologically activated hull-less barley grain and malt extract could be competitive.
Key words: yoghurt, nutritional value, sensory properties.

Introduction

Growing interest of consumers in healthy eating has provided the development of new range of food. The dairy sector is the one that has undergone the greatest change, with many new products claiming healthy characteristics, not all of which are equally successful (Bayarri et al., 2011). The wide variety of macronutrient-modified foods available to consumers has enabled people to eat a more healthy diet, along the lines of the recommendations, and so reduce the risk of diseases such as obesity, cardiovascular disease and cancer (Clugston and Smith, 2002). There is increased consumer demand for low fat yoghurt, due to their potential health and nutritional benefits (Prasanna et al., 2013). The nutritional image of milk fat suffers from its content of saturated fatty acids increasing serum cholesterol, which is considered as a risk factor for coronary heart disease (Steijns, 2008). Therefore customers have an interest in yoghurt with low or reduced fat content. Whereas milk proteins are potential ingredients of health-promoting functional foods targeted at diet-related chronic disease, such as cardiovascular disease, diabetes type II and obesity (Korhonen, 2009). The dairy proteins are the preferred choice in special nutrition formulas for (re)building tissues and muscle mass in infants, hospitalized individuals, performance athletes, dieters and the elderly (Steijns, 2001). Therefore it could be concluded that it is significant to produce a new dairy product with low or reduced fat and increased protein content. An important point to consider is that consumer acceptance of a new healthy product is unpredictable, because their benefits may provide added value to consumers but cannot outweight the sensory properties of foods (Siró et al., 2008). The reward value of food products by consumers depends on the sensory properties, e.g., taste, aroma, texture and appearance (Sclafani, 2004), metabolic effects, e.g., energy density and macro-nutrient composition (De Houwer et al., 2001) and learned reward association based on previous experience with the product (Zandstra and El-Deredy, 2011). These three factors influence the acceptance of a new product by consumers. Therefore the task of research was to determine the nutritional value, calculate energy value and investigate the sensory properties of yoghurt samples enriched with flakes from biologically activated hull-less barley grain and malt extract.

Materials and Methods

Materials and preparation of yoghurt samples

Pasteurized milk with a 2.5% fat content and the yoghurt culture YF-L811, containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Chr.Hansen, Denmark), were used for experiments. Yoghurt culture was stored in the freezer at -18 °C and used directly for milk fermentation.

Flakes from biologically activated hull-less barley grain (Latvia) were added to milk in concentration of 5% and malt extract (Ilgezeem, Latvia) in different concentrations (2%, 4% and 6%). Milk samples with flakes from biologically activated hull-less barley grain and malt extract were inoculated with yoghurt culture and fermented at 43 ± 1 °C for 4 hours. After fermentation the maturation of yoghurt samples was done at 5 ± 1 °C for 24 hours.

Five yoghurt samples were analyzed (Table 1). The control sample was prepared without the

flakes from biologically activated hull-less barley grain and malt extract for comparing results.

Determination of pH and lactic acid

pH of yoghurt samples was determined using pH-meter WTW series inoLAB pH 720. Lactic acid is calculated on the basic titratable acidity. The titratable acidity of yoghurt samples was determined by titration following the LVS ISO 6092:2003 using phenolphthalein as an indicator. The measurements of pH and lactic acid were carried out after yoghurt sample's fermentation, and on the 1st day.

Determination of carbohydrates content

The content of carbohydrates of the yoghurt samples was determined with high-performance liquid chromatographic (Shimadzu LC 20 Prominence). Determination parameters: detector: refractive index RID-10A; column: Alltech NH_2, 4.6 mm x 250 mm, 5µm; temperature +25 °C; isocratic elution regime, mobile phase: A – acetonitrile; B – deionized water (A70:B30); capacity of the injection sample: 10 µL; total time of the analysis: up to 25 min; rate of the flow: 1.0 mL min^{-1}.

Calculation of energetic value

The total energy of samples was calculated according to the following equations (Council Directive 90/496/EEC, 1990):

(1) Energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g lipid)

(2) Energy (kJ) = 17 × (g protein + g carbohydrate) + 37 × (g lipid)

Sensory analysis

Sensory evaluation of yoghurt samples enriched with flakes from biologically activated hull-less barley grain and malt extract was carried out on the 1st day. Eight assessors (females, aged 35–52) selected from Latvia University of Agriculture Faculty of Food Technology staff members, who consume different yoghurts and had previous taste panel experience, rated sensory properties of yoghurts. They were selected according to their willingness, availability, motivation, and previously demonstrated capability to work as a member of a sensory panel.

Four sensory properties – aroma, taste, consistency, appearance were evaluated. The intensity of each attribute was scored on a 5-point scale, according to ISO 4121:2003: 5 – excellent quality; 4 – good quality; 3 – passable, insignificant defects; 2 – bad, pronounced defects; 1 – very bad, hard pronounced defects. When evaluating the samples with 3 or lower score the assessors indicated the defects.

The characteristics of good quality yoghurt, enriched with flakes from biologically activated hull-less barley grain and malt extract correspond to the description presented in Table 2.

Table 1

Yoghurt samples description

Code	Sample
Control	Yoghurt without flakes from biologically activated hull-less barley grain and malt extract
YFBG5%	Yoghurt enriched with 5% of flakes from biologically activated hull-less barley grain
YFBG5% ME2%	Yoghurt enriched with 5% of flakes from biologically activated hull-less barley grain and 2% of malt extract
YFBG5% ME4%	Yoghurt enriched with 5% of flakes from biologically activated hull-less barley grain and 4% of malt extract
YFBG5% ME6%	Yoghurt enriched with 5% of flakes from biologically activated hull-less barley grain and 6% of malt extract

Table 2

Quality description of yoghurt enriched with flakes from biologically activated hull-less barley grain and malt extract

Sensory properties	Description
Taste	Pleasant lactic acid taste, yoghurt like with malt extract and cereals taste, clean, refreshing, slight acid taste
Aroma	Lactic acid aroma, intensive, clean, refreshing aroma
Consistency	Uniform and compact with cereals flakes, creamy not lumpy, without syneresis
Appearance	Intense white to slightly creamy/yellow/brown, if more of malt extract is added colour can be brown

Table 3

Effect of flakes from biologically activated hull-less barley grain and malt extract in yoghurt samples on lactic acid and pH

Yoghurt samples	Lactic acid, %		pH	
	After fermentation	1st day	After fermentation	1st day
Control	0.767±0.009	0.802±0.010	4.35±0.03	4.35±0.03
YFBG5%	0.721±0.007	0.787±0.008	4.47±0.04	4.54±0.03
YFBG5% ME2%	0.747±0.007	0.799±0.007	4.49±0.03	4.52±0.02
YFBG5% ME4%	0.777±0.010	0.901±0.012	4.42±0.02	4.44±0.03
YFBG5% ME6%	0.788±0.007	0.897±0.008	4.33±0.04	4.43±0.04

Table 4

Nutritional and energy value of yoghurt enriched with flakes from biologically activated hull-less barley grain and malt extract

Yoghurt samples	Protein (g 100 g^{-1}) (Beitane, 2013)	Fat (g 100 g^{-1}) (Beitane, 2013)	Carbohydrate (g 100 g^{-1})	Energetic value	
				kcal 100 g^{-1}	kJ 100 g^{-1}
Control	3.48	2.40	6.08	59.84	251.32
YFBG5%	3.81	2.31	7.28	65.15	274.00
YFBG5% ME2%	3.85	2.28	7.51	65.96	277.48
YFBG5% ME4%	3.77	2.23	8.52	69.23	291.44
YFBG5% ME6%	3.84	2.12	10.32	75.72	319.16

Samples of yoghurts for sensory evaluation were presented in coded glass containers (approximately 50 g products) and served at 12 ± 2 °C. Between one sample and the next assessors used warm black tea to cleanse their palates.

Statistical analysis

The measurements of pH and titratable acidity as well the analyses of carbohydrate content in yoghurt samples were performed in triplicate. The results of research were analyzed 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. Tukey's test was used for multiple comparisons of sensory attributes at $p<0.05$.

Results and Discussion

The pH and titratable acidity changes in the control (yoghurt without flakes from biologically activated hull-less barley grain and malt extract) and experimental yoghurt samples after fermentation and on the 1st day is shown in Table 3.

pH of commercial yoghurts is largely variable, ranging from 3.7 to 4.6 (Souza, 1991). Nevertheless, to avoid insipidness or excess acidity to the taste, the optimal value of pH should be in the range 4.0-4.4 (Oliveira et al., 2011). The obtained results showed that pH of all yoghurt samples ranged from 4.33 to 4.54, which is close to the optimal value. Evaluating the data obtained for lactic acid it could be concluded that after the yoghurt samples fermentation lactic acid continued to increase, which provided in yoghurt existent lactic acid bacteria (LAB), whose activity influenced the added flakes from biologically activated hull-less barley grain and malt extract in concentration of 4% and 6% in yoghurt. Then the highest value of lactic acid (YFBG5% ME4% – 0.897% and YFBG5% ME6% – 0.901%) was determined.

The significant result, obtained from the evaluation of the new product is this increased nutritional and decreased energy value. Therefore the nutritional and energy value of yoghurt enriched with flakes from biologically activated hull-less barley grain and malt extract are summarized in Table 4.

By adding flakes from biologically activated hull-less barley grain and malt extract it was possible to improve the nutritional value of yoghurt, i.e., increased protein and decreased fat content. However, the changes of total protein and fat content in yoghurt samples enriched with flakes from biologically activated hull-less barley grain and malt extract and control were insignificant ($p>0.05$), in common with effect of added malt extract in different concentrations was insignificant ($p>0.05$), too (Beitane, 2013). The changes of carbohydrate content in yoghurt samples enriched with flakes from biologically activated hull-less barley grain and malt extract were significant

comparing with control ($p<0.05$). Furthermore, the increase of carbohydrate content in yoghurt samples affected the concentration of added malt extract. The content of carbohydrate in yoghurt samples enriched with flakes from biologically activated hull-less barley grain and malt extract ranged between 7.51 and 10.32 g 100g^{-1}. Carbohydrate content in YFBG5% ME6% sample significantly differed from YFBG5% ME2% and YFBG5% ME6% samples ($p<0.05$).

The calculation of energy value of analysed samples showed that it is possible to produce new products with low energy value, which have significant point for acceptance by consumers. The energy value of yoghurt samples enriched with flakes from biologically activated hull-less barley grain and malt extract ranged between 65.96 and 75.72 kcal 100 g^{-1}, which is significantly lower comparing with mean energy value of commercial yoghurts (Table 5). However, it is known that consumer behaviour about food choice is determined not only by nutritional and energy value but also sensory evaluation of particular food product. Good quality yoghurt should possess pleasant odor and flavor and, especially with the set yoghurt, the defect of syneresis, which relates to the appearance and mouthfeel, can adversely affect acceptability or preference of consumers (Srisuvor et al., 2013). The changes of sensory properties in yoghurt samples enriched with flakes from biologically activated hull-less barley grain and malt extract are showed in Figure 1.

Evaluation of intensity of sensory properties of yoghurt enriched with flakes from biologically activated hull-less barley grain and malt extract shows that there is no significant difference ($p>0.05$) in appearance, aroma and consistence, but there exist significant difference in intensity of taste ($p<0.05$). The obtained results suggested that more intensive taste was established to samples YFBG5% ME2% and YFBG5%.

The changes of sensory properties were affected by the amount of added malt extract in yoghurt samples. The optimal amount of added malt extract for sensory evaluation in yoghurt samples was determined as 2%. Therefore the yoghurt sample enriched with 5% of flakes from biologically activated hull-less barley grain and 2% of malt extract (YFBG5% ME2%) was selected for nutritional and energy value comparing with equal commercial yoghurt (Table 5).

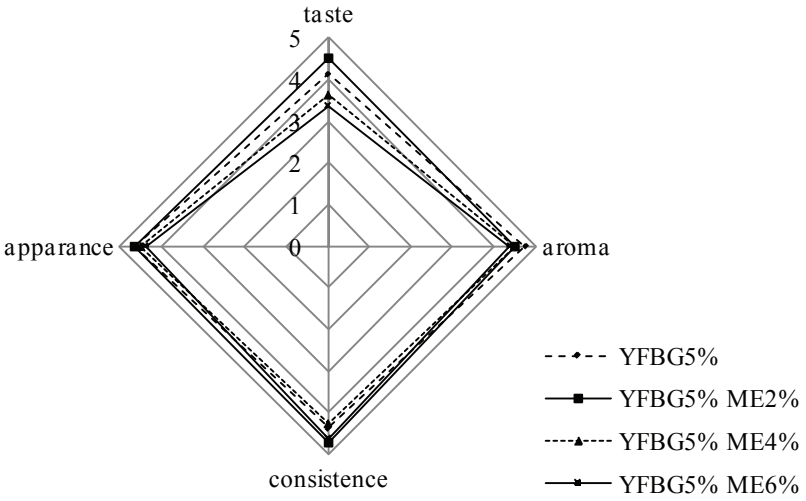

Figure 1. Intensity of sensory properties of yoghurt enriched with flakes from biologically activated hull-less barley grain and malt extract.

Table 5

Nutritional and energy value comparison of analysed and commercial yoghurts

Samples	Protein (g 100 g^{-1})	Fat (g 100 g^{-1})	Carbohydrate (g 100 g^{-1})	Energetic value	
				kcal 100 g^{-1}	kJ 100 g^{-1}
YFBG5% ME2%	3.85	2.28	7.51	65.96	277.48
Ecological yoghurt with apples and grain*	4.10	2.50	14.80	98.10	413.80
Drinking yoghurt with grain and seeds*	3.10	1.90	14.30	86.70	366.10

*Commercial yoghurts with declared nutritional value on label

During the study significant differences of carbohydrate content among YFBG5% ME2% sample and commercial yoghurts were determined. The content of carbohydrate in YFBG5% ME2% sample was two times lower as in commercial yoghurts. It affected the energy value decrease of YFBG5% ME2% sample. Therefore, it could be concluded that yoghurt enriched with flakes from biologically activated hull-less barley grain and malt extract could be competitive.

Conclusions

1. By adding the flakes from biologically activated hull-less barley grain and malt extract it was possible to change the nutritional value of yoghurt, i.e., increased protein, carbohydrate and decreased fat content.
2. The energy value of yoghurt samples enriched with flakes from biologically activated hull-less barley grain and malt extract ranged between 65.96 and 75.72 kcal 100 g^{-1}, which is significantly lower comparing with the mean energy value of commercial yoghurts. The carbohydrate content in yoghurt sample enriched with 5% of flakes from biologically activated hull-less barley grain and 2% of malt extract was two times lower as in commercial yoghurts.
3. The changes of sensory properties were affected by the amount of added malt extract in yoghurt samples. The optimal amount of added malt extract for sensory evaluation in yoghurt samples was determined as 2%.
4. Yoghurt enriched with flakes from biologically activated hull-less barley grain and malt extract could be competitive.

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THE EFFECT OF PRODUCTION AND STORAGE ON THE CONTENT OF VITAMIN C IN NFC ORANGE JUICE

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Abstract
The traditional consumption of citrus juices as a breakfast beverage has historical ties to obtaining one's daily requirement of vitamin C. Vitamin C is one of the most important vitamins found in citrus juices, including orange juice.
The aim of the study was to determine the effect of processing and storage on the content of vitamin C in orange juice varieties of 'Valencia' and 'Navel' of orange juice. The content of vitamin C (mg 100 g^{-1}) was determined with the iodine method in freshly squeezed orange juices and in pasteurized and packaged in aseptic bags orange juices of 'Valencia 'and 'Navel' variety, after processing and during 24 weeks of storage at 20 ± 1 °C and 5 ± 1 °C.
The study results showed that the average content of vitamin C in freshly squeezed orange juice 'Valencia' was found at 33.56 mg $100g^{-1}$ but in freshly squeezed orange juice 'Navel' the content of vitamin C was higher than in 'Valencia' variety orange juice average at 46.18 mg $100g^{-1}$. The loss of vitamin C for both orange juices on average about 7% during processing
The storage study showed that after 24 weeks of storage at 5 ± 1 °C and at 20 ± 1 °C, in pasteurized orange juice 'Valencia' the content of vitamin C decreased by 9.83% and 19.73 respectively. In the pasteurized orange juice 'Navel' the content of vitamin C decreased by 8.53% and 15.24% respectively.
Key words: 'Valencia', 'Navel' NFC orange juice, vitamin C, storage.

Introduction

The traditional consumption of citrus juices as a breakfast beverage has historical ties to obtaining one's daily requirement of vitamin C. Because of its refreshing taste and wholesome nature, orange juice dominates the fruit juice market. Orange juice has long been known to be an excellent source of vitamin C and is a product desired by many consumers who are interested in maintaining a healthy diet (Lee and Coates, 1999). Vitamin C is one of the most important water soluble antioxidants in citrus juices, including orange juice. It protects compounds in extracellular and intracellular spaces in most biological systems and reduces tocopherol radicals back to their active form at the cellular membranes (Kaur and Kapoor, 2001). Based on available biochemical, clinical, and epidemiological studies, the current recommended daily acceptance (RDA) for ascorbic acid is suggested to be 100–120 mg day^{-1} to achieve cellular saturation and optimum risk reduction of heart diseases, stroke and cancer in healthy individuals (Naidu, 2003). The vitamin C content in orange juices ranges from 150 to 450 mg per Litre; one glass of orange juice (200 mL) can deliver about 30 – 80% of recommended daily intake of vitamin C (Gliszczynska – Swiglo et al., 2004).

Factors influencing the vitamin C contents of citrus fruits include the production factor and climate condition, maturity state and position on the tree, type of fruits (species and variety), handling and storage, type of container (Nagy, 1980). High nitrogen fertilizer rates can lower vitamin C levels in citrus fruits. Vitamin decreases during the ripening process. Immature fruit has the highest levels of vitamin C. Since early season orange fruits have more vitamin C than oranges from later season, a noticeable difference in vitamin C in orange NFC from month to month was expected. In cans, which are not used very much today, it was found that enamel-lined cans had higher losses of vitamin C than plain tin cans. This was due to residual oxygen and vitamin C reacting with the tin. Glass packed orange juice provides poor retention of vitamin C, losing 10% after 4 months of storage. Older cardboard cartons lost up to 20%. (Today, most cartons have specially designed multi-layered oxygen and light barriers to protect both loss of vitamin C, flavour, and to enhance shelf-life.) Frozen concentrated orange juice (FCOJ) packed in foil-lined cardboard cans retained greater than 90% of their vitamin C after 12 months at -20 °C (Nagy, 1980). High storage temperatures combined with oxygen are the main factors involved in quality deterioration over time. Oxygen is the most destructive ingredient in juice causing degradation of vitamin C. During processing steps, the amount of oxygen present in system has an important impact on juice quality; the aerobic degradation of vitamin C predominates, whereas during orange juice storage both pathways must be taken into account. However, one of the major sugar found in orange juice, fructose, can also breakdown the cause of vitamin C. The higher fructose content, the greater the loss of vitamin C. Conversely, higher acid level of citric acid and malic acid stabilise vitamin C (Orange Book, 2004).

Citrus juices are sensitive to heat. Their vitamin C content and delicate fresh aroma and flavour may be

lost or damaged by undue exposure to heat, so they are usually pasteurized as rapidly as possible (Uelgen and Oezilgen, 1993). For an ideal theoretical process requiring four-long cycles of microbial reduction, the optimum pasteurization conditions are 12 minutes at 75 °C and pH 2.7. The natural pH of juices varies with the variety of oranges A.C. Polydera et al., 2003, reported that ascorbic acid degradation rates were lower for high pressurised juice, leading to an extension of its shelf life compared to conventionally pasteurised juice. Based on ascorbic acid retention, the increase of shelf life of high pressurised juice stored in bottles compared to thermally pasteurised one ranged from 11% (storage at 15 °C) to 65% (storage at 0 °C). Respective values of shelf life increase for juices in pouches were 24% and 57%. Concentration of vitamin C is a significant indicator that all processes, which ensure a high quality of product, have been applied in the production processes (Post, 1998).

The type of packaging materials has been shown to affect the orange juice quality and shelf life (Ros-Chumillas et al., 2007). Monolayer PET (polyethylene terephthalate) showed the lowest retention of ascorbic acid during storage compared with multilayer PET and glass. However, this difference in vitamin retention can be minimized by using oxygen scavengers, liquid nitrogen drop in headspace during filling, aluminium foil seal in screw cap, and the use of refrigeration. The presence of oxygen in the headspace of juice decreased the ascorbic acid, and darkened of colour during storage. The juice packaged in clear PET bottles when packed in carton had a shelf - life of 90 days to the 54 days in PET bottles when stored at 4 °C. In a subsequent study, Beltran-Gonzalez et al. (2009) suggested the use of Tetra pack carton for improved colour, ascorbic acid, and customer acceptance of orange juice stored at 4 °C.

The popularity of orange juice is certainly due to its pleasant and refreshing flavour plus consumers know that they get the nutritional benefits from vitamin C, folic acid and the dietary fibre in one serving. Not-From-Concentrate (NFC) juice is a product that today is the closest match to fresh juice in a convenient ready-to-serve package. It meets consumer's desires for improved flavour, for less-processed products, and for more natural juicy bits of orange. Year round supply of the product is possible as a large volume of juice is stored utilizing various storage technologies. Aseptic bulk storage in tank farms, Bag-In-Drum and Bag-In-Box systems, and frozen storage are commonly used. Aseptic storage of NFC became a necessity in the industry as the market expanded. NFC was first stored in frozen block form. For large volumes, aseptic storage is more viable economically because it is easily of handling and energy gains as compared to frozen storage. Aseptic juice handling technology will continue to grow, as new applications are required in juice blending and transfer operations (Johnson, 2008).

The aim of the study was to determine the effect of processing and storage on the content of vitamin C, in orange juice varieties of 'Valencia' and 'Navel' of orange juice.

Materials and Methods

The study was carried out in production laboratory 'Biofresh S.A.' producer in Laconia, Greece, from March 2010 till February 2011.

The objects of the study were juices obtained from orange summer variety 'Valencia'- and winter variety 'Navel'. Fresh-squeezed orange juice was prepared by special juice press. Samples of pasteurized orange juices, packaged aseptically (1L) in aseptic bags, (made from five-layer aluminizing materials with barrier property), were pasteurized in pasteuriser with a tubular heat exchanger (RossiCatelli) at 82 °C for 30 s.

The experiment was divided into two parts.

The content of vitamin C was analysed in freshly squeezed and pasteurized orange juice obtained from orange delivered for processing from orange delivered for processing at different times after storage.

The content of vitamin C was determined in fresh squeezed orange juice after pressing and of orange juice immediately after pasteurization.

The freshly squeezed pasteurized and packaged aseptically samples of each variety orange juice obtained on 18.06.2010 (Navel) and 18.02.2011 (Valencia) were stored for 2, 12 and 24 weeks at 20 ± 1 °C and 5 ± 1 °C and analysed to determine the vitamin C content. The analysis was carried out at 20 ± 1 °C, and was repeated 3 times.

The decrease of vitamin C was calculated as function of storage time (%) of initial concentration.

All chemical used were obtained from Merck in Athens, unless otherwise stated, and were of analytical grade purity. During the study double distilled water was used. The content of ascorbic acid (vitamin C) (mg 100 g^{-1}) was determined with iodine method (Moor et al., 2005).

Vitamin C standard solution was prepared by dissolving 0.250 g of vitamin C in 100 mL of water and then diluted to 250 ml with water in a volumetric flask.

Data were statistically elaborated using MS Excel variance analysis, significance level at $p < 0.05$.

Results and Discussion

The primary purpose of pasteurization in food processing is to destroy pathogenic organisms and also inactivate enzymes. Thermal processing continues to

Table 1

Changes of the content of vitamin C in orange juices 'Valencia' and 'Navel' during season and processing (mg 100g^{-1})

Date	'Valencia' orange		Date	'Navel' orange	
	Freshly squeezed juice	Pasteurized juice		Freshly squeezed juice	Pasteurized juice
	Vitamin C (mg 100 g^{-1})			Vitamin C (mg 100 g^{-1})	
19.03.2010	42.24 ± 0.05	39.34 ± 0.05	17.03.2010	32.26 ± 0.05	30.06 ± 0.05
20.04.2010	35.86 ± 0.05	33.62 ± 0.05	22.04.2010	40.48 ± 0.05	39.29 ± 0.05
18.05.2010	38.72 ± 0.05	36.02 ± 0.05	23.11.2010	49.28 ± 0.05	45.76 ± 0.05
18.06.2010	36.24 ± 0.05	33.56 ± 0.05	20.12.2010	48.72 ± 0.05	45.22 ± 0.05
15.07.2010	35.96 ± 0.05	33.03 ± 0.05	19.01.2011	59.84 ± 0.05	54.94 ± 0.05
18.08.2010	38.72 ± 0.05	35.84 ± 0.05	18.02.2011	49.64 ± 0.05	46.18 ± 0.05
20.09.2010	33.33 ± 0.05	30.83 ± 0.05	-	-	-
18.10.2010	30.46 ± 0.05	29.36 ± 0.05	-	-	-
Average in the season	36.44 ± 0.05	33.95 ± 0.05	Average in the season	46.7 ± 0.05	43.58 ± 0.05
Loses %	-	6.8	Loses %	-	6.7

be the most widely used method of preserving and extending the shelf-life of foods (Awuah et al., 2007). The thermal pasteurisation conditions used (82 °C, 30 s) were selected to be the same as in a conventional pasteurisation of industrially produced orange juice. The content of vitamin C in freshly squeezed orange juices and pasteurized orange juice of two different varieties of oranges ('Valencia' and 'Navel') delivered for processing at different times are presented in Table 1. In freshly squeezed orange juice 'Navel' the content of vitamin C is higher than in freshly squeezed 'Valencia' orange juice. Fresh squeezed orange juice 'Navel' the average content of vitamin C was 46.70 mg 100 g^{-1} while in 'Valencia' fresh squeezed orange juice the average content of vitamin C was 36.44 mg 100 g^{-1}. At the fruit processor, the loss of vitamin C from orange fruit to orange juice is generally negligible when the right processing conditions and short fruit storage times before extraction are used. But an important problem associated with orange juice quality is vitamin C loss during heat treatment (Lima et al., 1999; Manso et al., 2001). In the analysed orange juices the losses of vitamin C for both varieties of orange juices were average about 7% during processing. It depends on initial content of

Table 2

Changes of the content of vitamin C in orange juices during storage at 5 °C and 20 °C

Weeks of storage	Vitamin C content mg 100 g^{-1}	
	5 ± 1 °C	20 ± 1 °C
Orange juice 'Valencia'(18.06.2010)		
Freshly squeezed orange juice	36.24 ± 0.05	36.24 ± 0.05
Pasteurized orange juice	33.56 ± 0.05	33.56 ± 0.05
2	32.26 ± 0.05	32.06 ± 0.05
12	31.48 ± 0.05	30.26 ± 0.05
24	30.26 ± 0.05	26.94 ± 0.05
Orange juice 'Navel'(18.02.2011)		
Freshly squeezed orange juice	49.64 ± 0.05	49.64 ± 0.05
Pasteurized orange juice	46.18 ± 0.05	46.18 ± 0.05
2	45.02 ± 0.05	44.56 ± 0.05
12	43.74 ± 0.05	42.82 ± 0.05
24	42.22 ± 0.05	39.14 ± 0.05

vitamin C in fruits. The obtained results indicate little changes in the content of vitamin C in fruits during storage.

During storage, vitamin C decreased gradually with storage time. After analysing vitamin C content in pasteurized orange juice 'Navel' and 'Valencia' it was found that vitamin C is the most affected by the temperature in storage. The increase of temperature caused a distinct decrease in the content of vitamin C. Initial vitamin C contents of 'Valencia' and 'Navel' orange juices were 33.56 and 46.18 mg100 g^{-1}. After twenty four weeks of storage at 5 ± 1 °C and 20 ± 1 °C the content of vitamin C decreased by 30.26 mg 100 g^{-1} and 26.94 of 'Valencia' pasteurized orange juice and to 42.22 and 39.14 mg 100 g^{-1} in 'Navel' pasteurized orange juice respectively (Table 2).

The storage study showed that after 24 weeks of storage at 5 ± 1 °C and at 20 ± 1 °C, in pasteurized orange juice 'Valencia' the content of vitamin C decreased by 9.83% and 19.73 respectively (Figure 1).

After 24 weeks of storage at 5 ± 1 °C and at 20 ± 1 °C, in pasteurized orange juice 'Navel' the content of vitamin C decreased by 8.53% and 15.24% respectively (Figure 2).

Ascorbic acid in both varieties of pasteurized orange juices decreased at all storage temperature during time. Orange juice Valencia had higher decrease than Navel orange juice samples at both storage temperatures. Although, a significant decrease in vitamin C in orange juices stored at 5 and 20 °C was observed, these juices still lose decrease appropriate quality with respect to vitamin C content. The results presented are in line with the data obtained by Klimczat et al., (2007) who reported a 21% decrease in ascorbic acid content in commercial orange juices after 6 months storage at 18 °C.

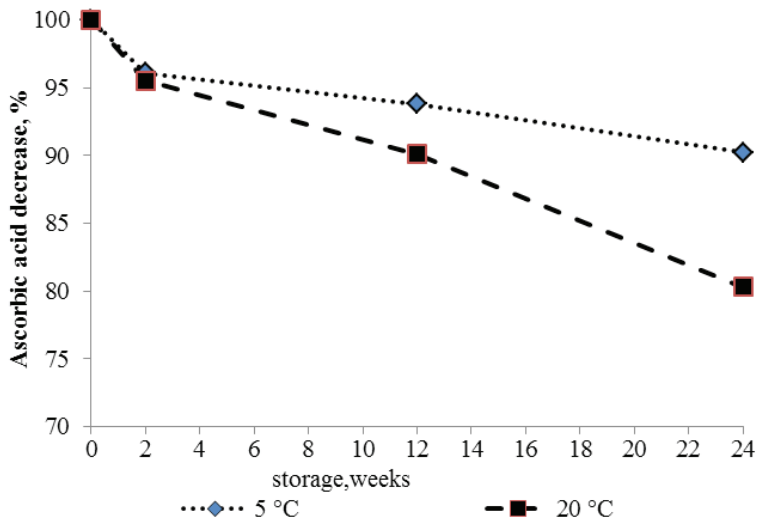

Figure 1. The decrease of vitamin C content in 'Valencia' orange juice during storage.

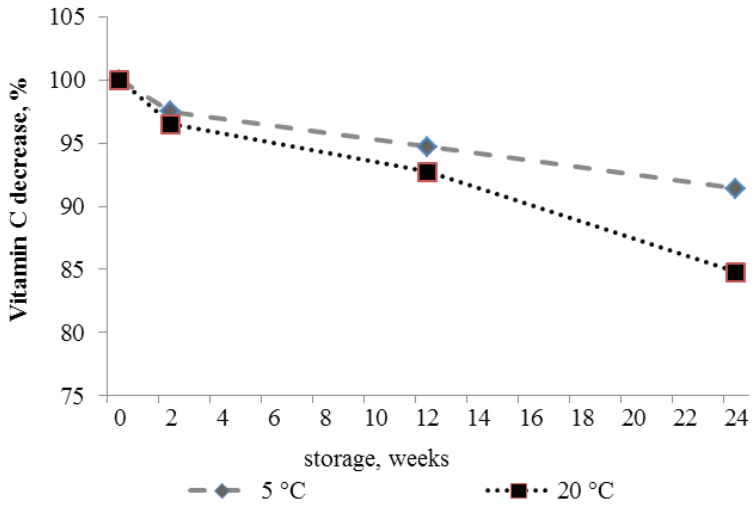

Figure 2. The decrease of vitamin C content in 'Navel' orange juice during storage.

Conclusions

1. The research data showed that the content of vitamin C in orange variety depends upon circumstances - processing and storage.
2. The study results showed that the average content of vitamin C in season in freshly squeezed orange juice 'Valencia' was found on average 36.44 mg 100 g^{-1} but in freshly squeezed orange juice 'Navel' the content of vitamin C is on average for at 46.7 mg 100 g^{-1}, which is higher than in freshly squeezed orange juice 'Valencia'.
3. The loss of vitamin C for both orange juices was on average about 7 % during processing.
4. The study showed that after 24 weeks of storage at 5 ± 1 °C and at 20 ± 1 °C, in pasteurized orange juice 'Valencia' the content of vitamin C decreased by 9.83% and 19.73 respectively.
5. In pasteurized orange juice 'Navel' the content of vitamin C decreased by 8.53% and 15.24% respectively.

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INTERACTION OF SELENIUM AND VITAMIN E IN EGGS AND EGG YOLK OIL

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Abstract
In modern life oxidative stress has a serious effect on human health, therefore, natural antioxidants play an important role in human wellbeing. Hen (*Gallus gallus domesticus)* eggs can be effectively enriched with selenium and vitamin E and can be used as a source of natural antioxidants. The objective of this study was to determine the interaction of selenium and vitamin E in eggs and egg yolk oil. Two groups of Lohman Brown-Classic breed laying hens from a real production facility were taken as an experimental object. The basal diet was the same for both groups and content 25 mg kg^{-1} of vitamin E added. 0.2 mg kg^{-1} of sodium selenite was used as an inorganic source of selenium in feed for one hen group and 0.3 mg kg^{-1} of organic selenium in the selenized yeast form for the other group. The selenium content was determined in eggs, egg yolk, egg white and egg yolk oil samples and vitamin E content in egg yolks and egg yolk oil. The results of this study indicate that there is no difference in selenium content in eggs from inorganic and organic selenium hen diet taking into account that selenium content in feed was different. The majority of selenium is located in egg yolk, but there was no selenium detected in egg yolk oil. Vitamin E content in egg yolks was not affected by the source of selenium ($p<0.05$). High vitamin E content in egg yolk oil effectively protects egg yolk oil from oxidation during storage.
Key words: egg yolk oil, selenium, vitamin E, lipid oxidation.

Introduction

Nowadays oxidative stress may play an important role in many chronic diseases, but natural antioxidants such as vitamin E can delay or prevent steps in atherogenesis. Results from large-scale human observational studies suggest that antioxidant consumption reduces the risk of developing cardiovascular disease (Gaziano, 2004).

Selenium is very important for life, and reasonable amounts of this element are required for good human health. Selenium plays an important role in several metabolic processes including thyroid hormone metabolism, antioxidant defense systems, and immune function. Selenium is incorporated into proteins to make selenoproteins. Selenoproteins are important antioxidant enzymes. These enzymes help to prevent cellular damage from free radicals. Free radicals may contribute to the development of dangerous diseases such as cancer and heart disease (McKanzie et al., 1998; Brown and Arthur, 2001; Finley, 2007; Papp et al., 2007).

Selenoproteins and vitamin E health benefits in its antioxidant properties are well known and it is good to have a product which contains both of them. Probably the best food product which can be enriched with selenium and vitamin E is a hen (*Gallus gallus domesticus*) egg. Eggs enriched with selenium and vitamins are the most popular 'designer food' thanks to very effective selenium and oil soluble vitamin transfer from the hen feed to the egg (Jiang et al., 1994; Grobas et al., 2002; Jiakui and Xialong, 2004; Bennet and Cheng, 2010). Usually, designer eggs are produced containing high concentration of vitamin E and selenium (Jacob and Miles, 2000).

Supplementation of the hen feed with organic selenium significantly increases selenium content in eggs comparing to inorganic source of feed supplement (Payne et al., 2005; Surai, 2006). The majority of the selenium in egg is located in the yolk (Surai and Dvorska, 2001; Jiakui and Xialong, 2004), but there was no information found about selenium content in egg yolk oil extracted from selenium enriched eggs. Selenium is incorporated into proteins (selenoproteins), so, theoretically, it cannot be found in egg yolk oil as organic selenium.

High vitamin E content and potential presence of selenium in egg yolk oil must significantly decrease egg yolk susceptibility to lipid peroxidation, prolonging high nutritional properties of egg yolk oil.

Due to the fact that egg yolk oil contains high levels of oil soluble vitamin, phospholipid and polyunsaturated fatty acid content it can be used in different food products increasing their nutritional value and giving characteristic egg flavor to the products like mayonnaise, dressings, bakery products and others. Egg yolk oil can be used as a supplement for infant nutrition resembling the fatty acid profile of human milk (Simopoulos and Salem, 1992).

A lack of information about the possible interaction of selenium and vitamin E in eggs and egg yolk oil prompted for this research. The aim of this study was to determine the interaction of selenium and vitamin E in eggs and egg yolk oil.

Materials and Methods

The eggs for experiment were collected from two groups of Lohmann Brow-Classic breed laying hens from production facilities at Balticovo AS, Iecava

area, Latvia. Each group contains one hundred fifty thousand hens. Bird age (60 weeks), productivity and housing conditions were the same for both groups. The basic feed was equal for both groups and contained 25 mg kg^{-1} of vitamin E added. Feed of the first group contained 0.2 mg kg^{-1} of inorganic selenium (sodium selenite) and other group feed contained 0.3 mg kg^{-1} of organic selenium (selenized yeast 'Sel-Plex' from Alltech). Hens were fed both diets from age of 28 weeks. From each hen group ninety eggs were collected as a representative sample. Sixty eggs from each group were cracked to separate egg yolks from egg whites. Egg yolks were pooled together and homogenized getting one pooled sample. The same was done with egg whites. Egg whites were separated from egg yolk manually to be sure that egg whites are totally free from egg yolk traces. For the whole egg sample thirty eggs from each group were cracked and homogenized getting a pooled sample.

A part of egg yolk was taken for egg yolk oil extraction, the other part and other samples of whites and whole egg were frozen at -18 ºC temperature until selenium and vitamin E were analyzed.

For egg yolk oil extraction one part of ethanol was mixed with two parts of chloroform by volume and poured in the beaker. Homogenized egg yolks were added to the solvent mixture with a thin squirt vigorously mixing. The ratio between the solvent mixture and egg yolk was 2:1. Extraction was done at +21 ºC for 30 minutes. All chemicals and solvents used in the egg yolk oil extraction were with analytical grade from Sigma Aldrich. The solvent mixture was filtered through a filter paper and collected in a clean container. The oil was recovered by evaporation off the solvent mixture using rotary evaporator Laborora 4000 – efficient (Heidolph Instruments GmbH and Co. KG) at +55 ºC temperature under the vacuum.

Selenium in whole egg, egg yolk, egg white and egg yolk oil was determined in accordance with DIN EN ISO 17294-2 (E29), ICP-MS (inductively coupled plasma mass spectrometry).

Vitamin E in egg yolks and egg yolk oil was determined in accordance with standard method EN ISO 12822:2000, HPLC-FLD (high performance liquid chromatography with fluorescent detection).

Selenium and vitamin E analysis were carried out in Eurofins, WEJ Contaminants GmbH laboratory, Germany.

Egg yolk oil peroxide value was determined by iodometric titration method (Standard method EN ISO 3960:2010: E29) and was carried out in Balticovo AS laboratory, Latvia. To speed up the egg yolk oil oxidation, the egg yolk oil samples were kept at +37 ºC in an open beaker (Dvorska et al., 2003), the control samples were kept in the refrigerator at +4 ºC in a hermetically closed container.

Values represented are the means and standard deviation for three replicates. Means were compared by T-test and analysis of variance (ANOVA). Significance was defined at $p<0.05$. Statistical analysis was carried out by Microsoft Excel 2010 version software.

Results and Discussion

A lot of studies reveal that supplementation of hen diet with organic selenium results in higher selenium content in eggs comparing to inorganic (sodium selenite) selenium (Payne et al., 2005; Surai, 2006). Our results (Figure 1) show that there is a difference of selenium content in eggs ($p<0.05$), but there was also difference in the selenium content in the feed. Selenium content in both samples was low, on average 0.19 mg kg^{-1} with inorganic selenium supplementation and 0.24 ± 0.01 mg kg^{-1} with organic selenium supplementation.

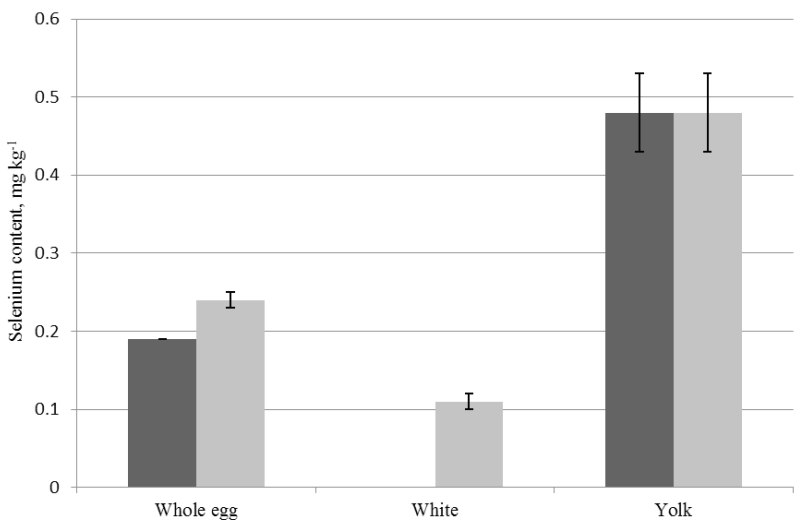

Figure 1. Selenium distribution in eggs: ■ - sodium selenite, ■ - organic selenium.

Due to the low selenium content in feed the selenium content in eggs was not so high as that was mentioned in other studies (Jiakui and Xialong, 2004; Mohiti-Alsi et al., 2008; Bennet and Cheng, 2010; Aljamal, 2011). In other studies organic selenium supplementation was at the level from 1 mg kg^{-1} to 5 mg kg^{-1} (Bennet and Cheng, 2010) in comparison with our level of 0.3 mg kg^{-1}. The samples of eggs for this research were collected from real production facilities when laying hen groups were 150,000 each, comparing to other researchers (Jiakui and Xialong, 2004; Mohiti-Alsi et al., 2008; Bennet and Cheng, 2010; Aljamal, 2011) who used experimental groups from fifty to one hundred fifty hens divided by 2 or 3 birds in separate cages with perfectly controlled environment and feeding. There are many factors which can affect results of analysis from the real production, like bird health, stress and others. In many studies the age of birds were around sixty weeks, the same as in our research, so the age of birds cannot be the main factor of difference.

D.C. Bennet and K.M. Cheng (2010) declare linear correlation of supplemented organic selenium in hen feed and selenium content in eggs. They admit that production of eggs containing 0.8, 1.6, or 3.7 mg kg^{-1} of selenium would necessitate feeding hens diets containing 1.3, 2.8, or 5.7 mg of selenium kg^{-1}, respectively. Our results show 80% transition level of organic selenium from feed to the egg, where 0.3 mg kg^{-1} of organic selenium in feed gave 0.24 ± 0.01 mg kg^{-1} in eggs, but in case of inorganic selenium transition level was 95%, where 0.2 mg kg^{-1} in feed gave on average 0.19 mg kg^{-1} in eggs. Based on these results we cannot confirm that organic selenium in feed results in higher selenium content in eggs than inorganic selenium. But the presence of selenium in both egg samples allowed us to analyze selenium distribution in eggs depending on selenium source.

Selenium distribution in egg is showed in Figure 1. Selenium content in egg whites from sodium selenite hen diet was < 0.05 mg kg^{-1} (below quantification level) and 0.11 ± 0.01 mg kg^{-1} from organic selenium diet, which was in line with the results from M. Mohiti-Asli et al. (2008) and R.L. Payne et al. (2005), where organic selenium gave higher selenium content in egg white compared with selenite.

Like the other researchers (Surai and Dvorska, 2001; Jiakui and Xialong, 2004) we can confirm that selenium content from both sodium selenite and organic selenium diets was higher in egg yolk than in egg white ($p<0.05$) and the result was 0.48 ± 0.05 mg kg^{-1} in both egg yolk samples. As the egg yolks were used for extraction of egg yolk oil, we could admit the possible effect of selenium on egg yolk oil vitamin E content.

The results of selenium analysis using ICP-MS show that selenium content in both samples of egg yolk oil, extracted from inorganic and organic selenium enriched yolks, were below quantification level <0.05 mg kg^{-1}. It confirms our expectations about the absence of selenium in egg yolk oil, but it does not mean that there are no selenium traces in egg yolk oil. Because selenium is incorporated in proteins, it was left in protein part after the extraction of egg yolk oil from the egg yolks.

As the results confirm the absence of selenium in egg yolk oil, it means that the interaction of selenium and vitamin E could be possible only in egg yolk. So it was important to understand whether selenium and vitamin E contents in egg yolk are affected by each other.

Vitamin E content in egg yolk and egg yolk oil samples is presented as a tocopherol profile and is given in Table 1.

A. Aljamal (2011) reports that when the hen feed was supplemented with selenium, the yolk selenium content also increased with increasing vitamin E content. In our experiment selenium content in both egg yolk samples was the same and it was not possible to define the effect of selenium content in egg yolk on vitamin E content in egg yolk. But we could admit that the vitamin E content in egg yolk is not affected by the source of selenium, the same amount of vitamin E was detected in egg yolk enriched with inorganic and organic selenium.

The major form of vitamin E in egg yolk and egg yolk oil was presented by α-tocopherol and γ – tocopherol, but β-tocopherol was determined in egg

Table 1

Tocopherol profile of egg yolk and egg yolk oil

Parameters	Sodium selenite		Organic selenium	
	Egg yolk	Egg yolk oil	Egg yolk	Egg yolk oil
α-tocopherol, mg 100 g^{-1}	13.20 ± 2.01	25.40 ± 3.05	11.40 ± 1.73	24.2 ± 2.9
β-tocopherol, mg 100 g^{-1}	n.d.*	6.50 ± 0.08	n.d.*	n.d.*
δ-tocopherol, mg 100 g^{-1}	n.d.*	n.d.*	n.d.*	n.d.*
γ-tocopherol, mg 100 g^{-1}	2.20 ± 0.44	3.85 ± 0.46	2.06 ± 0.41	3.60 ± 0.43

*- not detectable

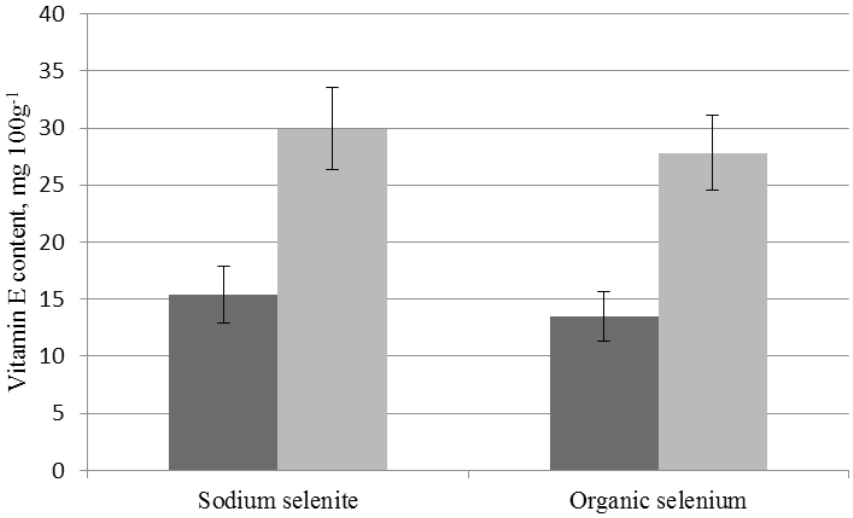

Figure 2. Vitamin E content in egg yolk and egg yolk oil: ■ - egg yolk, ■ - egg yolk oil.

yolk oil extracted from inorganic selenium enriched egg yolk, too.

The vitamin E (sum of tocopherols) content in egg yolks and egg yolk oil is showed in Figure 2. In our study the supplementation of hen feed with vitamin E was low. Vitamin E content in feed was 25 mg kg^{-1}; in both diets that gave 15.4 ± 2.5 mg 100 g^{-1} of vitamin E content in egg yolk with inorganic selenium diet and 13.5 ± 2.2 mg 100 g^{-1} of vitamin E in egg yolk with organic selenium diet which is 2 times higher in comparison to A. Mohiti-Asli et al. (2008) where vitamin E content in egg yolks was 8.8 mg 100 g^{-1} from the hen diet without vitamin E.

A. Mohiti-Asli et al. (2008) in their research supplemented hen feed with 200 mg kg^{-1} of vitamin E and as a result they received 485.37 mg kg^{-1} of vitamin E in egg yolk. It means that vitamin E content in egg yolk was 2.4 times higher than the supplemented vitamin E in feed. Our results show lower vitamin E content in egg yolk than in feed. We can conclude that vitamin E transition level from feed to the eggs decreases with a lower vitamin E supplementation level in feed.

The determined vitamin E content in egg yolk oil was 29.9 ± 3.6 mg 100 g^{-1} from inorganic selenium enriched eggs and 27.8 ± 3.3 mg 100 g^{-1} from organic selenium enriched eggs. Egg yolk can be enriched with vitamin E through higher tocopherol supplementation of hen feed (Galobart et al., 2001; Mohiti-Asli et al., 2008). Due to the linear correlation of supplemented vitamin E and its content in egg yolk (Grobas et al., 2002), egg yolk oil can be probably the richest source of vitamin E for human diet. Vitamin E content in egg yolk oil was high and close to vitamin E content in sunflower (*Helianthus annuus*) oil which contains approximately 45 mg 100 g^{-1} of vitamin E (Tuberoso et al., 2007) and considered as a food product with the highest vitamin E content.

The determined vitamin E content in egg yolk oil was two times higher than in egg yolks. It was predictable because vitamin E is oil soluble vitamin and it was extracted from egg yolk together with oil.

High vitamin E content in egg yolk oil acts as a strong antioxidant. According to S. Grobas et al. (2002) the oxidation of fresh egg yolk lipids was low and was not affected by storage time. In our experiment egg yolk oil was extracted from fresh egg yolks that result in very good oxidative stability of oil. The peroxide value of egg yolk oil was not determined during oil storage for 21 days in both storage conditions – in a closed container at +4 ºC and in an opened container at +37 ºC. G. Cherian et al. (1996) and J. Galobart et al. (2001) observe that inclusion of tocopherols in the hen diet resulted in a significant protection of egg yolk lipids from oxidation. We made the same conclusion as J. Galobart et al. (2001) that dietary supplementation of hen feed with α-tocopherols is an effective way to prevent egg lipids from oxidation. Much longer time period (several months) is needed to see oxidation caused changes in egg yolk oil.

In our study we did not detect selenium in egg yolk oil, therefore, we can conclude that selenium did not have any influence on egg yolk oil oxidative stability. Oxidative stability was achieved by vitamin E and, probably, other natural antioxidants like vitamin A and lecithin.

Conclusions

From the results of this study we cannot confirm that organic selenium increases egg selenium concentration more than sodium selenite. Selenium content in egg yolks was significantly higher than in

egg whites ($p<0.05$) in both inorganic and organic selenium enriched eggs. There was no selenium determined in egg yolk oils. Selenium content in egg yolks did not affect vitamin E content in egg yolks or egg yolk oils ($p<0.05$). Vitamin E in egg yolks and egg yolk oil was presented by α-tocopherol and γ – tocopherol. Egg yolk oil is a good source of vitamin E which effectively protects egg yolk oil from oxidation during storage.

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BLOOD PRESSURE AND AORTIC LUMEN DIAMETER CHANGES AFTER REPLACING AORTA ABDOMINALIS WITH PROSTHESIS

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Abstract
The objective of this study was to observe and evaluate the ability of innovative vascular prosthesis, made in Riga Technical University (RTU), to incorporate in a canine model. The research has been performed in Veterinary Medicine Faculty of Latvia University of Agriculture since July 19. 2011. The research was approved by the Food and Veterinary Service of the Republic of Latvia. The research is realized within the framework of European Social Fund co-financed project 'Establishment of interdisciplinary research groups for new functional properties of smart textiles development and integrating in innovative products' (ESF No 2009/0198/1DP/1.1.1.2.0./09/APIA/VIAA/148). RTU produced aortic grafts 5-8 mm in diameter and 8 mm to 18 mm long were implanted retroperitoneal in 9 female, 1-3 years old Beagle dogs. Once before and regularly after the surgery, abdominal aortic and vascular graft diameter was measured in cross sections. Blood pressure was measured before and regularly after the procedure. Results show no significant differences in aortic and graft diameter before and 12 months after the operation and there are no significant differences in systolic and diastolic blood pressure before and 6 months after the aortic surgery at a significance level α 0.05. The study shows that the innovative aortic prostheses do not change in diameter and abdominal aorta transplantation surgery does not cause significant variations in blood pressure. Study is continued to find out later reactions to synthetic vascular graft.
Key words: vascular graft, polyurethane and polyester prostheses, canine model.

Introduction

The research about vascular prostheses are still outstanding worldwide, since the incidence of cardiovascular diseases is high and it is growing. In 2008, 17.3 million people in the world died from the heart and vascular disease and that is 30% of all deaths (http://www.who.int/cardiovascular_diseases/about_cvd/en/). In order to prevent dangerous consequences of various cardiovascular diseases, in clinical practice synthetic vascular grafts are often used because there is no alternative. There is a continuous progress in the science and more than 450 different synthetic vascular graft types had been developed in the last 30 years (Weselovs, 2008). However, there are still various complications in biointegration observed after the blood-vessel transplantation. Most of them are often associated with vascular lumen overgrowth with neointimal cells, thrombus formation, foreign body reaction, infection and aneurysms formation at the later stages (Lukyanchkovs et al., 2010). One of the causes of the problems mentioned may be related to inappropriate material and/or incomplete constructions using vascular prostheses or there are incomplete preclinical studies on experimental animals.

For successful introduction of new vascular prosthesis with better rheological and immunological properties in clinical practice, these properties must be studied comprehensively using experimental animals *in vivo*. Such experimental studies have no alternative methods *in vitro* (Podlaha et al., 2009).

At Riga Technical University (RTU), under the professor's V. Kantsevičas management a new structure aortic implant has been created. They use weaving technology, biocompatible with the surrounding tissue, inert, non-toxic polyurethane and polyester filament yarn. In order to implement the aortic implants in medicine, it is necessary to study their effects on the body and the potentially possible complications using laboratory animals.

The aim of this study was to explore changes in the lumen of abdominal aorta cranially from graft, in grafts place and caudally from it, as well as the blood pressure and its changes during postoperative period in dogs.

Materials and Methods

The study was performed at the Latvia University of Agriculture, Faculty of Veterinary Medicine from 2011 to 2013, using 9 female, 1 to 3 years old beagle dogs, purchased from the experimental animal kennel in France. Experiment has been confirmed by the Food and Veterinary Service of Republic of Latvia, and has received a permission. RTU elaborated aortic prostheses 5 - 8 mm in diameter and 8 mm to 18 mm in length were implanted retroperitoneal in dogs. Dogs were divided into two research groups. Dogs from group A were euthanized 6 months after surgery, but from group B -12 months after surgery.

Before surgery the animals were clinically examined, weighed and according to their mass 'Enroxil' 5 mg 100 mL^{-1} or 10 mg 100 mL^{-1} (enrofloxacin) 5 mg kg^{-1} was injected intramuscularly to prevent infection penetration. Oral administration of non-steroidal anti-inflammatory drug 'Loxicom' (meloxicam) was completed, appropriate to the

animal's weight to reduce post-operative inflammation and pain. As premedication 0.1 mg 100 mL^{-1} atropine sulfate in dose of 0.02 mg kg^{-1} and 1 mg 100 mL^{-1} acepromazine maleate in dose of 0.1 mg kg^{-1} were injected intramuscularly (i.m.). For induction anesthesia diazepam 0.5 mg 100 mL^{-1} in dose of 0.25 mg kg^{-1} and 10 mg 100 mL^{-1} ketamine hydrochloride in dose of 10 mg kg^{-1} were used intravenously (i.v.) and during the operation 'Isoflurane' was used as inhalation anesthesia.

Animals on the operating table were positioned in the right lateral position and surgery area - lumbar vertebral area was prepared according to the generally accepted principles of aseptic technique. Skin was cut parallel to the lumbar vertebrae below the longest dorsal muscle (*M. longissimus dorsi*) and caudally from the, last, left rib. Next, abdominal external oblique muscle (*M. obliquus externus abdominis*), internal abdominal oblique muscle (*M. obliquus internus abdominis*) and abdominal transverse muscle *(M. transversus abdominis*) were cut to access the abdominal aorta and to dissect it from the surrounding tissue, without cutting peritoneum.

Blood flow in the abdominal aorta was stopped with two vascular clamps, about 4 to 5 cm from each other. After that a perpendicular cut to the longitudinal axis of the aorta was done and some of its segment dissected and RTU manufactured aortic graft was implanted. Three minutes before this action, heparin was administered intravenously. Then clamps one after another were removed, controlling bleeding from the anastomoses. In the presence of bleeding from connection place between aortic wall and prostheses extra sutures were used to provide tightness. The total ischemic time ranged from 30 - 60 min. Aortic and graft ends were sutured with no absorbable thread 'Premilene' 7/0, muscles and subcutaneous tissues with absorbable thread 'Serafit' 2/0, but the skin with 'Supramide' 3/0 thread.

After surgery the animals were observed daily for a period of one week. The sutures were cleaned one time per day using 3 mg 100 mL^{-1} hydrogen peroxide or sodium chloride 0.9 mg 100 mL^{-1} solution. Once per day the dogs received antibiotics 5 mg 100 mL^{-1} or 10 mg 100 mL^{-1} 'Enroxil' 5 mg kg^{-1} by intramuscular injection and oral anti-inflammatory agent typed 'Loxicom' appropriate for the animal's weight. Next five days after the surgery animals received 'Tramadol' 4 mg kg^{-1} twice a day by subcutaneous injections to relieve pain. Fourteen days after the surgery the sutures were removed.

Diameter of aorta and prosthesis were controlled under ultrasound guidance. The first measurement of aorta's diameter was done before the surgery, but the next one - two weeks after the replacement of prosthesis and then once per month till the end of the experiment. When the transplantation of the aortic grafts was done, the measurements were made cranially from the graft, in the graft place and caudally from it. Ultrasound examination was performed using Philips HD11 ultrasound system. For the precise measurements animals had to be sedated by intramuscular injections of acepromazine maleate 0.1 mg kg^{-1}. For ultrasound examination the area 10×20 cm was clipped caudally from the rib arc and ventrally from the *processus transversus* of lumbar vertebrae. During the examination the dogs were placed in the right lateral position. To measure diameter of abdominal aorta and aortic graft, cursor was placed in the middle of aorta and aortic graft. The length of prosthesis was measured in longitudinal section. As an additional measurement the speed/rate of blood stream was taken using Doppler section and M-mode function.

The blood pressure was measured in six dogs. This manipulation was done by High Definition Oscillometry (HDO) equipment and the measurements were taken before the surgery and once every month during the six month period after the surgery. Before the blood pressure measurements, animals were relaxed, they were taken in a separate room without any noise and measurements were done when they had calmed down. The blood pressure was measured by first size cuff that was placed on the tail and on the metacarpal area as it was written in the instruction. During the manipulation, those parts of body where the cuffs were on, were placed on cardiac level ± 10 cm. Five measurements in each investigation were performed and the average volume was calculated.

For statistical analysis, descriptive statistics in Mc Excel program and Wilkokson test for paired samples in SPSS program were used.

Results and Discussion

In the literature mainly the histological and immunohistochemical analysis of vascular prostheses are described. One of the problems is the hyperplasia of the intimal cells that is found in vascular prostheses in a variety of materials after explantation (Ao et al., 2000; Podlaha et al., 2009). This is the main reason, why the narrowing of the lumen develops.

Our study shows that diameter of aortic prostheses, made of polyurethane and polyester yarn, six and 12 months after implantation, was not occluded, but there is a tendency for it to tighten (Fig 1.). The diameter of the prosthesis prior to the surgery was in an average of 0.600 cm, after six months 0.540 cm and after one year 0.505 cm. Statistically comparing the diameters of the prostheses prior to surgery and 12 months after it, the significant differences were not observed at α 0.05. On the contrary, other studies described that in the commercial polyester prostheses three

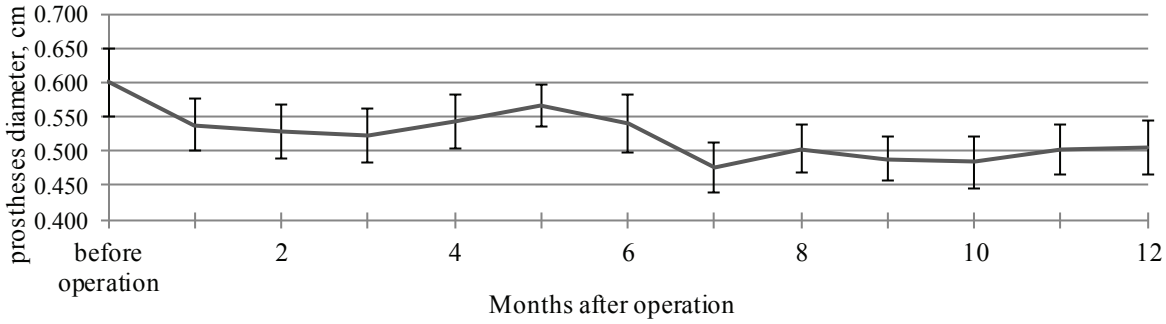

Figure 1. Aortic prostheses diameter changes in dogs (means ± st. error) in postoperative period.

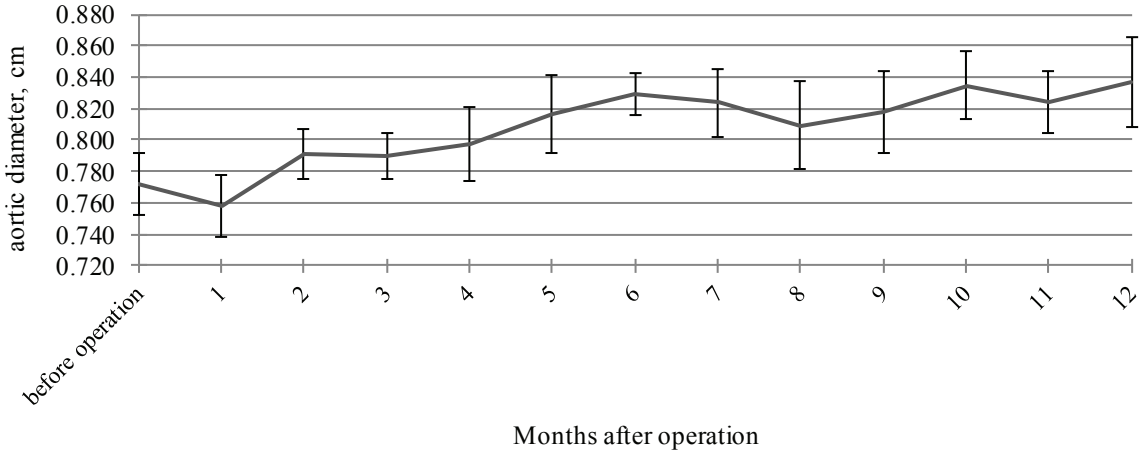

Figure 2. Aortic diameter cranial from aortic prostheses changes in dogs (means ± st. error) in postoperative period.

months after implantation neointimal hyperplasia in various grades was observed and in individual animals prosthetic lumen had occluded (Uberrueck et al., 2004). Authors explained theses changes by differences in experimental animal species. In general, polyester, polytetrafluoroethylene, poliurethane prosthesis have been used for many years and found to be suitable for transplantation of large diameter blood vessels, but the problem begins when blood vessels < 5mm in diameter are transplanted (Rashid et al., 2004; Alcantara et al., 2005).

The second most common complication after the implantation of an artificial blood vessel is aneurism. Authors have found thickening of capsule and narrowing of prostheses internal lumen, and in some cases there were aneurysm formations after explantation of polytetrafluoroethylene prosthesis in people (Formiche et al., 1988).

In this study, we measured aortic diameter cranially and caudally from the prosthesis, because we believe that there are morphological changes in postoperative period and it is possible that the diameter can change not only in prosthesis, but also in abdominal aorta which is connected to it. Until six months after the RTU manufactured aortic prostheses implantation, there were no aneurysm formation detected by ultrasound in prosthesis or in the aorta cranially and caudally from it. Six months after the surgery in two dogs, aneurysm signs in the aortic prosthesis were detected.

The diameter of abdominal aorta before aortic prosthesis implantation was varying from the minimum of 0.688 cm to the maximum of 0.882 cm, with an average of 0.772 cm in experimental animals (Fig 2.). During the postoperative period we observed increase in aortic diameter cranially from the prosthesis. The difference in abdominal aortic diameter before and 12 months after the surgery is 0.065 cm. Comparing the diameter of the aorta before surgery and cranially from the prosthesis 12 months after the surgery, there was no significant differences observed at the α 0.05.

Similar changes in the abdominal aortic diameter can be observed distally from RTU manufactured prosthesis throughout the postoperative period. If the diameter of abdominal aorta before prosthesis implantation was an average of 0.772 cm, then 6

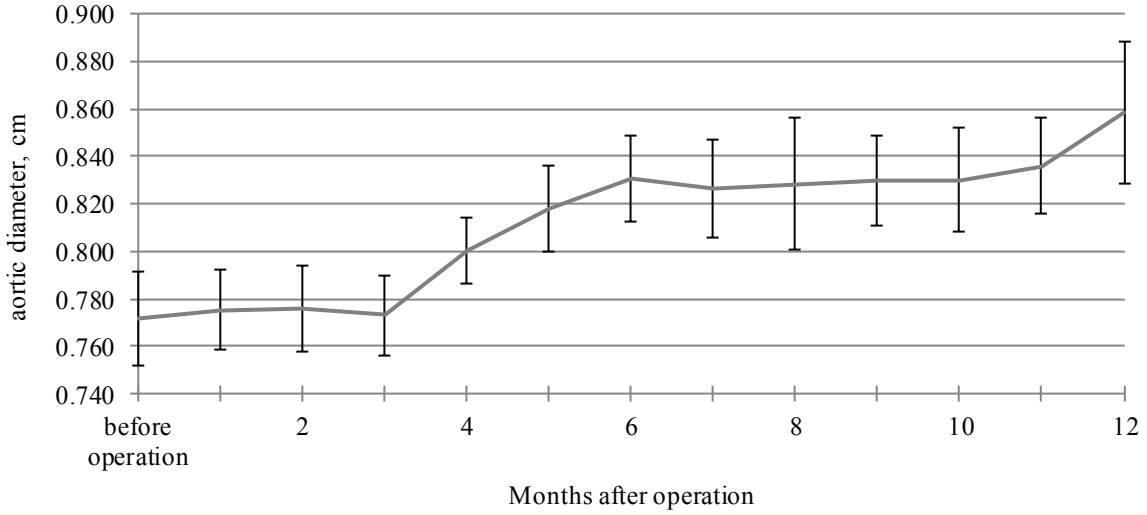

Figure 3. Aortic diameter distal from aortic prostheses changes in dogs (means ± st. error) in postoperative period.

months later - 0.831 cm, and 12 months after the surgery 0.859 cm (Fig 3.). Hence, the diameter of the aorta at this place was increased by 0.087 cm, but there was no significant difference between the aortic diameter before surgery and aortic diameter distally from the prosthesis 12 months after the operation at α 0.05. By comparing the abdominal aortic diameter distally from prostheses with abdominal aortic diameter cranially from it, results show that distally it is 0.022 cm wider than cranially. These changes can be explained by blood flow and resistance changes after the artificial aortic segment surgery.

Summarizing the results of blood pressure deviation we obtained the following data. Before aortic transplantation, systolic blood pressure was an average of 156.88 ± 5.01 mmHg and minimal from 140.80 mmHg to a maximum 166.80 mmHg (Fig 4.). After the operation, it ranged from an average of 162.13 ± 10.12 to 140.83 ± 2.78 mmHg and minimal from 117.8 mmHg to a maximum of 186.0 mmHg (Fig 4.). Significant differences between the systolic blood pressure before surgery and 6 months after it were not observed at α 0.05. Normal systolic blood pressure in dogs is 120 mmHg (Reece, 1997; Garančs, 2006). However, the blood pressure is a variable rate and it varies greatly between dog breeds. In Beagle dogs systolic blood pressure rate is an average of 140 ± 15 mmHg, but even among individuals of the same breed normal blood pressure can vary and each animal have its own (Egner et al., 2007).

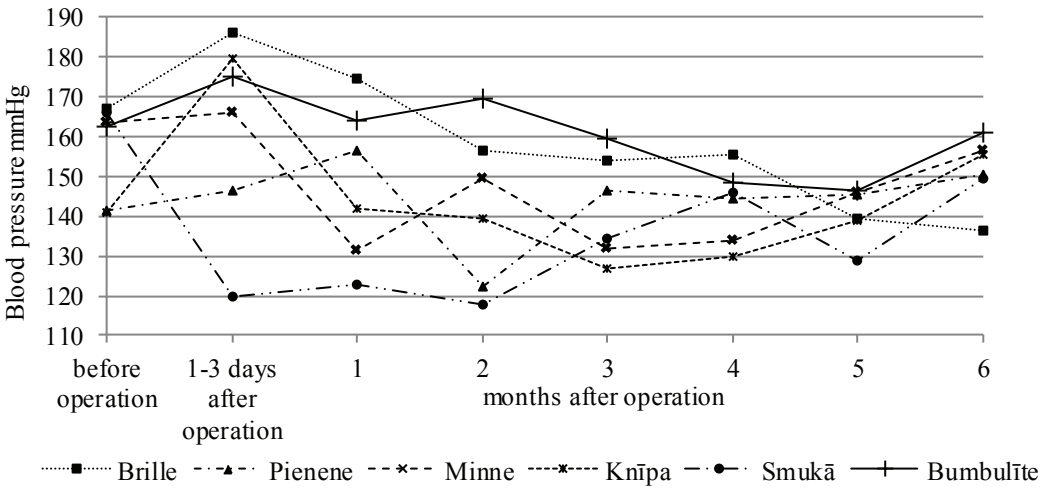

Figure 4. Systolic blood pressure dynamics in dogs in postoperative period.

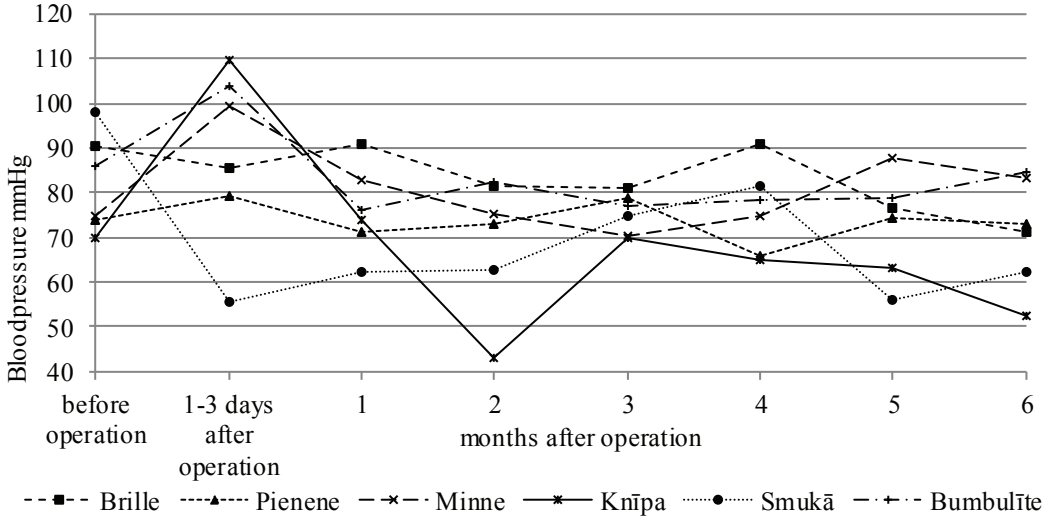

Figure 5. Diastolic blood pressure dynamics in postoperative period.

Based on our results the blood pressure corresponds to be normal. Before surgery the systolic pressure in most experimental animals is higher than normal, but that can be explained by the additional stress, because this kind of manipulation was done for the first time. It should be noted that animals with high blood pressure respond well to strangers such as presence of a veterinarian (Marino et al., 2011). Increased systolic pressure 1-3 days after the surgery can also be explained by the additional stress in animals since in postoperative period they were in the other room. Of course, after surgery there was some tissue damage and pain response (despite the use of pain relievers) that can lead to increased blood pressure (Egner et al., 2007; Reece, 1997).

Before aortic transplant diastolic blood pressure was in an average of 82.16 ± 4.50 mmHg and a minimum 69.8 mmHg to a maximum 98 mmHg (Fig 5.). After the surgery, it ranged from an average of 88.83 ± 8.14 to 69.57 ± 6.05 mmHg and from a minimum of 43.00 mmHg to a maximum of 109.6 mmHg (Fig 5.). Normal diastolic blood pressure in dogs is referred to 70 mm Hg (Reece, 1997; Garančs, 2006). Similarly to the systolic blood pressure the diastolic blood pressure varies between animal species. In Beagle it is 79 ± 13 mmHg, of course, the individual characteristics of the animal and the circumstances surrounding them can impact influence it (Egner et al., 2007).

Diastolic blood pressure the same as systolic exceeded norms 1-3 days after surgery, but later returned to normal and corresponds to norms described in the literature. The reasons for this phenomenon are the same as for the systolic blood pressure, because both of these pressures and changes in them are affected by the same causes. Figure 5. shows the above mentioned animal's individual response to external and internal conditions. Individual animal diastolic blood pressure curves go greatly low and high. Comparing the mean values before and after the surgery we found no significant differences at α 0.05.

Conclusions

1. The study shows that the innovative aortic prostheses do not particularly change in diameter 12 months after the surgery.
2. Surgery, like abdominal aorta transplantation, does not cause significant variations in blood pressure during 6 months period.
3. Further observations are recommended to find out later reactions to synthetic vascular graft.

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SEROLOGICAL ASPECTS OF AVIAN METAPNEUMOVIRUS INFECTION IN KAZAKHSTAN

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Abstract
Avian metapneumovirus (AMPV), formerly known as avian pneumovirus (APV) is epizootic agent of turkey rhinotracheitis (TRT) and swollen head syndrome (SHS) in turkeys and chickens. The infection primarily affects the upper respiratory tract of young birds (broilers), while also decreases egg production of adult hens. Thus, the development of infection in susceptible birds of any age can cause serious economic losses.
The purpose of this study is to test serums from broilers and hens for the presence of antibodies against the avian metapneumovirus. In this series of studies 317 serum samples taken from one 1 day to 75 weeks old birds were tested. Thus, on the basis of serological tests of blood serum and of chicken flocks and broilers, we had a preliminary diagnosis on the presence of avian metapneumovirus infection. Serological studies of unvaccinated against avian metapneumovirus infection bird flocks using the ELISA method showed antibody titers on average at 22 859 ± 4133. Avian metapneumovirus infection in birds was accompanied by a decrease in egg production of chicken flocks by 8.0 - 12.8%.
Key words: Rhinotracheitis of turkeys, swollen head syndrome, bird's metapneumovirus, seropositivity, antibodies.

Introduction

Avian metapneumovirus (AMPV), formerly known as avian pneumovirus (APV) is epizootic agent of turkey rhinotracheitis (TRT) and swollen head syndrome (SHS) in turkeys and chickens. The infection primarily affects the upper respiratory tract of young birds (broilers), while also decreases egg production of adult hens. Thus, the development of infection in susceptible birds of any age can cause serious economic losses.

In recent years, early unknown infectious disease was observed among chicken population of Kazakhstan poultry farms, mainly of respiratory character, later diagnosed (Assanov et al., 2012; Assanov et al., 2012) as an avian metapneumovirus infection of poultry (AMPV). The first signs of rhinotracheitis were observed in 1970 in South Africa (Buys et al., 1980), while on the European continent it was firstly discovered in 1981 in France in turkeys (Buys et al., 1989). Later the disease spread rapidly in the UK, France, Spain, Germany, Italy, the Netherlands, Israel and Asia (Jing et al., 1993).

Causative agent is avian metapneumovirus, an RNA virus of the *Paramyxoviridae* family, Metapneumovirus genus (Pedersen et al., 2000).

Virus genome is presented by the linear not segmented molecule of not infectious RNA and contains 8 genes. There are four subtypes of the metapneumovirus of birds: A, B, C and D. Viruses of subtypes A and B are spread in Europe, Asia, Africa, Southern and Northern America whereas this virus of a subtype C circulates mainly among turkeys in the USA Cook J.K.A., 2000. The metapneumovirus of birds of subtype D has been revealed only once in France (Bäyon-Auboyer et al., 2000).

The problem of antigenic structure of the metapneumovirus besides the big theoretical value now represents also essential practical interest in the period of mass vaccination against this illness and also at studying of replication of vaccinal and epizootic strains of a virus in an organism and cell cultures and their spread among birds.

According to our observation, clinical symptoms of metapneumovirus infection in chickens are characterized by rhinitis, conjunctivitis, swollen feathers and infraorbitals, while individual chickens have complete complex of symptoms of swollen head syndrome (SHS). Such symptoms are little observed in adult hens, but the infection development is characterized by the reduction of egg production, lower average daily increase and feed conversion ratio deterioration. In addition, immunosuppression caused by pneumovirus makes poultry sensitive to other agents including conditionally pathogenic microflora.

Urgency of the problem lies in the fact that up to now, epizootic peculiarities of avian metapneumovirus infection, diagnosis and serotyping of epizootic virus strains as well as effectiveness of preventive maintenance methods were not studied in Kazakhstan.

The purpose of this study is to test serums from broilers and hens for the presence of antibodies against the avian metapneumovirus. In this series of studies 317 serum samples taken from one day to 75 weeks old birds were tested.

Materials and Methods

The work was carried out in 2011-2012 in the laboratory of virology and bird illnesses of

Kazakh National Agrarian University, laboratory on prophylaxis of special dangerous illnesses of animals of Republican State Enterprise "Scientific research institute of problems of biological safety" and serological laboratory of Univet Limited partnership.

The following materials were used:

317 samples of chicken blood serum have been sampled. For serological testing a set for detection of antibodies to agent of metapneumovirus infection of birds BioCek, manufactures of firm "Avian Rhinotracheitis Antibody Test Kit" (Holland) has been used. Procedure of test and the analysis of results were made according to recommendations of the manufacturer (Svanova Biotech, Lyon, France).

The positive and negative control of antiserum has been used in each period. Absorption has been read on length of a wave of 650 nanometers on ELX 800 ® ELISA reader (Bio-Chek, Winoski, VT, USA). The relative level of antibodies has been defined by calculation of the sample to positive (S/P) ratio. Serum samples with S/P ratio are more 0.2 (titres more than 396), in re-testing it is considered positive of AMPV. Statistical processing was conducted using the student's t-test.

Samples from each poultry yard with 18-25 birds were taken randomly. Blood samples were taken regardless of whether there are any signs of respiratory or other clinical disease in the herd. A total of 317 blood samples were taken from 1 day to 75 week old hens. The presence of antibodies against avian metapneumovirus in each serum sample was tested twice using immune-enzymatic analysis, which was able to identify antibodies against A, B and C subtypes of avian metapneumovirus.

For comparison, serological studies were conducted in three different poultry farms and in different age groups.

Results and Discussion

The results of this study may indicate the possible involvement of avian metapneumovirus in respiratory diseases that are observed in chickens in Kazakhstan. Its prevalence has to be investigated in other parts of the Republic. It meets the information of different authors in other countries. According to Gharaibeh SM et al., (2007) in 100% of cases in Jordan it has been confirmed by ELISA method that birds have positive antibodies to AMPV. In Poland

Table 1

These serological studies in poultry farms No. 1

Number of subgroups	Age in days and weeks	Number of samples	Min titer	Max titer	Mean titer	Positive	%	Negative	CV%
1	1 day	10	1	623	253	-	-	10	79
2	90 days	10	106	497	254	-	-	10	40
3	20 weeks	10	437	5278	1326	3	30	7	116
4	26 weeks	15	596	3384	1393	4	26	11	64
5	32 weeks	13	798	8901	4377	11	84	2	55
6	61 weeks	14	642	11229	3933	12	85	2	66
7	64 weeks	14	4825	20086	12173	14	100	-	35

CV- coefficient of variation.

Table 2

These serological studies in poultry farms No. 2

Number of subgroups	Age in days and weeks	Number of samples	Min titer	Max titer	Mean titer	Positive	%	Negative	CV%
1	2 day	10	1	623	249	-	-	10	106
2	90 day	10	228	1477	650	-	-	10	66
3	20 weeks	10	480	5517	1226	1	10	9	124
4	27 weeks	15	1841	7997	4133	15	100	-	46
5	32 weeks	13	1454	12924	5671	13	100	-	48
6	45 weeks	11	4093	30126	15303	11	100	-	57
7	56 weeks	10	1864	18517	9728	10	100	-	62
8	60 weeks	20	3702	24778	11280	20	100	-	52
9	68 weeks	18	4033	17567	9896	18	100	-	42

CV- coefficient of variation.

Table 3

These serological studies in poultry farms No. 3

Number of subgroups	Age in days and weeks	Number of samples	Min titer	Max titer	Mean titer	Positive	%	Negative	CV%
1	4 day	10	1	1233	5094	8	80	2	72
2	95 day	15	1	4063	849	3	-	12	136
3	27 weeks	15	2182	20464	11472	15	100	-	60
4	39 weeks	10	9159	18517	16023	10	100	-	24
5	42 weeks	10	13421	18649	16400	10	100	-	11
6	44 weeks	18	8143	21278	16844	18	100	-	23
7	63 weeks	22	9874	27848	22859	18	100	-	24
8	75 weeks	24	520	23477	11771	17	-	7	73

CV- coefficient of variation.

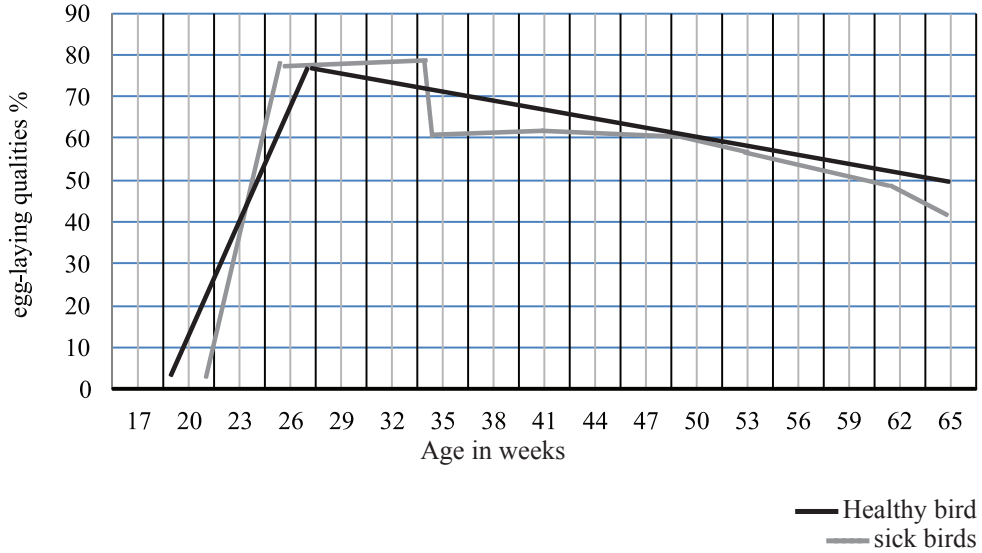

Figure 1. Chicken egg production curve unfavorable for AMPV.

according to Minta et al., (1995) 56.4% of birds have positive results.

As can be seen from Table 1 in young birds up to 90 days of age, specific antibody titers were minor, but in the poultry farm number 3 (Table 3) in 4-day old chicks 80% of the samples were positive.

Studies of blood sera of birds poultry number 1 showed that for 32-week old chicks antibodies were detected in 84% of individuals flocks, and at 64 weeks AMPV antibodies were found in 100% of cases.

The highest antibody titers were detected in the study of sera from birds in poultry farm No. 2, where the individuals of 27 weeks of age, 100% of the positive results.

Similar results were obtained in a study of sera from chickens in poultry farm No. 3. The average antibody titer to metapevmovirusny infection of birds was 22859, with 45% of birds antibody titers being very high of more than 10,000. The maximum titre was 27848 at 63 weeks of chickens.

During these studies the egg production decrease was observed in adult hens. Egg production of 32 weeks old laying hens declined by 63.6%, which is below the accepted standard of 12.8%. When these hens reached 50 weeks, their egg production was at the standard level, but at the end of the observation period 60 weeks figure was lower than the norm by 80% Figure 1.

Birds in these poultry farms have not been vaccinated against AMPV, while the presence of antibodies in the serum of birds indicates the circulation of epizootic virus in bird flocks.

Thus, on the basis of serological tests of blood serum and of chicken flocks and broilers, we had a preliminary diagnosis on the presence avian metapneumovirus infection.

Conclusions

Serological studies of unvaccinated against avian metapneumovirus infection chickens flocks using the ELISA method showed antibody titers on average at 22 859 ± 4133. Avian metapneumovirus infection in birds was accompanied by a decrease in egg production of chicken flocks by 8.0 - 12.8%.

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IMMUNOCORRECTING THERAPHY OF ALLERGIC DERMATITIS

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Abstract
This article summarizes data of determining a therapeutic dose of a new immunocorrecting drug, dermatocytoglobulin, developed by the authors of this study. It has been established that this drug has a therapeutic immunocorrecting effect on allergic dermatitis. Subjects of the study cattle, horses, of Raimbek – Commercial Diary Farm, 56 dogs and 35 cats admitted to a veterinary clinic. It has been established that administration of dermatocytoglobulin in trial groups reduces autoantibody titers until their elimination on the 12th day, dose of 0.2 cm^3 per 10 kg while autoantibody titers in control groups were identified on the 30th day.
Key words: allergic dermatitis, dermatitis simulation, dermatocytoglobulin, therapeutic doses, treatment immunocorrection.

Introduction

Allergic dermatitis quite frequently affects animals. It is a chronic allergic disease. It frequently affects animals with genetic predisposition. The course of the disease is relapsing and has age-related specifics. Relapses occur in clear time intervals. They occur in winter, while in the warm season it is disease-free survival (Pieters et al., 2002; Goloviznin, 1996).

In pathogenesis of atopic dermatitis high importance is attached to genetically determined processes of cytomembrane activation which entails dysfunction of exchange of cyclic nucleotides and phosphoinositols, incomplete response to stimulation of beta-adrenergic, H1-H2- histamine, E1-prostaglandin, cholinergic receptor systems, exchange of interleukins, eicosanoids. Integrally sensitivity threshold to different exo and endogenic influences in atopic dermatitis is reduced (Khaitov and Pinegin, 2000; Speranskiy and Ivanova, 2002)

From among additional links of pathogenesis of atopic dermatitis the following are known: malfunction of thymus, renal glands, decreased hormone protection of organism, level of gamma-interferone, cell-mediated reactions in the skin. Intrauterine immunization of skin when amniotic fluid is contaminated, foci of chronioinfections, secondary immunosuppression which in patients with atopic dermatitis is not only skin-mediated but also systemic, autoimmune reactions (Peterson et al., 1997; Leung, 2000).

The use of interferons and their inducing substances is pathogenically justified for treatment of atopic dermatitis. The drug is used for hyperergic and proliferative types of atopic dermatitis with high content of reagin, IL-1, 4 against the background of falling level of IL-2, reduced content of CD4/CD8. It is recommended to administer affinoleukine, favorable results are indicated from synthetic immunocorrector of a wide range of action – immunofane which is given by 1 cm^3 of 0.005 mg L^{-1} solution intramuscularly twice a week, and some certain hopes in treatment of atopic dermatitis are connected with administration of tacrolimus (Suvorov, 1998; Leung, 2000).

However, all applied therapies do not produce any inhibiting effect on synthesis of autoantibodies which are a leading factor in dynamics of pathogenesis of allergic diseases. The objective of the study were to develop method of directed immunocorrecting treatment of allergic dermatitis by means of dermatocytoglobulin, - it is a new drug developed by us.

Materials and Methods

Studies were conducted at the chair of midwifery, surgery and biotechnology of reproduction, in the clinic of the faculty of the Kazakh National Agrarian University, in farms of Almaty region and veterinary clinic of Almaty city on rabbits, dog, cats, and cattle and horses (2012).

Animals of the first group with allergic dermatitis were injected dermatocytoglobulin 0.2 cm^3 per 10 kg of body weight, intramuscularly, twice with a 3-day interval. Animals in the second group were injected prednisolone 0.4% solution, 0.1 mg per kg of body weight, intramuscularly, during 5 days. Animals in the third group were injected azathioprine 0.25 mg intramuscularly on days 1, 2, 4, 6 and 8. The fourth group of animals was a control group. Throughout the experiment animals were exposed to continuous clinical supervision.

A further object of our studies was administration of dermatocytoglobulin at production field. Test subjects were sick animals with allergic dermatitis: cattle, horses of Raimbek – Commercial Diary Farm dogs and cats admitted to veterinary clinic. Animals were divided into two groups: test and control. Test animals

were injected dermatocytoglobulin 0.2 cm^3 per 10 kg of weight body twice with a 3-day interval. Blood samples were studied before drugs were injected and on days 3, 6, 12, 21 and 30 after it was administered to determine the titers of autoantibodies against skin in the process of inhibiting allergic pathologies.

Rabbits in the first group were injected dermatocytoglobulin subcutaneously 0.1 cm^3 per 10 kg of body weight, once; rabbits in the second group were injected the same dose of the test drug twice on the first and third day. Animals in the third group were injected dermatocytoglobulin 0.2 cm^3 per 10 kg of weight body, once; animals in the fourth group were injected the same dose of the test drug twice on the first and third day. Animals in the fifth group were injected dermatocytoglobulin 0.3 cm^3 per 10 kg of weight body, once; animals in the sixth group were injected the same dose of the test drug twice on the first and third day. Animals in the control group were not injected dermatocytoglobulin.

The study has covered a total of 311 heads of cattle with allergization identified in 125 animals, 86 horses with allergization identified in 58 animals, and 50 cats with allergization identified in 32 animals. Results of conducted studies have showed that allergization is widely spread in animals with dermatitis. Antibodies that resulted in response to such damaging action of dermatitis can react not only with modified proteins but also with antigens of intact tissues due to cross reaction, since significant portion of determinate groups of autoantigens can to a certain degree be similar to that of undamaged elements.

21 chinchilla breed rabbits of 6-8 months were used for determination of therapeutic doses of dermatocytoglobulin. The animals were kept in similar conditions and were divided into 7 groups of 3 animals each. Animals in the seventh group were control ones. All test animals were induced allergic dermatitis and 2, 4-dinitrochlorbenzene was used as an allergen. Therapeutic doses of dermatocytoglobulin were determined provided that animals had expressed clinical signs of allergic dermatitis and a titer of autoantibodies of at least 1:640.

Results and Discussion

Dermatocytoglobulin for treatment of allergic and autoimmune dermatitis is obtained in the following manner: animal donors (donkeys, sheep and rabbit) are vaccinated three times by suspension of tissues of epidermis, derma and hypoderm of horse or cattle. Blood is drawn on the 21st day after last vaccination, obtained hyperimmune serum – idiotype against tissue of epidermis, derma and hypoderm, is checked for activity which must be within 1:5120 and 1:10240. Immunoglobulins are excreted from the obtained idiotype by method of ethanol fractionation. Suspended immunoglobulin is dissolved in isotonic solution of sodium chloride containing 1.8% sodium hyposulphite (stabilizer and sorbent). The drug is filled into 2 or 5 ml ampoules and exposed to lyophilization. Before use ampoule content is dissolved into 2 mL of purified water. The use of drug obtained in such a manner conditions curative effect on allergic dermatitis of animals.

Specific autoantibodies against skin were identified in immunologic studies by identifying autoantibodies with application of serum test of indirect hemagglutination.

Statistical processing of results was carried out using method of Saiduldin T.S. (1992). Test of validity of obtained results was determined by Student-Fisher method (1997).

Findings of the study

In the first group (Table 1) pre-dose titers of autoantibodies against skin made 811 (+6.1% - 2.8%), on the third day they reduced down to 728 (+22.3% - 14.5%), on the 6th day titers made 574 (+32.8% - 19.1%) and on the 15th they made 87 (+6.4% - 5.1%) and on the 21st day they were not identified. In the second group, on the third day they totaled 653 (+55.8% - 44.7%; p=0.025), on the 12th day - 98 (+11.8% - 8.3%; p=0.025) and on the 18th day no autoantibodies were identified. In the third group, on the third - 549 (+63.2% - 45.8%), on the 9th day - 218 (+22.8% - 19.4%) and on the 15th day they were not identified. In the fourth group, on the third day - 568 (+36.7% - 32.5%; p=0.025), on the 9th day - 87 (+15.7% - 5.9%; p=0.025) and on the 12th day they were not identified. In the fifth group, pre-dose titers of autoantibodies made 692 (+21.0% - 7.3%; p=0.025) and on the 9th day they were not identified. In the sixth group, initially titers of autoantibodies were equal to 755 (+47.5% - 36.1%), on the third day they made 238 (+14.7% - 9.2%), on the 6th day - 54 (+3.7% - 1.6%) and were not identified on the 9th day. In the control group the titers of autoantibodies stayed high until the 21st day, on the 28th day they made 367 (+23.5% - 15.7%) and on the 30th day – 288 (+21.6 – 9.7).

The study has established that an optimum inhibiting dose of dermatocytoglobulin is 0.2 cm^3 per 10 kg of body weight injected twice with a 3-day interval.

Study of therapeutic effect of dermatocytoglobulin in comparative aspect with generally accepted methods of treatment was conducted on dogs experimentally induced with allergic dermatitis when their titers of autoantibodies reached at least 1:1280. Test animals were divided into 4 groups.

Before the injection of dermatocytoglobulin dose (table 2), the titers of autoantibodies against skin were identified in dissolution 1738 (+9.0% - 6.8%; p<0.05),

on the 3rd day level of antibodies lowered down to 964 (+22.3% - 18.1%; p<0.05) and on the 12th day no autoantibodies were identified.

Before the injection of prednisolone, the titers of autoantibodies were 1863 (+34.6% - 28.8%; p<0.05), on the 3rd day after treatment - 1206 (+33.7% - 29.4%; p<0.05), on the 21st day - 131 (+14.5% - 11.3%; p<0.05) and on day 24 - 86 (+7.3% -3.2%). Before the injection of azathioprine, the titers of autoantibodies to skin were 769 (+44.1% - 35.9%; p<0.05), on the 6th day after treatment titers made 1267 (+15.4% - 8.3%; p<0.05), o the 27th day - 53 (+14.7% - 7.4%; p<0.05).

Table 1

Determination of therapeutic dose of dermatocytoglobulin

Subgroup numbers	Number of animals	Dosis (cm³)	The multiplicity	Day study and autoantibody titers (cm³)										
				0	3	6	9	12	15	18	21	24	28	30
1	3	0.1	1	811 +6.1 -2.8	728 +22.3-14.5	574 +32.8-19.1	328 +36.5-32.4	126 +52.4 -38.1	87 +6.4 -5.1	28 +14.1-6.5	-	-	-	-
2	3	0.2	1	815 +19.3 -14.8	653 +55.8 -44.7	383 +51.8-37.3	211 +36.9-18.5	98 +11.8 -8.3	57 +6.7 -3.5	-	-	-	-	-
3	3	0.3	1	795 +45.1-28.5	549 +63.2-45.8	428 +9.2 -5.3	218 +22.8 -19.4	49 +12.5 -6.6	-	-	-	-	-	-
4	3	0.1	2	829 +52.8 -42.6	568 +36.7 -32.5	281 +16.2 -6.71	87 +15.7-5.9	-	-	-	-	-	-	-
5	3	0.2	2	692 +21.0 -7.3	288 +17.6 -8.2	71 +34.1 -25.9	-	-	-	-	-	-	-	-
6	3	0.3	2	755 +47.5-36.1	238 +14.7 -9.2	54 +3.7 -1.6	-	-	-	-	-	-	-	-
7	3	-	-	761 +39.4 -33.8	757 +41.8 -36.7	748 +33.1 -19.3	738 +53.5 -39.1	711 +15.8 -8.1	681 +33.8 -27.4	548 +6.4 -5.1	495 +37.6 -18.5	417 +54.1 -38.7	367 +23.5 -15.7	288 +21.6 -9.7

Notes
1 Deviation rates are specified in per cent;
2 Titers are specified in reciprocal variables.

Table 2

Influence of dermatocytoglobulin on genesis of autoantibody against dog skin

Groups	Preparation	Number of animals	Day study / titer in indirect hemagglutination reaction (cm³)										
			0	3	6	9	12	15	18	21	24	27	30
1	Dermatotsito-globulin	17	1738 +9.0% -6.8%	964 +22.3% -18.1%	247 +11.2% -8.5%	71 +11.8% -6.3%	-	-	-	-	-	-	-
2	Prednisolone	9	1863 +34.6% -28.8%	1206 +33.7% -29.4%	862 +32.9% -24.1%	604 +9.6% -5.8%	418 +16.7% -11.3%	257 +3.4% -2.7%	168 +5.8% -4.7%	131 +14.5% -11.3%	86 +73% -3.2%	-	-
3	Azathioprine	12	1769 +44.1% -35.9%	1538 +25.8%-17.5%	1267 +15.4% -8.3%	951 +38.5% -26.8%	763 +15.8% -13.6%	529 +18.6% -15.3%	361 +8.3% -7.2%	215 +6.1% -1.8%	94 +12.8% -8.6%	53 +14.7% -7.4%	-
4	Thecontrol	7	1815 +36.1% -18.5%	1785 +23.5% -18.4%	1647 +43.2% -28.5%	1568 +38.5% -22.8%	1461 +25.4% -18.8%	1387 +25.3% -16.8%	1274 +36.8% -33.1%	1122 +24.5% -16.8%	1025 +18.7% -15.3%	968 +35.1% -28.7%	893 +23.8% -17.4%

Notes - titers are specified in reciprocal variables.

On the 6th day of study the titers of autoantibodies against skin in animals in the control group totaled 1815 (+36.1% - 18.5%; $p<0.05$), on the 30th day of study the titers of autoantibodies were identified in dissolution 893 (+32.8% - 17.4%; $p<0.05$).

Administration of prednisolone and azathioprine reduces the titers of autoantibodies against skin and skin on days 24-28, whereas administration of dermatocytoglobulin normalizes the titers of autoantibodies on the 9th day. In control groups high titers of autoantibodies against skin and skin remained up to 30 days.

Biological product that we have developed - dermatocytoglobulin - has caused immunocorrecting therapeutic effect on immune-associated condition and has completely suppressed synthesis of autoantibodies.

The conducted study has established (Table 3) that in trial groups the titers of autoantibodies against skin were halved on the third day of administration, further reduction of titers of autoantibodies against skin was observed on the 6th day and on the 12th day of study no autoantibodies against skin were identified. In the control group animals demonstrated an insignificant reduction of titers of autoantibodies against skin until day 6, on days 12 – 21 there was further moderate decline of titers and they were identified until day 30.

The conducted experiments have demonstrated that the administration of cytoglobulins in test groups reduces the titers of autoantibodies until their elimination on day 9-12, while in the control group the titers of autoantibodies were identified until day 30.

Therefore, the use of our method for therapeutic immunocorrection of allergic dermatitis leads to a full recovery of sick animals with disappearance of all symptoms of disease and elimination of underlying pathology.

Notion of autoallergy (autoaggression) includes immune reactions that develop against cells and tissues of their own organism. As far as immunologic study methods are introduced in clinic practice it becomes more obvious that autoaggression mechanisms underlie a number of diseases or they are important in chronic progression of their course.

To establish the nature of allergization we have simulated that pathology on laboratory animals (rabbits, dogs) with 2.4-dinitrochlorbenzene used as an allergen rubbed into shaved surface of skin once with a 3 to 5 day interval.

Test rabbits demonstrated peak of antibodies on the 21st day in indirect hemagglutination test up to 1:1280, which gradually were reduced and were not identified on day 45. Test animals demonstrated expressed symptoms of allergic pathology.

Experiments conducted on laboratory and production animals have established that autoantibodies are excreted in high titers in case of allergic pathologies. Products of autoantibodies are viewed as a mediator of immune-associated process. Out of the studied livestock allergization was

Table 3

Dynamics of autoantibody titers during use of dermatocytoglobulin

Groups	Species of animals	Groups	Number of animals	Day study autoantibody titers (serial dilution)					
1	Cattle	Control	63	0	3	6	12	21	30
		Eperimental	62	1280 1280-640	640 640-320	160 160 -80	40 40-20	-	-
2	Horse	Control	22	1280 1280-640	1280 1280-640	1280 1280-640	1280 1280-640	640 640-320	640 640 16
		Experimental	12	640 640-320	320 320-160	80 80-40	-	-	-
3	Dogs	Control	38	640 640-320	640 640-80	640 640 320	640 640 -320	640 640- 320	320 320 80
		Experimental	20	1280 1280-640	640 640 -320	320 160 -80	80 80-40	-	-
4	Cats	Control	18	1280 1280-640	1280 1280-640	1280 1280-640	1280 640- 320	1280 640-320	640 640- 320
		Experimental	14	1280 1280-640	320 640 -160	80 80 -40	-	-	-

Notes - titers are specified in reciprocal variables.

identified in Raimbek – Commercial Diary Farmas follows: cattle – 33.3%, horses – 39.1%, in Almaty city: dogs – 60.3%, cats – 69.4%.

Damages of tissues that occur with the disease condition autoantigenic determination of their tissues and cells, which causes occurrence and development of subsequent stages of allergization. Azathioprine, prednisolone and dermatocytoglobulin were evaluated for inhibiting allergization of dermatitis (Omarbekova et al., 2013).

Administration of azathioprine lessened the inflammatory process and decreased the titers of autoantibodies down to normal parameters on the 28th day; with administration of prednisolone autoantibodies against skin were not identified on day 24, while administration of dermatocytoglobulin entailed complete suppression of synthesis of autoantibodies on the 9th day of treatment. High titers of autoantibodies in the control group were observed up to the 30th day.

We have substantiated administration of dermatocytoglobulin to inhibit allergization processes with dermatitis by fact that the best immunodepression is achieved with the use of idiotypic elements. Dermatocytoglobulin is specific to idiotypes skin and therefore it causes inhibiting synthesis of autoantibodies and performs homeostatic function participating in regenerative growth and elimination of caused damages. Idiotypes contained in their composition can, as antigens, cause immunosuppressive action, inhibiting synthesis of specific antibodies to their own tissues.

We have developed methods immunocorrection allergic dermatitis.

Conclusions

1. We have developed techniques aimed immunocorrective treatment of allergic dermatitis.
2. Using dermatotsitoglobulinis for the treatment of atopic dermatitis is effective for therapeutic immunomodulation, the drug is used twice within three days 0.2 cm^3 10 kg of body weight of the animal.

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CORRELATION BETWEEN PRODUCTIVITY OF COWS SIRED BY DIFFERENT BREEDING BULLS AND BIOCHEMICAL PARAMETERS OF THEIR BLOOD

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Abstract
The objective of the study was to examine how the circumstances of different bull daughters – Latvian Brown cow - group productivity levels correlate with the blood biochemical composition indices in Latvian conditions. The daughters of seven bulls, selected for our research trial, were kept in the same barn and under rearing conditions. Their blood samples were taken in a single sampling activity and analyzed for blood serum biochemical averages. Also Cu, Fe, Zn, Mn and Mg were detected in blood serum and the values found were compared with values fixed in regulatory documents. There were changes in Cu levels (daughter groups of four bulls), in Zn levels (one group); Mn level was lower in all groups and Mg level was higher in 6 groups. The altered biochemical parameters of blood were assessed from the pathogenetical point of view and compared with productivity averages in the progeny groups. The correlation factors suggested that there exist a strong positive correlation ($p<0.05$) among a number of indicators, such as aspartate aminotransferase (ASAT) and protein $r = 0.823$; ASAT and somatic cell count (SCC), $r = 0.737$; blood protein and milk protein $r = 0.903 - 0.917$; albumins and protein $r = 0.964$; blood urea and milk protein $r = 0.931 - 0.984$ along with additional correlations. The correlation factors for the progeny group of each bull differed. The data obtained shows that the Latvian Brown cows crossed with other breeds produce cows with new genetic traits.
Key words: cow's milk, cow's blood, correlation, genetic predisposition.

Introduction

Fast and effective genetic improvement of cows depends on superior genetic quality sires having a high reliability of passing the productivity traits to their daughters. When scoring a bull, the scores of health, body stature and other characteristic traits of his daughters are taken into account. Over the productive lifetime of a cow, certain correlations between the amount and composition of milk, influenced by different environmental factors and the genetic variety of cattle, can be observed (Zutere, 2008). The milk yield, that is, the content of fats and proteins in milk, as well as the level of somatic cells, is determined by genes that animals inherit from their ancestors. By and large, the milk yield of a cow depends on the following factors: 50% feed, 30% management and 20% genetics (Jemeļjanovs, 2001). Milk as the main indicator of productivity, it is made up of substances brought to the cow's udder via the blood stream. Proteins, fats and lactose as components of milk are synthesised in alveoli and epithelial (milk-secretory) cells in milk ducts (Grings et al., 1991). Milk proteins are formed from amino acids and polypeptides of blood, milk fats – from plasma amino acids, triglycerides, acetate, propionic acid and butyric acid while lactose is formed from blood glucose and is the only known process of disaccharide synthesis that occur in animal bodies. It is proven that for ruminants there is a close correlation among different blood biochemical and productivity factors, e.g. a high positive correlation between the concentration of urea in milk and that in the cows' blood (Roussel and Whitnery, 1997; Marenjak et al., 2007; Liepa et al., 2008), as well as a notable correlation between urea and the milk yield (Stoop et al., 2007). The amount of energy to be metabolized increases along with the increase of the amount of the protein to be digested in the *rumen*, and the concentration of urea in blood and in milk increases as well (Gustaffson et al., 1987; Hoffman and Steinhofel, 1990). The detected content of urea in milk allows to assess and control the level of the protein ingested by feed which affects productivity of cows (Osītis, 2005). These coefficients can be used to diagnose diseases and predict changes in health condition two months in advance (Liepa et al., 2008). Since elemental cofactors are required for enzyme activation, their presence ensures healthy metabolism in cows. The lack of elemental cofactors results in decreases in cows' productivity and, as a consequence, health problems and reproduction dysfunctions occur (Jemeļjanovs, 2001; Osītis, 2005).

The aim of this research: study productivity of the daughters sired by different bulls and establish correlations between their productivity coefficients and biochemical parameters of their blood.

Materials and Methods

Fifty-two clinically healthy dairy cows, daughters of 7 different bulls, were included in the research. The venous blood samples were drawn from the cows on monthly milk recording days immediately after the morning milking. Biochemical blood tests were performed once a quarter over a 15-month period in 2008 and 2009 of SIA Palsa in Variņi parish, Smiltene county. The animals included in the trial were all located in the same barn, handled with the same

Table 1

The blood composition indicators of bulls included in the trial

Traits	Bull						
	Alters Disaks LB31007	Primats Punčs LB29804	Hojbru Bits LB31365	Aks Moments LB31470	Lanis Moments LB31368	Ryttargard Kvarnakre LB31394	Orkels Rudi LB31349
Blood composition %	DS 68.75 ŠV 31.25	DS 75.00 ŠV 25.00	DS 59.37 ŠV 4.38 HS 6.25	HS 50.00 DS 43.75 ŠV 6.25	HS 50.00 DS 25.00 ŠV 25.00	ZS 87.50 NS 12.50	AN 50.00 HS 50.00
Number of cows	4	5	6	5	10	7	15

Table 1 shows the pure-bred status in percentage indicators of 7 groups of bulls. Variety names: DS – Danish Red, ŠV – Schwyz, HS – Red Holstein, ZS – Swedish Red and white, NS–Norwegian Red, AN–Angler variety (V/A LDC, 2009, http: //www.ldc.gov.lv).

technical facilities, under the same herd management conditions, (indoors during winter season, grazing during the summer season), received equal feed ration. Biochemical blood tests were performed at the Research Institute of Biotechnology and Veterinary Medicine 'Sigra' of the Latvia University of Agriculture following the generally accepted methods. In the blood samples the following indicators were measured by the method: alkaline transaminase (ALAT) BL- TM – 02 - 01, aspartate aminotransferase (ASAT) BL- TM – 03 - 01, gamma-glutamyl transferase (GGT) BL- TM – 04 - 01, alkaline phosphatase (SAP) BL- TM – 06 - 01, protein, albumins, Ca, P BL- TM – 08 - 01, creatine BL- TM – 07 - 01, blood urea nitrogen (BUN) LVS EN ISO 8968 – 4:2002, bilirubin, cholesterol BL-TM – 05 - 01, glucose, triglycerides, carotene ГОСТ 13496,17 - 95, copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), magnesium (Mg) LVS EN ISO 6869 - 2002. Mathematical data processing was performed using mathematical methods of statistics, MS Excel software (average and standard deviation), SPSS software. P – values less than 0.05 were considered to be statistically significant. The productivity factors of dairy cows (milk yield, protein, fat content, fat kg/protein kg), somatic cell count (SCC) (detected according to method ISO 13366 – 3:1997) and the genetic value scores of the sires were obtained from the data base of V/A 'Lauksaimniecības datu centrs' (V/A LDC) in 2008 and 2009.

Results and Discussion

The results of the average blood biochemical readings for the cows varied (Table 3) as their sires and productivity indicators of progeny were different (Table 2). The most productive bull, according to the average results of his 5-daughter group in 305 lactation days, fat kg x protein kg, is LB31470 Aks Moments with 509.5 ± 86.38 kg and with average daughter milk yield in 305 days of 7076 ± 560.2 kg. The second most productive bull is LB31007 Alters Disaks with the average 4-daughter group result in 305 lactation days of fat, kg x protein kg, is 507.0 ± 200.00 kg and average daughter group's milk yield 6365.3 ± 313.50 kg. In the pedigree group Latvian Red Cattle , LB cows with HS blood in their pedigree have shown the best milk yield (Skagale, 2011) which matches the average milk yield result for the daughters of the bull LB31470 Aks Moments showed by our studies. SV, DS and ZS varieties give the second best crossings for high milk yield (Skagale, 2011). This is confirmed in our trial by productivity indicators of the bull LB31007 Alters Disaks, while within the yield range of 6000 – 7000 kg and milk fat productivity around 70% and protein amount around 65% the importance of good genetics and mating increases (Jemeļjanovs, 2001). The average milk yields in 305 lactation days show statistically significant differences ($p = 0.039$ or $p < 0.05$). The daughters of bull LB29804 Primats Punčs and the daughters of bull LB 31349 Orkels Rudi showed the highest average milk fat content results in our studies, respectively, 49.5 ± 4.7 g kg^{-1} and 46.6 ± 4.5 g kg^{-1}. We established that the average fat content (g kg^{-1}) ratios of the daughters of different bulls were not statistically significant ($p = 0.100$ or $p > 0.05$) and they do not essentially differ. In 2003, Strautmanis had obtained analogous data. The fat and protein content in milk is a stable hereditary trait which can be increased by a targeted genetic improvement. In 2000, the recorded average milk fat content ratio in Latvia was 4.62 g kg^{-1} and the average protein content in milk was 34.0 g kg^{-1} (Zutere, 2008). The latest research in Latvia indicates that the highest fat content, 45.2 g kg^{-1}, has been obtained from the breed combination LB x DS as well as SV breed cattle. The protein content in milk can be improved by LB x SV, reaching 34.0 g kg^{-1} protein content in milk (Skagale, 2011).

Table 2

Average values of productivity and SCC of sire daughters

Bull No	n	Milk yield, kg	Fat, g kg^{-1}	Protein, g kg^{-1}	Fat kg/ Protein kg	SCC, thou-sand mL^{-1}
LB31470 Aks Moments	5	7076.6 ± 560.2	46.3 ± 2.3	33.3 ± 1.7	509.5 ± 86.38	125. 6 ± 93.69
LB31007 Alters Disaks	4	6365.3 ± 313.50	46.3 ± 4.8	33.6 ± 1.2	507.0 ± 220.00	258.8 ± 148.55
LB31365 Hojbru Bits	6	6187.3 ± 888.50	41.8 ± 2.4	33.4 ± 0.7	454.2 ± 77.24	244.3 ± 355.55
LB31368 Lanis Moments	10	6226.9 ± 996.7	45.9 ± 4.7	34.7 ± 1.8	499.6 ± 77.99	140.6 ± 128.85
LB31349 Orkels Rudi	15	5728.8 ± 730.90	46.6 ±4.5	33.9 ± 1.6	476.6 ± 83.93	44.5 ± 31.99
LB29804 Primats Punčs	5	5301.0 ± 1244.60	49.5 ±4.7	34.8 ± 1.8	472.7 ± 62.82	312.0 ± 340.75
LB31394 Ryttar-gard Kvarnakre	7	5782.3 ± 935.20	45.6 ± 2.7	32.3 ± 1.0	449.5 ± 68.41	106.9 ± 136.32
On average in the observation group	52	6095.5 ± 809.94	46.0 ± 3.7	33.7 ± 1.4	481.3 ± 96.68	176.1 ± 176.52

In our trial, the daughters of bull LB29804 Primats Punčs and the daughters of bull LB 31368 Lanis Moments showed the highest average milk protein content ratios, respectively 34.8 ± 1.8 g kg^{-1} and 34.7 ± 1.8 g kg^{-1}. We established that differences of the average protein content (%) ratios of the daughters of different bulls were statistically significant ($p = 0.047$ or $p < 0.05$). In order to identify, apart from the genetically most superior bulls, also the healthiest sires, we evaluated the off-the-norm average blood biochemical parameters of their daughters group by group (Table 3). The protein level in blood serum of the daughters of six bulls (all except bull LB31007) was elevated, namely, 82.60 ± 6.59 g L^{-1} up to 85.05 ± 6.01 g L^{-1}. The daughters of all bulls had an increased average level of urea in blood serum, namely, 8.87 ± 3.60 mmol L^{-1} up to 12.84 ± 3.55 mmol L^{-1}. The biochemical and normative ratios of cows' blood serum are shown in Table 3 and Table 4 (Liepa, 2000; Jemeļjanovs et al., 2007). Increased concentration of BUN in blood and milk occur in clinically healthy cows in the event of overdosing protein in their feed or failing to balance the amount of protein with easily digestible carbohydrates (Osītis, 2005). The concentration of urea in milk can be genetically influenced through the sire's line (Kureoja and Kaart, 2004). The daughters of bull LB31007 Alters Disaks showed normal average protein and P (phosphorus) levels in blood serum, respectively, 75.55 ± 6.42 g L^{-1} and 2.37 ± 1.08 mmol L^{-1}. Nevertheless, these cows react with increased SAP 154.66 ± 37.61 IU L^{-1}, BUN 9.76 ± 2.98 mmol L^{-1} and lowered glucose 2.25 ± 1.00 mmol L^{-1} in blood serum, but the daughters of LB31470 Aks Moments showed increased protein 182.6 ± 6.59 g L^{-1}, SAP 153.00 ± 35.48 IU L^{-1}, GGT 33.00 ± 23.63 IU L^{-1}, phosphorus 2.60 ± 1.12 mmol L^{-1}, BUN 10.60 ± 2.17 mmol L^{-1} and cholesterol 5.06 ± 1.48 mmol L^{-1} levels in blood serum. GGT belongs to the ferment group that catalyzes hydrolysis of phosphate esters; it is found in liver, bones, intestines, and placenta, and provides indication of the liver diseases. In cases of hypoglycaemia (2.25 ± 1.00 mmol L^{-1} for the daughters of LB31007 Alters Disaks and LB31349 Orkels Rudi), due to stimulation of hepatic glycogenolysis and inhibition of gluconeogenesis, the synthesis of glycogen is delayed, while glucagon increases *proteolysis* and breakdown of the fat cells – *lipolysis*. The concentration of free fatty acids increases and becomes the source of glucose. (The increase of SAP in blood serum signals the development of a pathological process outside liver in the cases of rachitis and osteodistrophy when the Ca level in blood serum is lowered (Jemeļjanovs et al., 2007).

Malfunction of liver, fasting, ketosis, sepsis, fatty liver degeneration, *E. coli* (mastitis) and endotoxemia are causes of hypoglycemia (Liepa, 2000). The progeny groups of the remaining 6 bulls showed increased protein level in blood, namely, from 82.60 ± 6.59 *g* L^{-1} up to 85.05 ± 6.01 g L^{-1} which had promoted the increase in urea level from 8.87 ± 3.60 mmol L^{-1} up to 12.84 ± 3.55 mmol L^{-1}. All blood serum proteins are synthesised in liver therefore increased levels of protein in test results signal chronic inflammation

Table 3

Biochemical results, average values of the blood of bulls' daughters

Bull No.	Gammma-glutamyl transferase IU L^{-1}	Alkaline phosphortase IU L^{-1}	Total protein g L^{-1}	Ca mol L^{-1} Calcium	Phosphorus mol L^{-1}	Urea mol L^{-1}	Cholesterol mol L^{-1}	Glucose mol L^{-1}
LB31470 Aks Moments	↑**33.0** ± 23.63	↑**153.00** ± 35.48	↑**82.60** ± 6.59	2.6 ± 0.17	↑**2.60** ± 1.12	↑**10.60** ± 2.17	↑**5.06** ± 1.48	2.99± 1.79
LB31007 Alters Disaks	24.27 ± 7.20	↑**154.66** ± 37.61	75.55 ± 6.2	2.3 ± 0.30	2.37 ± 1.08	↑**9.76** ± 2.98 ↑**9.76**	4.00 ± 1.25	↓**2.2**± 1.00
LB31365 Hojbru Bits	25.44 ± 6.56	↑**162.92** ± 46.57	↑**84.00** ± 10.30	2.7± 0.16	↑**2.53** ± 0.79	↑**12.84** ± 3.55	4.13 ± 1.19	2.35± 1.08
LB31368 Lanis Moments	↑**26.5** ± 8.63	↑**165.94** ± 39.23	↑**82.67** ± 8.15	2.7 ± 0.31	↑**2.63** ± 0.48	↑**10.63** ± 3.42	4.59 ± 1.21	2.32± 1.48
LB31349 Orkels Rudi	25.62 ± 6.02	130.56 ± 28.30	↑**83.14** ± 8.98	2.5 ± 0.13	↑**2.53** ± 0.61	↑**11.45** ± 2.84	↑**5.21** ± 1.10	↓**1.9**± 0.86
LB29804 Primats Punčs	21.51 ± 7.65	149.34 ± 28.55	↑**83.78** ± 4.71	2.6± 0.13	2.08 ± 0.68	↑**12.41** ± 3.34	4.85 ± 1.37	2.54± 1.02
LB31394 Ryt-targard Kvarnakre	23.00 ± 5.84	150.89 ± 32.52	↑**85.05** ± 6.01	2.5± 0.31	↑**2.85** ± 0.70	↑**8.87** ± 3.60	4.82 ± 1.07	2.29± 0.92
On the average in the trial group	25.62 ± 9.36	152.47 ± 35.47	82.40 ± 7.28	2.6± 0.22	2.51 ± 0.78	10.94 ± 0.78	4.67 ± 1.24	2.39± 1.16
Regulatory indicators	4.9 - 25.7	17.5 - 152.7	61.6 - 82.2	2.1 - 2.8	1.4 - 2.5	2.8 - 8.8	1.6 - 5.0	2.3 - 4.10

processes and dehydration of the body. A part of BUN enters the blood and kidneys, and is secreted with milk and urine (Liepa, 2000; Jemeļjanovs and Dūrītis, 2009), while the remaining part is returned to the rumen wall and involved in the turnover of nitrogen in the body. The daughters of four bulls, Aks Moments LB31470, Alters Disaks LB31007, Hojbru Bits LB31365, Lanis Moments LB31368, had high SAP indicators (the elevated indicators are highlighted in Table 3), the daughters of Moments' line (50% HS blood) also had high GGT which is a pathogenetic signal of changes in activity of cholestatic enzymes: fatty liver degeneration and acute malfunction of liver (Liepa, 2000). The daughters of Moments' line showed high productivity ratios (Table 2). The cows had a misbalanced average Ca : P proportions (according to the data analysis, Ca : P were in the same proportions in September and December of 2008 while in blood serum of several cows the level of P exceeded the level of Ca). The preferred Ca : P proportion for high producing cows is 1.6 – 1.7:1 (Osītis, 2005), while over dry periods the suggested Ca : P proportion in the feed is 1 – 1.5:1 (Jemeļjanovs, 2001).

Among the Red breeds, genetically the most highly valued bulls are the Red Holstein (HS), the Danish Red (DS) and the Swedish Red (ZS) (Zutere, 2008; Skagale, 2011) as were the bulls selected for our research, but their daughters are LB breed cows. In our study, comparing the productivity ratios of the sire daughters, the daughters of bull Ryttargard Kvarnakre of ZS breed showed the lowest ratios, namely, 5782.3 ± 935.20 kg in 305 lactation days. We carried out health tests of the sire daughters and evaluated correlation between their biochemical and productivity parameters of their blood using the correlation factor (r). We established a mutually close ($r > 0.7$) positive correlation ($p < 0.05$) among several ratios of the sire daughters under study. Correlation ratios show close genetic correlation between the average productivity ratios and blood biochemical parameters of daughters which is in line with the findings of the studies of other authors (Haile - Mariam et al., 2008; Mucha and Strandberg, 2011). In our research we established the concentration of Cu, Fe, Zn, Mn and Mg levels in blood serum of bulls' daughters (Table 4).

Table 4

Mean Values of the Biochemical Results Cu, Fe, Zn, Mn, Mg of the Blood of Bulls' daughters

Bulls No.	Cu, µmol L^{-1}	Fe, µmol L^{-1}	Zn, µmol L^{-1}	Mn, µmol L^{-1}	Mg, mmol L^{-1}
LB31470 Aks Moments	↓11.41 ± 5.67	22.75 ± 2.10	56.34 ± 18.51	↓1.41 ± 1.09	↑3.43 ± 1.41
LB31007 Alters Disaks	↑21.05 ± 8.18	19.74 ± 1.75	49.72 ± 14.54	↓1.67 ± 0.55	0.84 ± 0.50
LB31365 Hojbru Bits	↑17.65 ± 9.29	26.26 ± 12.08	55.00 ± 12.85	↓1.55± 0.66	↑3.64 ± 1.21
LB31368 Lanis Moments	15.01 ± 7.71	↓17.10 ± 0.50	49.36 ± 14.23	↓1.38 ± 0.58	↑3.36 ± 1.42
LB31349 Orkels Rudi	↓12.59 ± 11.96	25.5 ± 2.11	57.9 ± 12.85	↓1.30 ± 0.10	↑1.54 ± 1.09
LB29804 Primats Punčs	16.05 ± 9.44	25.46 ± 2.67	55.62 ± 11.94	↓1.41 ± 0.58	↑3.46 ± 1.15
LB31394 Ryttargard Kvarnakre	17.69 ± 8.34	23.18 ± 12.88	↓45.56 ± 8.87	↓2.19 ± 1.57	↑3.26 ± 1.34
On average in the observation group	15.31 ± 8.75	21.87 ± 4.74	52.79 ± 11.97	↓1.56 ± 0.73	↑2.79 ± 1.16
Regulatory indicators	14.1-17.3	18-28	47-76	2.73-4.55	0.82-1.23

Under the same feeding conditions, several cows had altered blood serum indicators: Cu was decreased for the daughters of LB31470 Aks Moments 11.41 ± 5.67 µmol L^{-1} and LB31349 Orkels Rudi 12.59 ± 11.96 µmol L^{-1}, increased for the daughters of LB31007 Alters Disaks 21.05 ± 8.18 µmol L^{-1} and LB31365 Hojbru Bits 17.65 ± 9.29 µmol L^{-1}. Fe content was decreased for the daughters of LB31368 Lanis Moments 17.10 ± 0.50 µmol L^{-1}, while Zn content was decreased for the daughters of LB31394 Ryttargard Kvarnakre 45.56 ± 8.87 µmol L^{-1}. All cows in the trial had reduced Mn levels, namely, from 1.30 ± 0.10 to 2.19 ± 1.57 µmol L^{-1} and elevated Mg levels, namely, from 1.54 ± 1.09 up to 3.64 ± 1.21 mmol L^{-1} (except LB31007 Alters Disaks - 0.84 ± 0.50 mmol L^{-1}) in blood serum. Table 5 shows relationships found in our study between the cow blood serum and productivity expressed as the correlation factor (r).

Daughters of bulls LB31349 Orkels Rudi r = 0.909 and LB31365 Hojbru Bits r = 0.917 indicate a correlation between the protein content in blood serum and protein content in milk, and daughters of bulls LB31368 Lanis Moments and LB31349 Orkels Rudi indicate a correlation between the urea content in blood serum and protein content in milk, respectively, r = 0.931 and r = 0.984. Daughters of bulls LB29804 Primats Punčs and LB31007 Alters Disaks indicate a correlation between cholesterol in blood serum and SCC in 1mL^{-1} of milk, respectively, r = 0.849 (SCC 312.0 ± 340.75 thousand mL^{-1} milk) and r = 0.726 (SCC 258.8 ± 148.55 thousand mL^{-1} milk). The daughters of bulls LB31368 Lanis Moments, LB31349 Orkels Rudi, LB31394 Ryttargard Kvarnakre indicate correlation between GGT in blood serum and fat content g kg^{-1} in milk, respectively, r = 0.812 (fat content 45.9 ± 4.7 g kg^{-1}), r = 0.787 (fat content 46.6 ± 4.5 g kg^{-1}) and r = 0.875 (fat content 45.6 ± 2.7 g kg^{-1}). The daughters of bulls LB31470 Aks Moments, LB31368 Lanis Moments, and LB31349 Orkels Rudi indicate correlation between carotene in blood serum and protein content in milk, respectively, r = 0.866, protein content 33.3 ± 1.7 g kg^{-1}), r = 0.855, (protein content 34.7 ± 1.8 g kg^{-1}) and r = 0.787, (protein content 33.9 ± 1.6 g kg^{-1}). Close positive and negative correlations can be inferred between the average enzymatic cofactor concentrations SCC in blood serum of daughters of various bulls as well as between productivity ratios which affirms the importance of enzymatic cofactors in biochemical processes of a cow. For instance, there is a correlation from r = 0.746 up to r = 0.894 between Cu and urea; there is a correlation from r = 819 up to r =0.889 between Cu and bilirubin. The blood serum average ratios of several bulls indicate a positive correlation between Zn and carotene within the limits from r = 0.869 up to r = 0.960, while there is a close negative correlation between Zn and triglycerides from r = - 0.780 down to r = - 0.826. There are close positive correlations between Fe and milk yield productivity ratios from r = 0.859 up to r = 0.994 and Fe and the average live weight of cows from r = 0.943 up to r= 0.957, while there is also a close correlation between Fe and SCC, namely, from r = - 0.736 down to r = - 0.936. In our research a close correlation between the productivity and blood serum biochemical parameters in the daughters of different bulls was established for all

Table 5

Correlation, Biochemical results of blood and productivity of bull's daughters

Bull breed	Genetic composition of blood %	Correlation		
		Blood	Milk	Correlation coefficient
Alters Disaks LB31007/DS	DS 68.75, SV 31.25	P	Protein content %	0.964
		carotene	Fat content %	0.981
Primats Punčs LB29804/ DS	DS 75.00, SV 25.00	Ca	Protein content %	0.982
		cholesterol	SCC	0.849
Aks Moments LB31470/ DS	HS 50, DS 43.75, SV 6.25	albumins	Protein content %	0.964
		carotene	Protein content %	0.866
Lanis Moments LB31368/ DS	HS 50.00, DS 25.00, SV 25.00	ALAT	SCC	0.899
		GGT	Fat content %	0.812
		urea	Protein content %	0.931
		cholesterol	Fat content %	0.733
		carotene	Fat content %	0.854
		carotene	Protein content %	0.855
		ASAT	Fat content %	0.721
		proteins	Protein content %	0.917
Hojbru Bits LB31365/ DS	DS 59.37, SV 34.38, HS 6.25	SAP	Protein content %	0.960
		albumins	Fat content %	0.815
		GGT	Fat content %	0.875
		triglycerides	Milk yield	0.888
Ryttargard Kvarnakre LB31394/ ZS	ZS 87.50, NS 12.50	ALAT	Protein content %	0.912
		GGT	Fat content %	0.787
Orkels Rudi LB31349/ AN	AN 50.00, HS 50.00	proteins	Protein content %	0.909
		urea	Protein content %	0.984
		carotene	Protein content %	0.995

groups of cows, while the average coefficients of daughters of each separate bull indicated a close correlation among different coefficients. This facilitates the evaluation of different hereditary genetic and pathogenetic trait potentials of each bull passed from sires to their daughters (Jemeļjanovs, 2001) expressed as an ultimate result of interaction of the genotype, environment and management (Hammani et al., 2009).

Conclusions

1. From all groups of cows chosen for the study, the daughters of LB31470 Aks Moments and LB31007 Alters Disaks demonstrate the highest productivity results expressed as fat kg x protein kg in 305 days.
2. Cows, that are daughters of different bulls, kept in the same management system, have slightly increased group average biochemical blood parameters, including protein, urea, GGT, alkaline phosphatise, cholesterol, phosphorous, Ca and P interrelations. The daughters of bulls LB31007 Alters Disaks and LB31349 Orkels Rudi have reduced glucose levels. The altered indicators suggest that there is an insufficient amount of easily digestible carbohydrates and increased amount of forage in feed ration resulting in the potential of liver diseases while dehydration causes a decrease in productivity of cows.
3. There is a close positive correlation ($r = 0.654 - 0.997$ at $p < 0.05$; $p < 0.01$) among various separate blood serum biochemical and productivity ratios in daughter groups of all 7 bulls'. For the cows under the trial, these correlations differed according to the genetic composition of blood of their sires.
4. For the daughters of sires LB31349 Orkels Rudi and LB31365 Hojbru Bits there is a correlation between protein concentration in blood serum and protein concentration in milk, respectively, $r = 0.909$ and $r = 0.917$, while for the daughters of sires LB31368 Lanis Moments and LB31349 Orkels Rudi there is a correlation between urea concentration in blood serum and protein concentration in milk, respectively, $r = 0.931$ and $r = 0.984$.

5. Close positive and negative correlations are found among the average readings of enzymatic cofactors Cu, Fe, Zn, Mn and Mg and productivity ratios in daughter groups of all bulls which influence the milk yield and the quality as well as health of the cow.

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THE CHEMICAL COMPOSITION AND NUTRITIONAL VALUE OF FISH MEAT WHILE USING AS A FEED ADDITIVE ZEOLITE OF CHANKANAY ORIGIN

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Abstract
This article presents the results of studies of the chemical composition of the fish meat while using as a feed additive zeolite of Chankanay origin. The research was conducted during 2011-2013 at the Department of Veterinary-sanitary examination and hygiene of the Kazakh National Agrarian University and at the laboratory of JSC 'Kazakh Academy of Nutrition' LTD 'NUTRITEST'. The material of study was the zeolitic tuff of Chankanay deposit (Almaty region) and two-year trout Oncorhynchus mykiss. For the research we used feed by recipes of GosNIORH - 12-80. The chemical composition of fish meat was determined by a set of methods: moisture by drying at 105 °C, fats by Soxhlet, total protein by modified Kjeldahl method (ISO 937:1974), and minerals by incineration in a muffle furnace. Calorie content of meat was determined by Alexandrov's formula: X = C - (F+A) ´ 4.1 + F ´ 9.3. Fatty acid composition of fish meat was determined by gas-liquid chromatography. Thus, the results are the indirect evidence that zeolites added to primary diet for growing fish, do not adversely impact proteolytic enzyme systems of fish, that is, have no negative effect on fish organism.
Key words: feed, feed additives, zeolite, trout, chemical composition.

Introduction

The increasing demands on environmental protection and production of food that does not endanger health require an increase in production of materials to be used in nature-based agriculture. As non-toxic, ecologically advantageous and affordable materials, the natural zeolites, due to their structure, ion exchange and sorption properties and also many other characteristics are well suited for agricultural uses – in animal as well as plant production (Van Bekkum et al., 2001; Allen and Ming, 1995; Colella, 2002).

Zeolites are crystalline hydrated aluminosilicates of alkali and alkaline earth cations, consisting of three-dimensional frameworks of SiO4 and AlO4 tetrahedra, linked through the shared oxygen atoms to form an open crystal lattice with approximately uniform pores of molecular dimensions. It is well established that the various applications of these naturally occurring or synthetic materials are based on their physical and chemical properties to act as ion exchangers, catalysts and adsorbents (Mumpton, 1999).

Adding natural zeolite of the clinoptilolite type to feed mixtures in low doses of about 1–2% has influences on very important functions heretofore not recorded by other natural compounds (Rimar and Gavalova, 2002; Bindas et al., 2002; Skalicka et al., 2002; Konakova, 2003a; Konakova, 2003b)

Addition of clinoptilolite to feed is assumed to have a similiar effect to that of antibiotics as the clay mineral is able to bind 135 meq ammonium equivalent to 1.89 nitrogen per 100 g clinoptilolite (Bernal and Lopez-Real, 1993).

The study of fish meat's chemical composition is an important part of veterinary sanitary evaluation since the nutritional value of fish and its physiological role as a source of biologically active substances to the human body depends on the ratio of moisture, proteins, fats and minerals. Supplementation of fish feed with different feed additives not only improves the aesthetic appearance of fish products, but also increases the shelf life and increases the amount of vitamins, minerals and nutrients.

The aim of this work was to study the possibility of using zeolite of Chankanay deposit in the diet of fish to increase their productivity, the quality of the fish meat, improving the chemical composition and nutritional value of fish meat.

The study addressed the following tasks: determining the chemical composition of meat of rainbow trout using zeolites in the diet; the study of the amino acid composition of meat of fish in the application of zeolites; the determination of fatty acid composition of meat trout; and the study of the mineral composition of fish meat for use in their diet zeolite of Chankanay deposit.

Materials and Methods

The research was performed during 2011-2013 at the Department of Veterinary-sanitary examination and hygiene of the Kazakh National Agrarian University and at the laboratory of JSC 'Kazakh Academy of Nutrition' LTD 'NUTRITEST'.

The material of study was the zeolitic tuff of Chankanay deposit (Almaty region). The two-year trout Oncorhynchus mykiss have been the experimental material and were grown in artificial ponds. For the research we used feed by recipes of GosNIORH - 12-80.

In the first batch of experimental feed 0.125 g kg^{-1} zeolite was introduced by replacing 0.125 g kg^{-1} of wheat. Zeolite was introduced into experimental feed in the shape of grit 0.01 to 1 mm in diameter. The studies were performed in triplicate. The standard 10 m^3 cages have been used for this purpose. Density of fish stock, feeding rations and other parts of their growing biotechnology complied fish-breeding regulations for industrial fish farms. In each case age groups have been formed of fish that weren't significantly different from initial individual weight. Before and after the test, all the fish in each cage were weighed, and in order to determine the average individual weight, 20% of the weighted fish has been counted. Two- year trout with body mass of 200-250 g were fed by Reflex 1000 automatic feeder.

Determination of moisture, protein, fat and mineral content in fish meat was performed by conventional methods.

The chemical composition of fish meat was determined by a set of methods: moisture by drying at 105 °C, fats by Soxhlet, total protein by modified Kjeldahl method (ISO 937:1974), and minerals by incineration in a muffle furnace. Calorie content of meat was determined by Alexandrov's formula: X = C - (F+A) ´ 4.1 + F ´ 9.3.

X - calorie content of meat, kcal kg^{-1}; C – number of dry substance, g; F – number of fat, g; A – number of ash, g. (Asanbayev, 2010).

The amino acid composition of fish meat was determined by ion exchange chromatography on an automatic amino acid analyzer AAA-881 "Czechiya".

Fatty acid composition of fish meat was determined by gas-liquid chromatography. Homogenized meat samples were prepared for GLC (gas-liquid chromatography) analysis using direct saponification with KOH/methanol followed by a derivatization with (trimethylsilyl) diazomethane by the method of Aldai et al. (Aldai et al., 2006; Osoro and Barron, 2006; Najero, 2006). An ACME, model 6100, GLC (Young Lin Instrument Co.) equipped with a flame ionization detector, an automatic sample injector, and an Alltech ATFAME analytical column (fused silica 30 m×0.25 mm i.d.) was used. As the carrier gas He was used with a flow rate approximately 2 L min^{-1}. Temperature conditions of the oven, injector and detector were the same as in the method of Aldai et al. Results were evaluated with the conventional integrator program (Autochro-2000, Young Lin Instrument Co.) The individual FAMEs (fatty acid methyl esters) were identified according to similar peak retention times using standard mixture Supelco 37 Component FAME Mix.

The mineral composition of fish meat of was investigated as follows: the iron was determined by colorimetrical method using the GOST 26928-86, and GOST R-09-066-02 was used for determining calcium and magnesium.

The statistical analysis was performed using SPSS 17. One way ANOVA was used for comparison of mean values.

Results and Discussion

The result shows that in the muscle tissue of the experimental group fish differences were minor compared with the control group: the moisture and ash content were normal, and the amount of protein and fat were slightly above normal (Table 1).

The results of meat assessment showed that the use of natural zeolites in fish nutrition does not lead to significant changes in chemical composition, however, meat of experimental fish had a higher amount of proteins by 2.73 ± 0.031. Comparing values of tenderness and water-holding capacity of the control and experimental meat samples one should note a tendency towards their improvement, however, the pH level and color intensity have similar values. Lipids and ash content are practically on the same level. On the basis of experimental studies it can be stated that zeolites have a positive impact on chemical composition and physical and chemical characteristics of meat. However negative impact of clinoptilolites

Table 1

Chemical structure of fish meat (control and experimental, g 100 g^{-1})

Description, Units of measurement	Actually received	
Nutritional value, g 100 g^{-1}	**Control group (n =20)**	**Experimental group (n=20)**
Protein	16.0±0.17	18.73±0.201
Fat	5.3±0.115	5.4±0.11
Moisture	77.4±0.159	76.9±0.201
Ash	1.43±0.001	1.43±2.877
Energy value, J 100 g^{-1}	112±0.616	113±0.657

Table 2

The content of essential and non-essential amino acids in muscle tissue of fish (control and experimental)

Amount of essential amino-acids, mg 100 g^{-1}	Control (n=20)	Experimental (n=20)
Valine	1100±1.65	1096±3.17
Histidine	311±1.531	305±1.504
Isoleucine	801±0.965	804±0.821
Leucine	1804±0.519	1901±0.671
Lysine	1904±0.501	1907±0.785
Methionine	502±0.587	503±0.439
Threonine	904±0.226	910±0.233
Tryptophan	180±0.348	180.6±0.173
Phenylalanine	803±0.375	803.2±0.069
Replaceable, among them: Alanine	1006±0.113	1006.7±0.123
Aspartic	1711±0.417	1717±0.308
Glycine	602±0.274	604±0.179
Glutamic	2706±0.459	2721±0.86
Proline	503±0.257	487±0.312
Serine	801±0.113	803±0.22
Tyrosine	512±0.252	521±0.168
Cysteine	154±0.126	161±0.174

Table 3

Fatty acid composition of fish meat (control and experimental, g 100 g^{-1})

Description, Units of measurement	Actually received	
Fatty acid composition, g 100 g^{-1}	**Control group (n =20)**	**Experimental group (n=20)**
Saturated fatty acids: $C_{14:0}$ myristic	0.04±0.004	0.039±0.004
$C_{16:0}$ palmitic	0.78±0.005	0.77±0.005
$C_{18:0}$ stearic	0.32±0.005	0.33±0.005
Monounsaturated fatty acids: $C_{16:1}$ palmitoleic	0.38±0.005	0.39±0.005
$C_{18:1}$ oleic	2.08±0.005	3.13±0.049
Polyunsaturated: $C_{18:2}$ linoleic	0.27±0.005	0.3±0.005
$C_{18:3}$linolenic	0.03±0.005	0.4±0.005

on the studied parameters hasn't been detected. These findings are in agreement with studies of others (Mumpton, 1999; Konakova, 2003a).

The research of amino acid composition showed no significant effect of zeolite from Chankanay deposits on the amino acid composition of meat. The meat proteins of fish that received clinoptilolites with primary ration are characterized as complete, and containing all the essential amino acids, while meat of experimental fish displayed a higher content of essential amino acid - leucine and lesser content of non-essential - proline (Table 2).

From the results shown in the (Table 2) we can see that addition of natural zeolites into fish does not reduce nutritional value of meat. Tryptophan content in muscle tissue of fish did not change, but cysteine is somewhat increased.

It is known that fish meat does not contain significant amounts of lipids, however, research of their fatty acid composition has great interest not

Table 4

Mineral composition of fish meat (control and experimental, g 100 g^{-1})

Description, Units of measurement	Actually received	
Mineral content, in 100 g	**Control group (n =20)**	**Experimental group (n=20)**
Macronutrients, mg: K	451±0.68	458±21.8
Ca	35±0.61	36±0.61
Mg	25±0.64	27±0.62
Na	55±0.68	57±22.3
P	210±0.005	211±0.005
Micronutrients, mg: Fe	0.8±0.089	1.32±0.115
Zn	2.08±0.005	1.79±0.005
Mn	0.15±0.005	0.11±0.005

only for determining their biological value, but as an indicator, a change of which indicates the irregularities of biochemical processes in cells. Our data shows that feeding fish with zeolites leads to a tendency of rising levels of oleic acid (Table 3).

In the lipid fraction of the experimental fish we also noted higher levels of polyunsaturated fatty acids: linoleic, linolenic, which is probably associated with increased fat metabolism in organism of the experimental group fish.

Minerals perform multiple functions in the body. As structural elements, they are part of the bones, found in many enzymes that catalyze the metabolism of the body (Bondarenko, 2000; Ostroumova, 2002). Mineral substances are the important group of substances belonging to the essential nutritional factors and influencing quality and nutritional value of meat and meat products. Their amount is not significantly different for the experimental and control samples of meat with the exception of manganese and zinc, which are slightly less in meat of fish that received zeolite (Table 4). In our opinion, this could be due to high adsorption capacity of the latter.

Thus, the results are the indirect evidence that zeolites added to primary diet for growing fish, do not adversely impact proteolytic enzyme systems of fish, that is, have no negative effect on fish organism.

Conclusions

1. The chemical composition assessment of muscle tissue of fish, whose ratio included zeolites of Kazakhstan origin, showed no significant changes. A slight moisture reduction and rise of protein amount has been noted.
2. The obtained data indicates that amino acid composition in meat from fish, whose diet included local origin zeolites, shows a tendency towards increase of the essential amino acid leucine, and reduction of proline – non-essential amino acid.
3. Fatty acid composition in meat of fish that received zeolites as feed additives, undergoes changes: there is an increase of polyunsaturated fatty acid amount, including linoleic, linolenic acids.
4. When zeolites of local origin were added to the diet minor changes were noted in the mineral composition of fish muscular tissue, and a tendency towards decrease of certain elements' amount: zinc, which is likely due to their adsorption properties.

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VETERINARY SANITARY CHARACTERISTICS OF CATTLE MEAT INFECTED BY LEPTOSPIROSIS

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Abstract
This article presents data on veterinary and sanitary evaluation of cows' *(Bovis)* meat infected by leptospirosis. The material for the study was a sample of muscle tissue of m. *longissimus dorsi* taken at slaughter of 10 cows (Bovis) kept on farms in Almaty region. The following parameters have been examined in muscle tissue: water, protein, fat, ash, amino acid composition. Besides the biochemical studies we also measured pH of meat, put the reaction of neutral formalin (Formalin test) and determined the biological value of meat. It was determined that physical and chemical characteristics of sick animal meat have significant deviations from the norm. Such meat rapidly accumulates products of protein decay. The amount of essential and nonessential amino acids decreases, which indicates low nutritional value of meat. Meat of cattle infected by leptospirosis concedes by nutritional and biological value comparing to meat of healthy animals.
Key words: Leptospirosis, meat safety, meat quality, cattle.

Introduction

Leptospirosis is a serious worldwide zoonotic disease caused by infection with *Leptospira* spp., gram-negative spirochetes that comprise 24 serogroups and more than 250 serovars (Sun et al., 2010; Levett, 2001; Palaniappan, 2007). Although leptospirosis has a worldwide distribution, it has emerged as a major public health problem in the developing countries and it is most common in rural as well as in urban areas (slums) (Bharti et al., 2003; Cachay et al., 2005). Leptospirosis of cattle, according to many researchers, is pervasive in Kazakhstan and worldwide, and brings significant economic losses due to the high mortality rate in cattle (25 to 45% and more), reduced milk yield (23-37%), weight loss (18-28%), reduced thrift in young animals, calf mortality (90%), abortion (15-20%), reduction in commercial quality of leather from the affected animals and rejection of livestock products at meat processing plants, and reduced fertility, as well as expenditure of significant funds for diagnostic, preventive, curative, quarantine and restrictive measures (Кибасов и др., 2000; Ерубаев и др., 2001; Киркимбаева и др., 2003). The literature analysis allows to conclude that leptospirosis is often subclinical by nature, resulting in late diagnosis of this disease (Sang, 2010; Ezhkova, 1980; Anderson, 2007). It is known that quality of meat and meat products can change under the influence of various internal and external environmental factors on animals' organism. These changes gain special importance under various infectious diseases of animals (Глебочев, 2009).

Current 'Rules of veterinary inspection of slaughtered animals and veterinary-sanitary examination of meat and meat products' state that in case of diagnosing leptospirosis in slaughtered animals, their carcasses in presence of degenerative changes in muscles and presence of icteric coloration on tissues that persists for 24 hours, become subject to technical utilization with internal organs, but in the absence of degenerative changes and the disappearance within 24 hours of icteric coloration become subject to decontaminating by boiling or salting (Позняковский, 2007).

Meanwhile, manuals and handbooks on veterinary and sanitary examination have no data on nutritional and biological value of meat obtained from animals infected with leptospirosis. There are no developed methods of decontaminating products of slaughtered sick animals under various forms of leptospirosis. The hazardous level for humans from meat and products is still undetermined. Information on the results of comprehensive research of cattle meat under leptospirosis has not been found by us in the available literature. Therefore, the aim of the research was to study the nutritional value of beef infected by leptospirosis.

Materials and Methods

The research was conducted at the laboratory of the 'Quality control and safety of livestock products', Kazakh Academy of Nutrition, as well as at the department of 'Veterinary-sanitary examination and Hygiene' of Kazakh National Agrarian University from 2010 to 2012. Meat samples (m. *longissimus dorsi*) were collected from 10 cows *(Bovis)* slaughtered on farms in Almaty region. Leptospirosis has been diagnosed by serological and bacteriological methods. Control samples were selected from three healthy animals that have been selected on the basis of analogues (origin, live weight, age at time of research, productivity). Because the aim of this study was to

investigate the chemical composition of the meat of sick animals infected with leptospirosis, it was not intended to use a representative number of animals in the country. More details about the animal origin could not be obtained. The following parameters have been examined in the muscle tissue: water, protein, fat, ash, amino acid composition. Protein content was determined as total nitrogen content by Kieldahl method (ISO 937:1974); intramuscular fat was measured with the ISO 1443–1973 method. Assessment of the amino acid composition of meat was carried out by means of automated amino acid analyzer (AAA-881).

Sample preparation was made in 24 hours after slaughtering. Meat samples of about 300 g were homogenized with BUCHI B-400 (ISO 3100-1). Along with this biochemical study the pH of meat was measured and reaction of the neutral formalin (formalin test) was performed. pH of the meat was measured by puncture electrode (LoT406-M6-DXK-S7/25). Electrode is entered into the meat sample (m. *longissimus dorsi*) to a depth of 5 cm, near the adipose tissue and the pH value is read on the display.

Reaction with neutral formalin allows to recognize meat of animals slaughtered in state of agony or disease. Such meat accumulates globulin decay products - polypeptides and free amino acids. The reaction is based on the interaction of formaldehyde with them and with toxic substances that pass into the extract. The extract of meat from animals slaughtered in state of agony or during disease becomes murky while extracts from meat of healthy animals remain transparent (Позняковский, 2007).

The biological value of meat was studied in infusorians '*Tetrachymena pyriformis*'. A 2.4 mg sample of meat was placed in a mortar and ground for 2-3 minutes. Then added 8 mL assay medium of the following composition 100 mL distilled-Rowan water, glucose - 0.5 g, yeast extract - 0.1 g, sodium chloride - 0.1 g (pH 7.0-7.5). It was homogenized and 2 mL of substrate poured into a test tube. Then 2 mL substrates were poured in each of three bottles. The samples were closed with a rubber stopper and placed in a rack and put in boiling water for 30 minutes for inactivation of foreign microflora. Then cooled, and in sterile conditions added in them at 0.05 mL three-five-day culture of ciliates *Tetrachymena pyriformis.* The bottles were left at room temperature for 4 days. The bottles were shaken 2-3 times a day for better aeration medium and resuspension of food substrate. After 4 days the cells were counted. The relative biological value determined by ratio of the number of cells grown on the investigated product to the number of cells in the control product (Долгов, 2000).

The statistical analysis was performed using SPSS 17. One way ANOVA was used for comparison of mean values.

Results and Discussion

The research showed that although the sensory characteristics of meat obtained from animals infected with leptospirosis after 24 hours of storage in refrigerated state (maturity period) had no significant difference from the control by color, smell, consistency, but it significantly varied from the norm by physical and chemical parameters.

It was noted that meat obtained from infected animals quickly undergoes spoilage during storage. In our studies, the pH of the meat after 24 hours was equal to 5.97 in the control group, while the experimental group had 6.47-6.54, an average 6.5 ± 0.07. Meat extract from the experimental group gave positive result on neutral formalin reaction. These data indicate that the meat from animals infected with leptospirosis reduced its good quality.

The study of the chemical composition of cow muscle revealed that the moisture content in the control group was 65.8 ± 1.8 g 100 g^{-1}, in the experimental group 71.1 ± 0.8 g 100 g^{-1}, i.e. a slight increase in moisture seen therein - 5.3 ± 1.0 g 100 g^{-1}. The amount of protein in muscles of cows of the control group was 20.4 ± 0.9 g 100 g^{-1} and in the experimental group 18.8 ± 0.5 g 100 g^{-1}, i.e. decreased by 1.6 ± 0.4 g 100 g^{-1}, as there is a decrease in the fat of experimental group by 4 ± 0.9 g 100 g^{-1} fat. The amount of ash elements slightly increased (Figure 1). Calorie content of meat control group was 182.7 ± 2.5 g 100 g^{-1}, and in the experimental group, 164 ± 1.6 g 100 g^{-1} that is decreased by 18.7 ± 0.9 g 100 g^{-1}. These data indicate that the moisture of sick animal meat increases, while the amount of essential nutrients reduces, that is, the nutritional value of meat somewhat decreases.

Changes in amino acid composition of meat proteins indicate decline of product's nutritional value. In our research we compared 19 amino acids of experimental and control groups. Our studies showed that the amount of essential amino acids of the experimental group was slightly more reduced than of the control group. If the total amount of essential amino acids in healthy animals is 7662 ± 71.11 mg 100 g^{-1} in infected animals it was 7169 ± 63.64 mg 100 g^{-1}, i.e. decreased by 498.9 mg 100 g^{-1} (Figure 2). Also, the total amount of nonessential amino acids in meat of experimental group decreased by 740 mg 100 g^{-1}compared to the control group (Figure 3).

Research of amino acid composition revealed that meat of animals with leptospirosis has decreased amount of essential and nonessential amino acids compared to the control group. The result of this

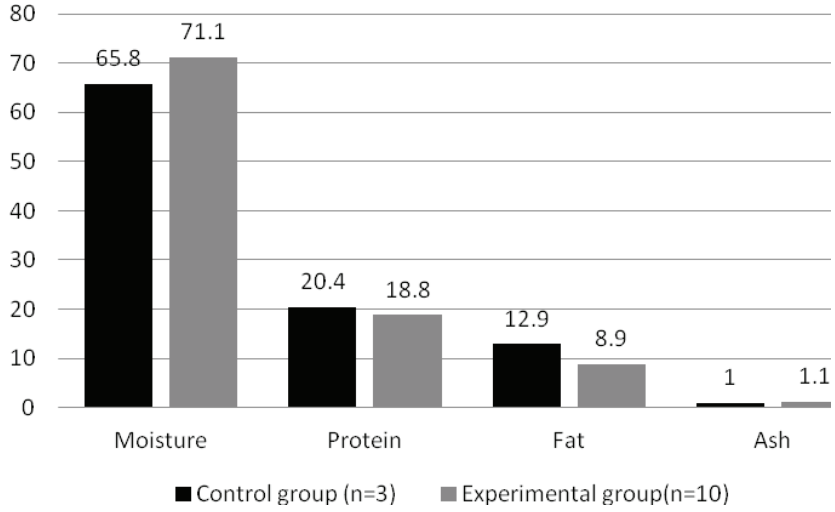

Figure 1. Chemical composition of muscules in lesions of leptospirosis (control and experimental, g 100 g^{-1}).

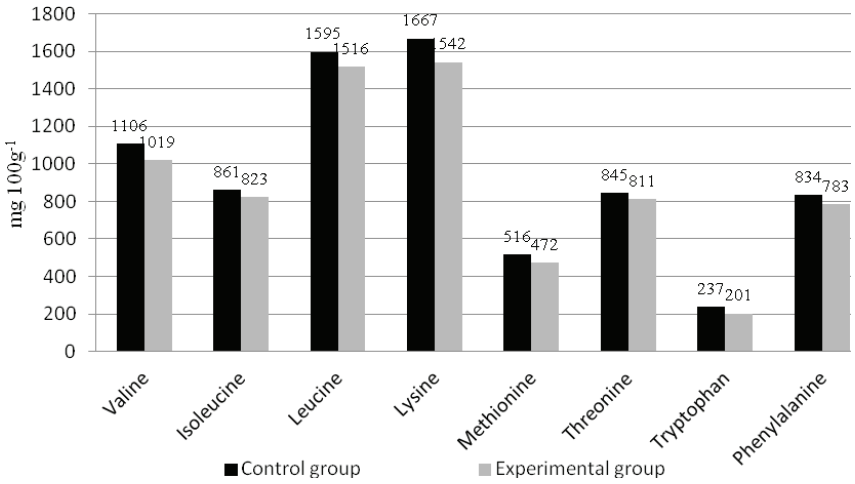

Figure 2. The amount of essential amino acids the defeat of meat leptospirosis (control and experimental, g 100 g^{-1}).

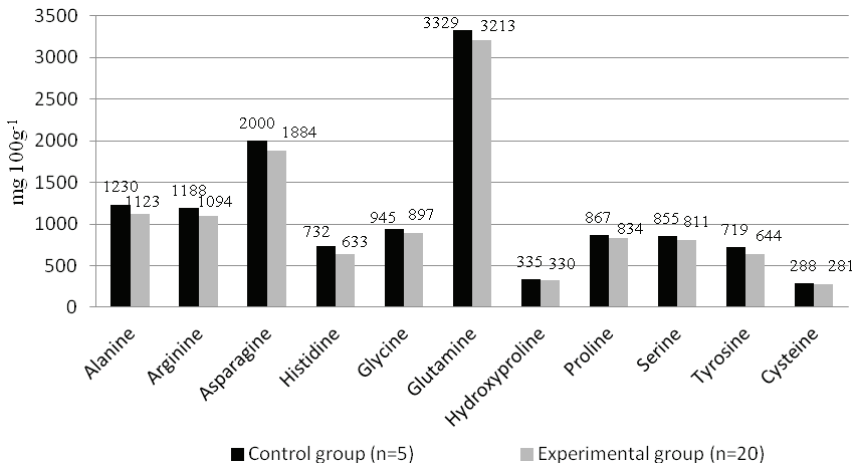

Figure 3. Amount of nonessential amino acids the defeat of meat leptospirosis (control and experimental, g 100 g^{-1}).

research also suggests that the consumer properties of meat of animals with leptospirosis is not a valuable source of proteins.

Additional protein test by Kjeldahl method showed that the total amount of amino acids correlates well with data obtained from amino acid analyzer.

A comparative histological analysis of muscle tissue of animals infected by leptospirosis and control animals showed no marked differences. No signs of pathological changes were found in skeletal and cardiac muscle tissue of experimental cows. However, various expressions of dystrophic and necrobiotic processes, marked bleeding diathesis and proliferative processes were noted in parenchymal cells of liver and kidneys.

One of the main problems in assessing food quality is to determine the product harmlessness, i.e. lack of its toxicity. One should not exclude presence of *Leptospira* waste products in meat. The meat toxicity was determined by means of test- organism on *Tetrachymena pyriformis* ciliates observing the amount of deceased infusorians, change in shape, nature of movement and presence of unusual inclusions in cells of *Tetrachymena pyriformis*.

Studies showed that the amount of cells with different variations was equal to 11.5% in the experimental group, whereas this value was 3.4% in the control group.

The study of technological influence on *Leptospira* survival by processing products from meat of animals infected by leptospirosis, showed that the existing regimes for roasting and smoking carcasses, the production of cooked, boiled and roasted sausages and wieners, as well as existing modes of sterilization and pasteurization of canned goods provides a 100% death of *Leptospira*.

Conclusions

1. The cow meat has slightly increased moisture amount under leptospirosis, whereas the amount of protein and fat is somewhat reduced, showing a decline in consumer properties of meat.
2. Meat of animals from experimental group showed changed reaction to the alkaline side, products of initial breakdown of proteins and decreased activity of tissue enzymes. This creates a favorable environment for the development of putrefactive microflora, resulting in product spoilage.
3. The amount of essential and nonessential amino acids in infected animals is somewhat reduced.
4. The experiments on infusoria *Tetrachymena pyriformis* showed harmlessness of meat of animals infected by leptospirosis, but noted decrease of its biological value.
5. Meat of diseased animals has much lower nutritional and biological value than meat of healthy animals, and can have a negative effect on human body when consumed. We suggest that even in the absence of pathological changes meat should be removed from the product line and should be send to an industrial processing - production of cooked sausages, canned food.

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CALCIUM AND PHOSPHORUS CONTENT IN ROMAN SNAIL (*HELIX POMATIA*) MEAT AND SHELL

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Abstract
The aim of the study was to determine and compare calcium and phosphorus content in pedal mass, visceral mass and shell of wild and bred trial groups' snails (*Helix pomatia*). The trial was performed from May to September of 2011 at the Roman Snail Research Facility of the Research Institute of Biotechnology and Veterinary Medicine „Sigra", of Latvia University of Agriculture (LLU). Snails were divided in 4 trial groups depending on the diets. Samples were collected from the local natural population of snails and experimental breeding farm in spring (May), summer (July) and autumn (September). Significant differences ($p<0.05$) were found between the levels of the calcium and phosphorus content in the edible part (pedal mass) and visceral mass in wild snails and experimental trial groups.
Key words: snail meat, calcium, phosphorus contents.

Introduction

The meat of Roman snails (*Helix pomatia*) is a popular product in many European countries, the demand of which continues to grow. Different data on quality indicators of the snail meat can be found in the research literature: on crude protein and crude fat content (Miletic et al., 1991; Ligaszewski et al., 2005; Zymantiene et al., 2006), and the composition of amino acids, fatty acids and minerals (Milinsk et al., 2003; Özogul et al., 2005; Milinsk et al., 2006; Çağiltay et al., 2011). At the same time, there are relatively little research data available on the impact of feed materials on the calcium content of the *Helix pomatia* meat and shell. It is known that snail shell basically is made of calcium carbonate**.** Calcium plays a variety of roles in the body of land snails, including in the fluid regulation, in the cell wall function, muscle contraction, and egg laying. The ion calcium is one of the most important elements in the shell of snails, which helps to protect the animal against predators and to avoid the dehydration (Soido et al., 2009).

Recently the cultivation of Roman snails (*Helix pomatia*) is rapidly growing in Latvia, as one of the alternative types of the non-traditional agricultural production. To ensure the competitiveness of locally produced snail meat on European markets, the research needs to be carried out on the quality of the obtained product and ways of improving it. Development of a quality product requires setting up of the snail feeding trials and performing in-depth research of the biochemical composition of the snail meat which up to now has not been done in Latvia. Research Institute of Biotechnology and Veterinary Medicine „Sigra", of Latvia University of Agriculture has set up a research facility of Roman snails (Reg. No. 051827) and undertaken the evaluation of the snail meat quality through biochemical studies.

The following hypothesis has been set forth for the present research: the calcium and phosphorus content in meat and shell of Roman snails found in Latvia depends on both, the cultivation conditions and the feed materials used.

The objective of the study: determination and evaluation of the composition of calcium and phosphorus in pedal mass, visceral mass and shells of Roman snails found in Latvia in the wild versus that of snails cultivated in a trial farm with the aim of using the results obtained for acquisition of high quality product with excellent organoleptic features.

Materials and Methods

In spring of 2011, a research facility of Roman snails was set up: an enclosure with the partitions (5.5 m^2 each). Snails were distributed among the partitions with the density of 500 snails per partition. Depending upon the diets fed to them, snails were divided into 4 groups (Table 1).

Table 1

Feeding trial scheme of Roman Snails

Groups	Feed materials used
Control	Wild plants
A	Wild plants and garden plants
B	Wild plants and special supplementary feed
C	Wild plants, wheat meal and wheat bran

The mix of wild plants included common dandelion (*Taraxacum officinale*), stinging nettle (*Urtica dioica*), common sowthistle (*Sonchus oleraceus*), greater burdock (*Arctium lappa*), creeping thistle (*Cirsium arvense*), white clover (*Trifolium repens*), coltsfoot (*Tussilago farfara*), common chickweed (*Stellaria media*) etc. The mix of garden plants included lettuce (*Lactuca sativa*), leaves of red beet (*Beta* vulgaris *L. subsp. conditiva*) and fodder beet (*Beta* vulgaris),

cabbage (*Brassica oleracea var. capitata*), cucumbers (*Cucumis sativus*), carrot tops, (*Daucus carota subsp. Sativus*) etc.

The samples were collected from the local natural population of snails and an experimental breeding farm in spring (May), summer (July) and autumn (September). The sampling was performed simultaneously from all partitions of the snail enclosure and from the wild. One aggregate sample consisted of 40-50 snails. After sampling, the snails were refrigerated for 24 hours (+ 4 °C). Post refrigeration the snails were slaughtered by mechanically breaking the shell and separating the pedal mass and visceral mass. The mineral compositions were analyzed from solutions obtained first by dry-ashing the samples at 550 °C and then dissolving the ash in standard flasks with distilled, de-ionized water containing a few drops of concentrated hydrochloric acid (Preparation of test samples ISO 6498). Phosphorus was determined by the spectrometric method ISO 6491-1998.

Statistical processing of data was performed with the software SPSS 17.0. (probability 95% or significance level – $p<0.05$). For the evaluation of crude fat level differences in different snail groups of the two sampled populations, T-test was used.

Results and Discussion

The trial results of the calcium and phosphorus composition in pedal and visceral mass of the wild and breeding snails are summarised in Figure 1.

The research data revealed that there are significant differences ($p<0.05$) between the levels of the calcium and phosphorus in the edible part (pedal mass) and visceral mass in wild snails and A, B and C trial groups. Results showed that there are not any significant differences ($p=0.33$) between the levels of the calcium in pedal and visceral mass in the control group. The most calcium and phosphorus (g 100 g^{-1}) was determined for the control group of snail meat (Ca 0.85 ± 0.08; P 0.25 ± 0.09) and visceral mass (Ca 1.99 ± 0.12; P 0.35 ± 0.03).

A. Gomot (1998) in her studies observed the opposite trend. Calcium in *Helix pomatia* viscera is two times less than in meat (pedal mass). The result of the mineral profile of four different breeds of snail (Babalola and Akinsoyinu, 2009), *Archachatina Marginata*, *Achatina Achatina*, *Achatina Fulica* and *Limicolaria* species showed that meat of *Archachatina Marginata* recorded the highest value (mg 100 g^{-1}) in calcium (126.40), phosphorous (22.01), while *Limicolaria* species had the least values in calcium (36.20) and phosphorus 0.008 respectively. Data of other scientists (Uboh et al., 2010) shows that calcium content varies from 199.26 ± 15.32 mg 100 g^{-1} (*Archachatina Marginata*) to 201.36 ± 12.45 mg 100 g^{-1} (*Achatina Achatina*).

Özogul et al., 2005 has identified calcium in the wild snail *Helix pomatia* 726.2 mg 100 g^{-1}, which is similar to our results (0.73 g 100 g^{-1} A group snails). F. Çağiltay et al., 2011 in the garden snail *Helix aspersa* has identified only 135.7 mg 100 g^{-1}, which is significantly more than in Atlantic bonito (Őksűz et al., 2008): 10.4 – 24.1 mg 100 g^{-1} fish meat. Phosphorus was observed of 104.5 mg 100 g^{-1} in

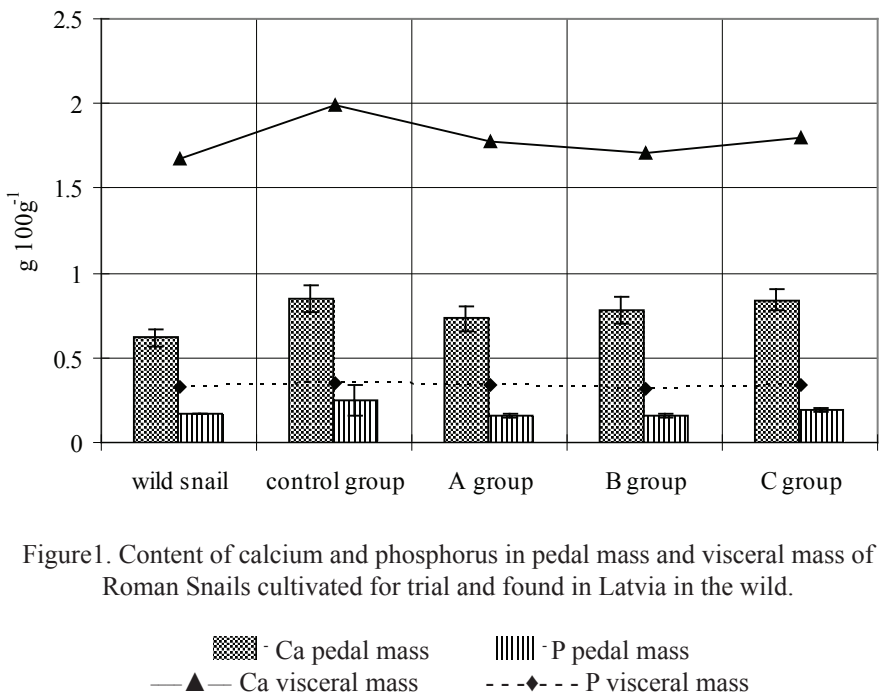

Figure1. Content of calcium and phosphorus in pedal mass and visceral mass of Roman Snails cultivated for trial and found in Latvia in the wild.

Helix pomatia meat (Çaġiltay et al., 2011), 96.7 mg 100 g^{-1} in the garden snails meat and 85.3 – 89.5 mg 100 g^{-1} in Atlantic bonito meat. In our study the most phosphorus was determined in the control group of snail meat P 0.25 ± 0.09 g 100 g^{-1}.

The results of the calcium and phosphorus composition in the shell of the wild and bred *Helix pomatia* snails are summarised in Table 2.

Table 2

Content of calcium and phosphorus (g 100 g^{-1}) in the shell of Roman Snails cultivated for trial and found in Latvia in the wild

Trial group (n=16)		Ca	P
1.	Control	36.30 ± 1.06	0.55 ± 1.06
2.	A	36.30 ± 0.99	0.55 ± 0.27
3.	B	36.43 ± 1.35	0.48 ± 0.24
4.	C	36.69 ± 1.65	0.51 ± 0.27
Wild snail (n=10)		39.14 ± 1.19	1.57 ± 0.26

Results expressed as g 100 g^{-1} in fresh matter

Values are presented as mean ± SEM – Standard Error of Means

Calcium content in the shell varies from 39.14 g 100 g^{-1} (wild snail) to 36.30 g 100 g^{-1} in the control and A group snails. The highest level of phosphorus level (1.57 ± 0.26 g 100 g^{-1}) was found for the wild snails. Our studies demonstrated that *Helix pomatia* shell contains significantly more calcium than phosporus.

Conclusions

Our data show, that *Helix pomatia* meat has similar levels of calcium as revealed in other studies. The phosphorus level in the control group of snails is higher than in other trial groups and other research studies. Latvian bred snail meat is richer in calcium than other species of snails.

In *Helix* snail shell the highest level of calcium (39.14 g 100 g^{-1}) and phosphorus (1.57 ± 0.26 g 100 g^{-1}) was found for the wild snails in Latvia.

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MORPHOMETRIC AND MASOMETRIC PARAMETERS OF THE OSTRICH (*STRUTHIO CAMELUS*) STOMACH IN POSTNATAL ONTOGENESIS

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Abstract
Stomach, as a part of the digestive canal, is important in the metabolic processes of the organism which affects the bird's general health condition, its growth and development, consequently also the increase of the body weight. The aim of the research was to find out the dynamics of weight, length, and area of the ostrich (*Struthio camelus*) stomach and its parts in postnatal ontogenesis from 4 to 12 months of age. The absolute weight of stomach, length of the greater curvature of the stomach glandular part, diameter of the muscular part, thickness of stomach wall was determined. To find out the differences of the mean values between various age groups, ANOVA and Post Hoch tests were used. The increase of the stomach absolute weight continued throughout the postnatal ontogenesis period, but the stomach weight in relation to the ostrich body weight decreased, especially rapidly at the age of 6 and 8 months. At the same time, proportions of the absolute and relative weight of the stomach glandular and muscular part changed. The length of the greater curvature of the stomach glandular part, length of the deep gland region and diameter of the stomach muscular part increased during the whole observed period of ontogenesis; moreover, these parameters increased more rapidly from 6 to 8 months of age. The area of the stomach glandular and muscular part mucosa increased throughout the observed period of ontogenesis. The area of the superficial gland region in all age groups was larger than the area of the deep gland region.
Key words: ostrich, stomach, growth, development.

Introduction

African ostrich *(Struthio camelus var. domesticus)* is the largest non-flying bird, which is raised in captivity mainly for meat production because it has a low fat and cholesterol content. The production yield depends on several factors, including digestive canal development, the stomach in particular. Stomach is important in the metabolic processes of the body, which, in its turn, affects the bird's general health condition, its growth and development, consequently also the increase of the body weight. In order to provide optimal conditions for ostrich keeping and feeding, further study is needed on development of separate parts of the digestive canal in postnatal ontogenesis.

The bird's stomach consists of two parts – glandular part (*proventriculus gastris; pars glandularis s. ventriculus glandularis*) and muscular part (*ventriculus gastris; pars muscularis s. ventriculus muscularis*) which are separated by a constriction (*isthmus gastris*) (Baumel, 1993; Rossi et al., 2005; Brūveris, 2007). The glandular part of the stomach in the ostrich is formed of two regions – the deep gland region and superficial gland region because of the different structure of their mucous membrane (Illanes et al., 2006; Порческу, 2007). The deep gland region (*regio glandularis*) is situated on the greater curvature of the glandular part, with a narrowed cranial end at the junction of esophagus (*oesophagus*) and with a widened rounded caudal end. The muscular part of the stomach is bilaterally curved with a complex structure. In most of the birds, it is developed from two layers of smooth musculature. A simpler structure of the muscular part is observed in the birds of prey and piscivorous birds. In the ostrich, the wall of the muscular part is thick, with a typical structure of herbivorous birds (Duke, 1997). The main caudodorsal and cranioventral thick muscle (*m. crassus caudodorsalis et m. crassus cranioventralis*) of the ostrich stomach are particularly well developed and 5.2-6.5 cm thick; in hens its thickness is 1.5-2.1 cm. Tendinous center (centrum tendineum) surfaces are joined (left/right) ventrolaterally and dorsolaterally, and divide the muscular part into cranial and caudal sack (*saccus cranialis et saccus caudalis*) (Baumel, 1993; Bezuidenhout, 1999; Sales, 2006; Порческу, 2007).

The aim of the study was to find out the dynamics of weight, length and area of the ostrich stomach and its parts in postnatal ontogenesis from 4 to 12 months of age.

Materials and Methods

In this research, 18 African ostriches of both sexes at the age of 4, 6, 8, and 12 months raised in Latvia on the farm Ozolini AB (Krustpils county Atasienes parish) and the farm Indrani (Sigulda county More parish) as well as in the premises of experimental animals of the Faculty of Veterinary Medicine, Latvia University of Agriculture were used. The temperature regime was maintained within the range of + 20 to 22 °C with air moisture of 43 - 50%, and light regime from 7 a.m. to 9 p.m. Ostrich chicks until two months of age were fed on the young birds feed Strus Premium – Strus 1, and over the further raising period, it was gradually changed to the wholesome young birds feed produced by the Latvian producer

Tukuma Straume, supplemented with oats and barley corn, barley meal, ground seashells, Dolfos D mineral substances, and vitamins. The feed, water and gravel stones were available ad libitum. After slaughtering, the body weight was determined, and necropsy performed for further examination. The absolute weight of the glandular and muscular part of the stomach was determined using scale Kern EW 420-3 NM (± 0.01 g), and the total and relative (in relation to the body weight) stomach weight were calculated. The stomach greater curvature length was estimated using a tape-measure (± 1 mm). The diameter of the muscular part and the thickness of the stomach wall in the deep gland region were measured with a digital slide gauge Limit-2000 (± 0.01 mm). By using the digital planimeter Sokkia KP-90N (± 0.1 cm), the total gastric mucosal area of the glandular part, area of the deep gland region and muscular part were estimated as well as their ratio was calculated. For the research data statistical processing SPSS 20.0 program was used. In each age group, the mean arithmetic value and standard error were calculated for all parameters. To find out the differences of the mean values between various age groups, a one-way variance analysis (ANOVA) and Post Hoch test were used.

Results and Discussion

The absolute ostrich stomach weight without its content increased ($p<0.001$) over the investigated period of ontogenesis; a significant increase was observed among 6, 8 and 12 months old ostriches ($p<0.001$). The absolute stomach weight with its content increased over the observed period of ontogenesis (see Tab. 1). However, the relative stomach weight with and without its content decreased ($p<0.001$) over the observed period of ontogenesis. A significant decrease of these parameters ($p<0.05$) was observed in 6 and 8 months old ostriches (see Tab. 2). Several researchers have noted that in ostriches at the age of 10 – 14 months the stomach relative weight with its content is 8.77 kg, i.e. 8.46%, but without the content it is 4.55 kg, i.e. 4.39% (Dijana et al., 2010). C.A. Moriss et al. (1995), in their turn, note that the relative stomach weight together with its content is lower – 5.8 kg, i.e. 6.05%. Even lower results have obtained K.D. Pollok et al. (1997) – 3.14 kg, i.e. 3.1%. P.A. Ilji and his colleagues (2003) have established that in younger birds the relative stomach weight is changing, namely, decreasing from 14.7% at the age of one month to 12.1% at the age of two months, but at 2.5 months of age the stomach relative weight has already increased to 15.1% (Ilji et al., 2003).

The absolute weight of the stomach glandular part increased ($p<0.001$) throughout the observed period of ontogenesis, but a more rapid increase ($p<0.05$) was observed in 6 and 8 months old ostriches, and in 8 and 12 months old birds (see Tab. 1). The relative weight of glandular part, in its turn, decreased with increasing of ostriches age ($p<0.001$). A significantly more rapid decrease of the relative weight ($p<0.05$) was observed from 4 to 6 months of age as well as in ostriches aged 6 and 8 months (see Tab. 2).

Table 1

Dynamics of the absolute weight of ostrich stomach and its parts from 4 to 12 months of age (g ± Standard error)

Age, month	Absolute weight of stomach glandular part	Absolute weight of stomach muscular part	Stomach absolute weight	Stomach absolute weight with its content	*Pars glandularis* and *pars muscularis* weight without their content
4	219.40±43.36	247.10±36.13	466.50±70.91	1218.55±220.99	1.20±0.15
6	274.50±36.83	422.75±43.98	697.25±68.88	1597.75±233.60	1.59±0.20
8	555.75±28.73	1605.75±61.53	2161.50±63.16	3691.00±385.37	2.92±0.20
12	785.00±62.81	2075.00±69.13	2860.00±112.84	5315.00±471.80	2.71±0.20

Table 2

Dynamics of the relative weight of ostrich stomach from 4 to 12 months of age (% ± Standard error)

Age, month	Stomach relative weight without content	Stomach relative weight with its content	Relative weight of stomach glandular part
4	7.92±0.77	20.52±2.78	3.71±0.58
6	6.44±0.64	14.66±2.20	2.51±0.29
8	4.28±0.35	7.15±0.34	1.09±0.05
12	3.84±0.63	7.27±1.50	1.07±0.22

Table 3

Dynamics of the ostrich stomach parts length and wall thickness form 4 to 12 months of age (mm ± Standard error)

Age, month	Length of *pars glandularis curvature major*	Diameter of *pars muscularis*	Wall thickness of *pars glandularis regio glandularis*	Wall thickness of *pars glandularis*	Wall thickness of *pars muscularis*	Length of *pars glandularis regio glandularis*
4	349.25±23.17	109.75±10.64	9.47±0.60	3.25±0.45	27.14±5.62	170.50±7.23
6	381.75±20.97	123.50±5.42	11.12±1.24	3.78±0.54	33.38±3.58	202.50±16.48
8	492.50±23.94	196.00±2.45	11.73±0.89	4.60±0.27	65.60±2.02	287.50±6.61
12	520.17±33.35	206.00±1.83	15.29±0.70	5.85±0.67	68.27±1.82	285.00±13.80

The absolute weight of the stomach muscular part increased ($p<0.001$) over the ontogenesis period studied, but a significant increase ($p<0.001$) was observed from 6 to 8 months of age, and in 8 to 12 months old ostriches (see Tab. 1). Although the ostrich stomach muscular part visually looks like a hen stomach muscular part, its weight without the content is even 12 times larger than that in a hen (52-81 g), and in an adult ostrich it reaches 960-1025 g (Порческу, 2007). The ratio between the absolute weight of stomach muscular part and glandular part increased from 1.19 ± 0.2 to 2.89 ± 0.2 throughout the observed period of ontogenesis ($p<0.001$), but significantly more rapid increase ($p<0.01$) was observed in 6 and 8 months old ostriches (from 1.59 ± 0.2 to 2.89 ± 0.2) (see Tab. 1).

The length of the greater curvature of the glandular part of the ostrich stomach increased ($p<0.01$) throughout the observed period of ontogenesis, but a significant increase of length ($p<0.05$) was observed in 6 and 8 months old ostriches (see Tab. 3). The wall thickness of the superficial gland region increased throughout the observed period of ontogenesis (see Tab. 3). Other scientists also have noted that in adult ostriches the wall thickness of the superficial gland region reaches 7–12 mm decreasing caudally to 1.2–1.5 mm (Порческу, 2007). The specific structure of the ostrich stomach with a relatively large glandular part and thin wall provides ability to intake a large amount of dry feed (Cho et al., 1984). It could be explained by the fact that in ostriches and emu, contrary to other bird species, there is no crop that is why its functions are carried out by the glandular part of the stomach. Consequently, the glandular part is large, because it is used as a reservoir for feed and water storage even up to 20 hours. In older birds and in larger young birds with a slower metabolism, these water reserves may be stored even for a longer time (Degen et al., 1994).

The length of the deep gland region of the glandular part of the stomach increased throughout the period of ontogenesis studied. A significant increase ($p<0.01$) was observed from 6 to 8 months of age (see Tab.3). The stomach wall thickness of the deep gland region increased throughout the investigated period of ontogenesis ($p<0.01$), but significantly more rapidly ($p<0.05$) this parameter increased from 8 to 12 months of age (see Tab.3). The obtained results on the length of the deep gland region and wall thickness changes in 12 months old ostriches correspond to the length of an adult ostrich gland region of glandular part, as indicated also by other authors – M.E. Fowler (1991) and G.S. Porchesku (Порческу, 2007). For instance, M.E. Fowler (1991) noted that the length of the deep gland region of glandular part was 240 mm, width – 4–7 mm, and wall thickness – 10 mm. According to G.S. Porchesku (Порческу, 2007) studies, the length of glandular part is 180–270 mm, width at the cranial end 45–60 mm, at the caudal end 70–130 mm, in the middle part 31–38 mm, and the maximal thickness is 15 mm. In this region of adult ostriches, around 750–1200 deep gland excretory ducts open (Порческу, 2007). The area of glandular part of ostriches differs from other ratites: in emu, the deep gland zone occupies all gastric mucosa of glandular part while in rhea only half of it (Fowler, 1991).

The diameter of the stomach muscular part increased throughout the observed period of ontogenesis ($p<0.001$), but a more rapid increase ($p<0.001$) was observed from 6 to 8 months of age (see Tab. 3). The wall thickness of the stomach muscular part increased ($p<0.01$) throughout the investigated period of ontogenesis, but significantly ($p<0.001$) it increased from 6 to 8 months of age (see Tab. 3). D. Swart and his colleagues (1993) indicated that the diameter of the ostrich stomach muscular part was 120±10 mm at 4 months of age, which was also proved by the present study results. The length of an adult ostrich stomach glandular part ranges from 141 to 155 mm, length 133–152 mm, and thickness is 79–92 mm (Порческу, 2007). M.E. Fowler (1991) observed 120–160 mm diameter of the muscular part in the adult ostrich while in emu and rhea it was larger than glandular part.

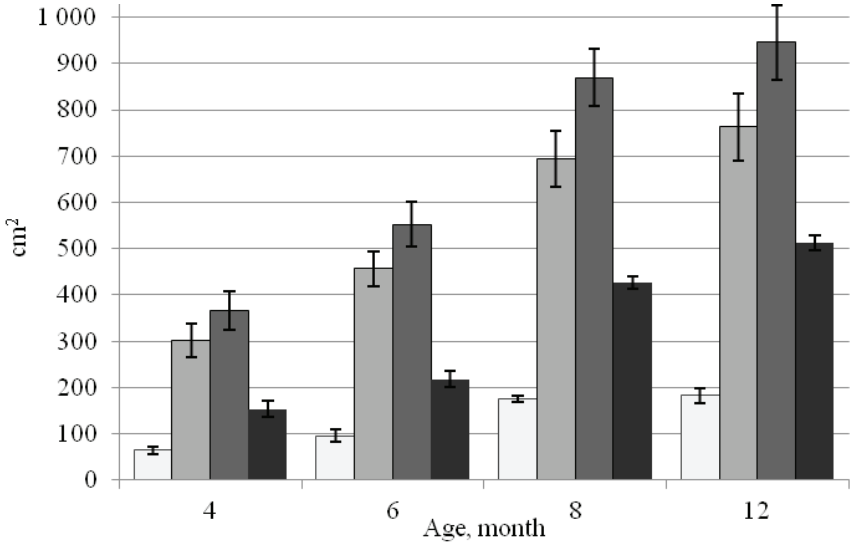

Figure 1. Dynamics of the area of ostrich gastric mucosa from 4 to 12 months of age:
□ Deep gland region of glandular part ■ Superficial gland region of glandular part
■ Stomach glandular part ■ Stomach muscular part

The mucosal area in both stomach parts increased with advancing of ostrich age ($p<0.001$). The area of the deep gland region of the stomach glandular part and the total mucosal area of glandular part increased significantly ($p<0.01$) from the age of 6 to 8 months. In turn, mucosal area of the stomach muscular part increased significantly ($p<0.05$) with advancing of ostrich age throughout the observed period of ontogenesis (see Fig. 1).

The area of the superficial gland region of the stomach glandular part exceeds the deep gland regional area by 4–4.7 times with a tendency to decrease with advancing of ostrich age. In adult ostriches, the superficial gland region exceeds the deep gland region of mucosal surface area by 2 -3 times (Порческу, 2007).

The area of the deep gland region of glandular part made 17.22 ± 1.37 – 20.4 ± 1.35% out of the total mucosal surface of glandular part throughout the observed period of ontogenesis. The area ratio of the stomach muscular part to the glandular part area from 4 to 6 months of age had a tendency to decrease from 1:3.56 ± 0.2 to 1:2.85 ± 0.2. Although in the ostrich, contrary to other running birds, the area of stomach glandular part is proportionally larger than muscular part area, the relative area of the deep gland of stomach glandular part is less than in other birds, only 25% of the total mucosal surface area in the adult ostrich (Cho et al., 1984; Fowler, 1991; Bezuidenhout, 1999; Cooper and Mahroze, 2004; Sales, 2006; Порческу, 2007).

Conclusions

1. The increase of the ostrich stomach absolute weight continued throughout the postnatal ontogenesis, while the stomach weight ratio to the ostrich body weight decreased rapidly at 6 and 8 months of age in particular, which indicates a more rapid increase of the ostrich body weight at this age. At the same time, proportions of the absolute and relative weight of the stomach glandular part and muscular part changed. If at the age of 4 months the absolute weight of both stomach parts was almost equal, then starting from 6 months of age, weight of the stomach muscular part continued to increase until it reached ratio 1:2.92 at the age of 8 months.
2. The length of the greater curvature of the stomach glandular part, the length of the deep gland region and the diameter of the stomach muscular part increased throughout the observed period of ontogenesis; however, a more rapid increase of these parameters was observed from 6 to 8 months of age.
3. The mucosal area of the stomach glandular part and muscular part increased throughout the observed period of ontogenesis. The area of the stomach superficial gland region of glandular part was larger than the deep gland region in all age groups.

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LOAD EFFECT ON THE DYNAMIC PARAMETERS OF THE WIND STATION

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Abstract
The research paper discusses simulation of the loading process of the vertical axis wind turbine with the permanent magnet synchronous generator. Experimental and analytical studies were done in the year 2013. The research results show that electrical load on the wind turbine generator is important for the wind turbine performance. If the load is above or below the power gained from the wind energy, the turbine operation cannot be considered efficient and its dynamic parameters fail to achieve the efficiency level at the proper operation load when the load is built up strictly in accordance with the wind energy input. In the research, 3 separate virtual electrical loading models with static, discrete, dynamic electrical load were established. MATLAB SIMULINK simulated virtual model shows the importance of usage of the right amount of electrical load in the vertical axis wind turbine with the permanent magnet synchronous generator. Final research results showed that the most effective loading process of wind turbine generator is a dynamic load.
Key words: wind turbine, vertical axis wind turbine, wind speed, simulation process.

Introduction

Renewable energy nowadays is one of the most widely discussed topics around the world and Baltic countries (Kuus, 2012). Wind energy as part of the renewable energy sources and its usage in the territory of Latvia is the most frequently researched and discussed. It takes a lot of time and financial costs to bring into the market a new generation wind turbine. Designing of the turbine is an important process, but in order to see how the real turbine operates in the wind area, the turbine should be built and tested in air tunnels or in good weather conditions with stable wind (Hu, 2009).

Modern computer software and applications help to conduct research in many areas. Wind industry can be one of the industries where simulation of the virtual models is of great help in testing turbines by making the use of the virtual turbine model (Thakur and Gupta, 2007). On a global scale, it becomes possible with the help of virtual models to fully reflect all the turbine components and the control process; however, the main problem that researchers come across when using virtual models is to make sure that the model is as much close to the real-life wind turbine as possible (Abbas and Abdulsada, 2010). Previous research has shown good results proving the possibility to build the virtual model for the total wind turbine process by including in the virtual model the real-life wind turbine aerodynamic parameters. However, the mentioned research proved to be insufficient to cover such aspects of the turbine dynamics as the control process and electric load control (Bati and Brennan, 2012).

Use of the vertical axis wind turbines with the permanent magnet synchronous generator (PMSG) may prove to be very useful and important for rural development (Sniders and Straume, 2008). The reason is that a very simple load control system can be constructed for such a type of the wind turbine. The system will consist of 3 main elements: the wind turbine, PMSG and the consumer of the generated power. In certain cases, the produced power can be used not only as electric energy, but as mechanical energy as well, for example, for pumping water or ventilation. Considering that in the rural area electric energy can be used directly for certain heating systems, it does not become necessary to transfer electric energy to the electric grid (Thakur and Gupta, 2012).

The main tasks of this work was to design and study the wind turbine load effect on the turbine performance, perform simulation of the virtual model operation with the help of the virtual model in MATLAB SIMULINK application as well as assess the possible turbine operation as a stand-alone turbine in the wind fields.

Materials and Methods

Research was established in the year 2013 on the personal computer software MATLAB SIMULINK. The object of the research is the vertical axis wind turbine, whose mechanical and aerodynamic parameters are described in the patent (Scerbina, 2009). It is important to mention that the vertical axis wind turbine does not have the pitch control system, which means that the wind turbine blade angle does not change. The patents provide the description of the main structure of the rotor, the blades and the blade cascade. For the purpose of discussing the wind turbine specification, it should be remembered that the most important parameter for the wind turbine is the coefficient of power C_p. In the modelled wind turbine C_p can be used as the turbine efficiency indicator; however, this is not true for all wind turbines. The C_p is the function of the wind turbine linear speeds ratio (TSR – Turbine Speed Ratio, λ) - $C_p = f(\lambda)$. C_p indicates the quality of aerodynamics of

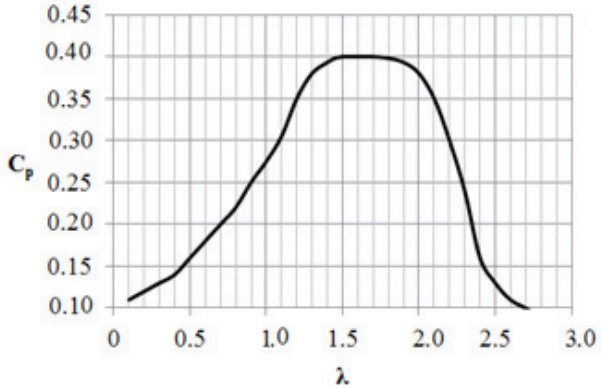


Figure 1. Turbine C_p curve as function C_p=f(λ).

the wind turbine. The C_p is calculated by formula (1): Ragheb, 2011).

$$C_p = \frac{P_e}{0.5 \cdot \rho \cdot v_w^{\ 3} \cdot A}, \quad (1)$$

where ρ = 1.225 kg·m^{-3} – air density
A – surface area of the wind turbine, m^2;
v_w –wind speed, m·s^{-1};
P_e – produced electrical power, W

TSR is important for a particular generator: when the blade set spins too slowly, most of the wind will pass by the rotor without being captured by the blades. If the blades are spinning too fast, they will always travel through the used/turbulent wind. This is because the blades will always travel through some point in space that the previous blade just moved through (and used up all the wind in that location). It is important to ensure that enough time elapses between the two blades travelling through the same point in space, so that new and unused wind can enter this spot. Thus, the next blade that passes through this point will be able to catch fresh and unused wind. To put it in a nutshell, if the blades are too slow they fail to catch all the wind they could. At the same time, if they are too fast, the blades go spinning through used/turbulent wind (Thakur and Gupta, 2012). For that reason, the TSR are employed when designing wind turbines to ensure that the maximum amount of energy is extracted from the wind when some particular generator is used. TSR is calculated by formula (2): (Ragheb, 2011).

$$\lambda = \frac{\omega_t \cdot R}{v_w}, \quad (2)$$

where ω_t – wind turbine angular speed, rad·s^{-1};
R – Turbine rotor diameter, m;

The mechanical dimensions for researched turbine: blade length L = 7.5 m, rotor diameter D = 7.5 m. For the researched wind turbine the C_p = f (λ) is achieved on the basis of experimental results (Fig.1). From the C_p = f (λ) of the researched turbine for the highest C_p the λ = 1.6. The higher is C_p, the more efficient is the wind turbine.

Produced energy serves as the indicator for the turbine performance. Since the researched turbine is without the pitch control system, the only parameter available to control the turbine is the electrical load. The wind turbine technical parameters for electrical load calculation can be described by the constant k_λ. For the purpose of experiment the turbine calculated k_λ = 1.75 kg·m^2. The k_λ is calculated by formula (3): (Ragheb, 2011).

$$k_\lambda = \frac{\pi \cdot \rho \cdot R^5 \cdot C_p \cdot i^3}{2 \cdot \lambda^3}, \quad (3)$$

where R = 3.75 m – wind turbine rotor radius;
i = 1:6 – gear ratio (multiplier);
C_p = 0.4 – wind turbine efficiency.

Simulation of the researched virtual model of the turbine will be done for 3 types of potential electric energy consumers: one constant load, discrete 5-stage load switched loads, dynamical load. The virtual model is built with the help of MATLAB SIMULINK. The model consists of 3 subsystems and 9 standard library blocks. Wind speed is simulated at the constant value or switched to real measured data. Wind speed data is linked to the subsystem 'VAWT'. Theoretical torque calculations are performed in the subsystem 'VAWT' (Fig. 3).

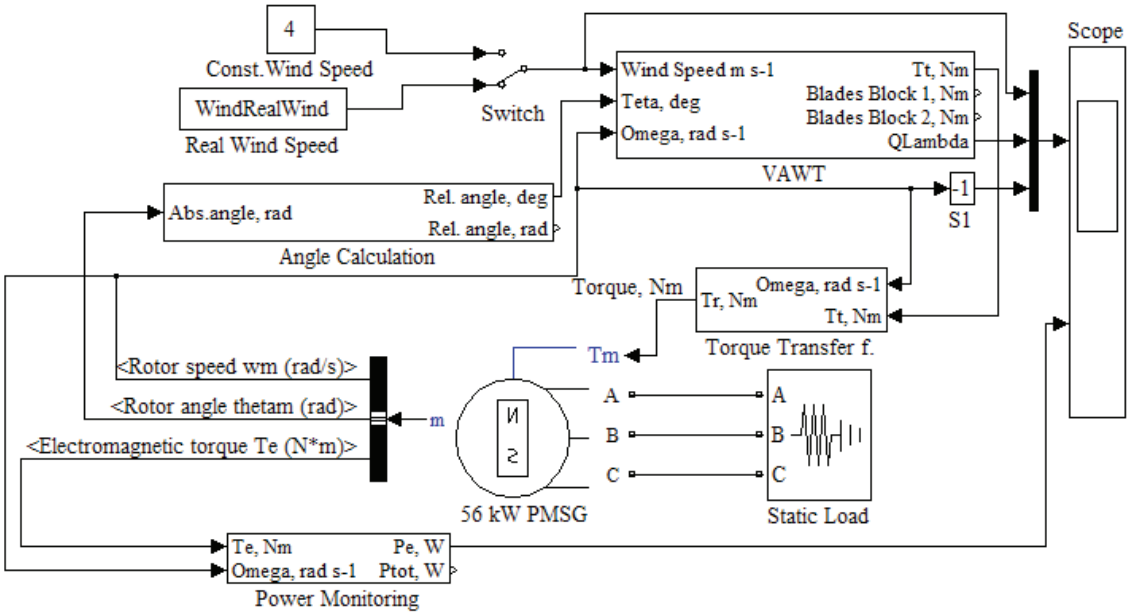


Figure 2. Wind turbine virtual model with static load.

For PMSG simulation the standard block from 'SimPowerSystems' library is used (Fig. 3). The block name in the virtual model is '56 kW PMSG'. To achieve more effective simulation results, real data according to specification of PMSG type 'M3BJ-315LKB-12' is used (Fedotov, 2011). It is important to take into account the fact that PMSG is represented by the second-order state-space model. One output of the block '56 kW PMSG' is the rotor angle (Fig. 3). The 3-fase static load block plays the role of a static electric energy consumer. The load block Three-Phase Series RLC Load' is imported from the standard library SimPowerSystems'. The block name in the virtual model is 'Static Load'. The problem with the static load is that there is no proper load control to ensure that the turbine works with the best dynamic parameters (ω_g, λ) called in wind industry 'maximum power point tracking for wind turbine' (Moor, 2004). If the load is too high for some particular wind speed, the turbine will rotate too slowly and if the energy from the wind is higher than electric load on the generator the system can go on overrun, which may damage the turbine and the PMSG.

There are two possible ways to control the load for the turbine. One of the solutions can be through discrete steps where, depending on the turbine angular speed, the load is controlled by connecting different loads to the turbine. The loading system with the discrete steps can be used to control the turbine. This solution can be used as the load sections with the switching devices to switch the right load on the electric line (Bati and Brennan, 2012). The flatter is the max point of the $C_p = f(\lambda)$, the more useful is the discrete load control solution. Electrical load connected to the PMSG from discrete load control block is calculated by formula (4):

$$P_{el} = \sum_{i=1}^{n} P_i + P_{const} \ , \qquad (4)$$

where P_i – the load for an individual load section, W; P_{const} = 2000 W– the constant load that is switched on the electric line all the time.

Discrete steps are switched according the wind turbine generator angular speed. The faster it is rotating, the more discrete steps are switched on. For discrete control method it is very important to ensure that the more steps in such multiple-step solution are used, the more accurate the control process is going to be. However, it is worth mentioning that increased number of steps is going to make the system more expensive. After the number of steps is selected, it is important to choose the right power connection for each of the steps. For this research the 5-step solution is used (Fig. 3).

Discrete load control will be not as accurate as the dynamic load control, which is the second possible control option. The dynamic load control is most accurate, in which case the system is calculating the required load depending on the dynamic and mechanical parameters of the turbine. For the purpose of dynamic load the standard block 'Three-Phase Dynamic Series RLC Load' from the standard library

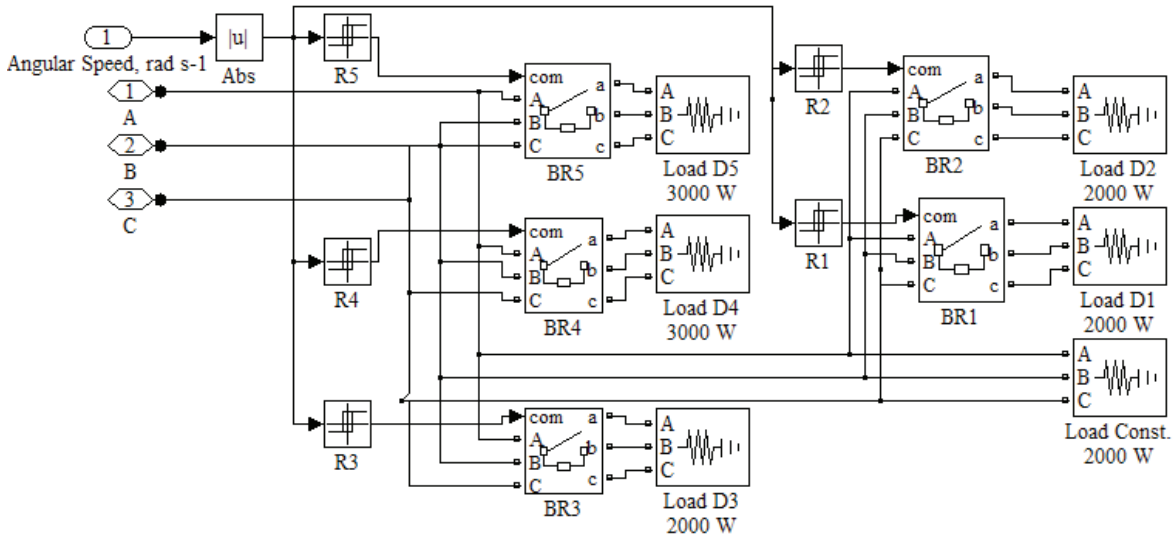


Figure 3. Discrete load control subsystem structure in virtual model.

SimPowerSystems (Fig. 4) is used. The block name in the simulation block program is 'Dynamic Load'.

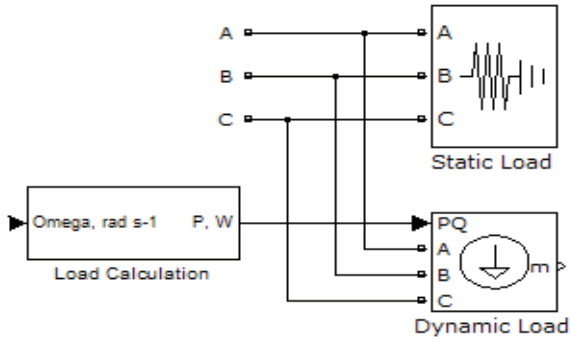


Figure 4. Dynamic load control structure in virtual model.

For calculation of the required load in the Dynamic Load block, it is possible to use two different sources of feedback. The first solution implies that we have measured the wind speed at some particular moment, and by using the power calculations based on the wind speed and turbine mechanical parameters, we are able to calculate the active electric power in the system (Barambones, 2012). Dynamic load reference active power P_r calculation also includes the wind speed data; it is calculated by formula (5): (Ragheb, 2011).

$$P_r = \frac{\rho \cdot A \cdot C_p \cdot v_w^{\,3}}{2}, \qquad (5)$$

The second possible solution is to use the K_λ parameter and the turbine angular speed ω_t. The second solution is good because it uses the turbine angular speed; thus, it is not affected by the wind speed measurements, which are too unstable, volatile and lack accuracy (Johnson, 2004). The wind speed is an unstable parameter, and it is very difficult to control anything by using this parameter. Dynamic load reference active power P_r calculated according to the turbine angular speed is calculated by formula (6): (Ragheb, 2011).

$$P_r = k_\lambda \cdot \omega_t^{\,3} \cdot i, \qquad (6)$$

It is better to use the turbine angular speed, which provides more information about the turbine power gained from the wind because of the high moment of inertia in the turbine. ω_t shows the received power from the wind while V_w shows the power that is likely to be received. When the angular speed is higher, the load should be higher too. Likewise, if the turbine rotates more slowly, the power should be lower.

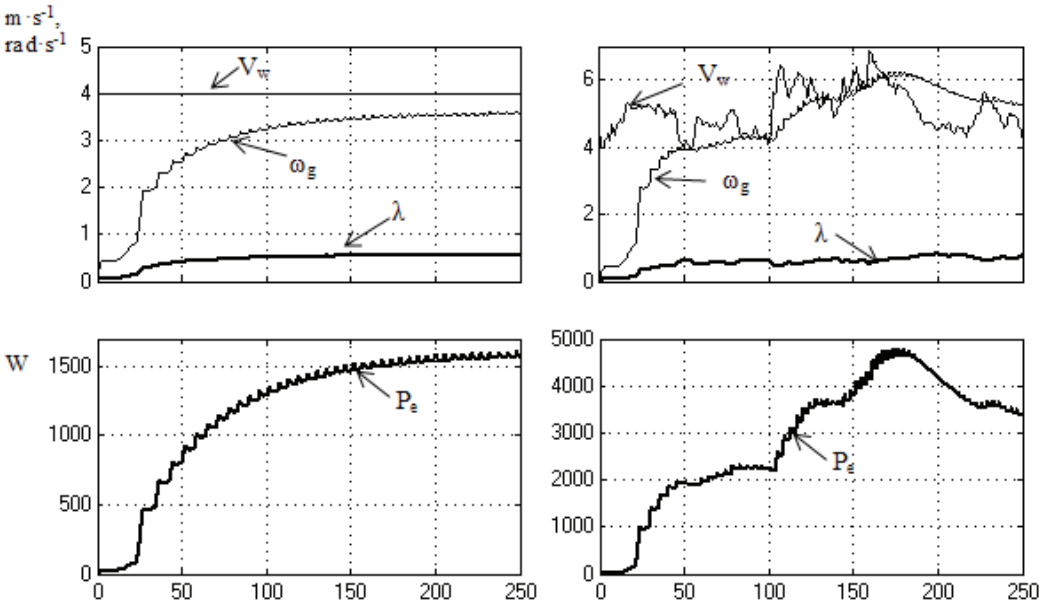
Results and Discussions

For all load simulations the wind speed is simulated as a constant - $v_w = 4$ m·s^{-1} or is transient from the real measured wind speed device data. At the static load simulation the static load is 56 kW, which is the rated electric power of PMSG. The results of simulation show that at the constant V_w the λ demonstrates high incongruity up to -55%, while the highest power coefficient $C_p = 0.4$ seems to be at the value λ = 1.6 ± 0.2. At λ = 0.7 the power coefficient of the wind turbine is $C_p = 0.18$, which is approximately 55% below the highest efficiency indicator $C_p = 0.4$. Power consumed from the wind turbine is $P_e = 1600$ W.

The wind turbine angular speed, reduced on the generator shaft is ω_g = 3.5 rad·s^{-1}. This constitutes 20 % of the rated angular speed of PMSG (Fig. 6).

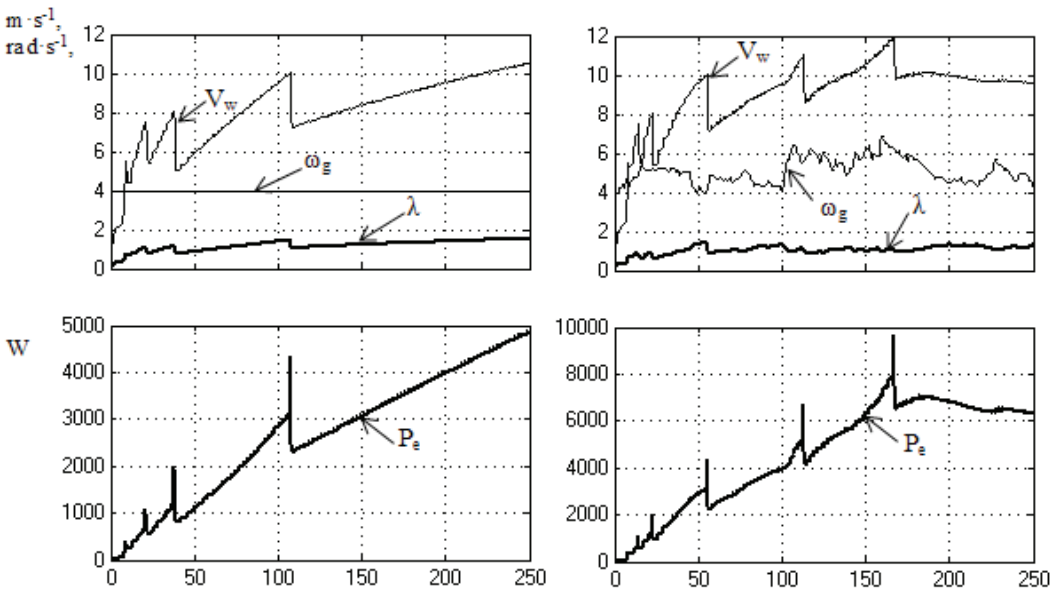
The results of the simulation, where V_w is simulated on the basis of the real-life data workspace in MATHLAB with average v_w = 5 m·s^{-1}. TSR demonstrates high incongruity of up to -35% of the most effective λ = 1.6 ± 0.2. C_p = 0.25 at the average being λ = 0.7 ± 0.2. The wind turbine P_e at the variable V_w is transient with the average value. The average power consumed form the Wind turbine is P_e= 2900 W. The average angular speed of the wind generator is ω_g= 5 rad·s^{-1}. The value of this ω_g constitutes approximately 27 % of the rated angular speed of PMSG.

For a discrete load simulation of the load on the wind turbine we use the PMSG consisting of 5 different loads (Fig. 3). The main results show that λ = 1.3 ± 1, which bring the approximate C_p = 0.35. The wind turbine PMSG angular speed line ω_g shows the places, where the load step is switched on. At load switching places the ω_g jumps down, which can be



a. b.

Figure 6. Simulation with static, rated load: a. constant wind speed, b. variable wind speed.



a. b.

Figure 7. Simulation with discrete load: a. constant wind speed, b. variable wind speed.

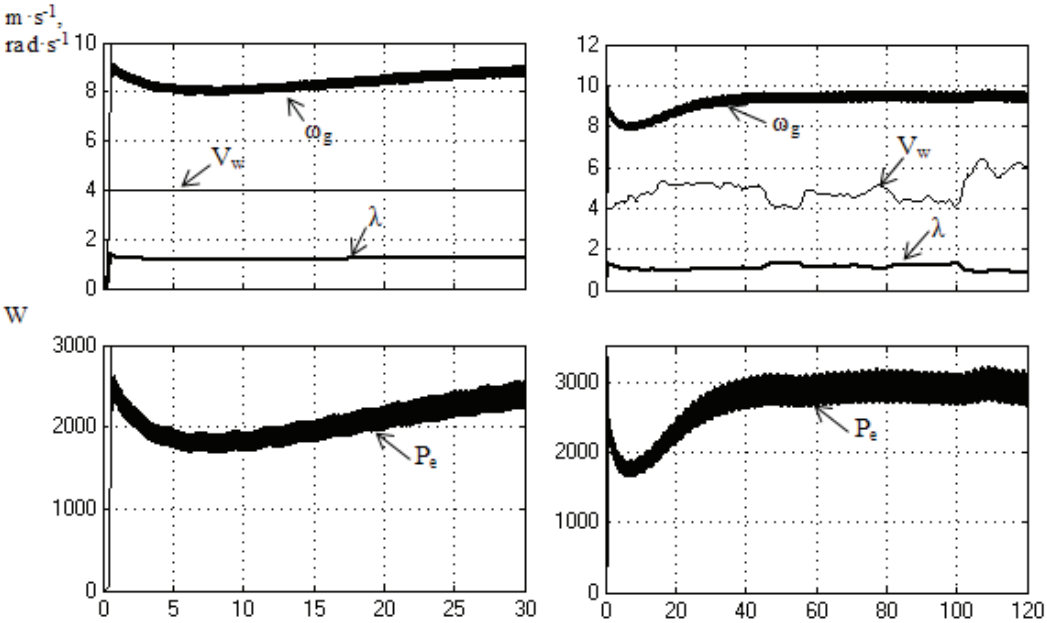


Figure 8. Simulation with the dynamic load: a. constant wind speed, b. variable wind speed.

described by the amount of the switched load. The smaller is the load, the less abrupt the downward jump of the angular speed ω_g will be. Very important jumps are registered in the P_e. At first, the power jumps up because of the bigger load on the PMSG, after that the power jumps down because the angular speed of the generator decreases (Fig. 7).

At the variable wind the speed is $\lambda = 1.2 \pm 1$, which produces the value of $C_p = 0.37$. The important role in the discrete load control is played by the discrete load step transient points, where the power is so high that it becomes necessary to switch on the additional load; however, when the load is switched on, the step appears too high, the turbine slows down to such an extent that the last switched load is switched off. This is shown in Figure 7 b, in the time range 0-50 s. Frequent switching can be the problem, and the reason for that is the choice of the load steps during the real-life wind turbine performance (Fig. 7).

For the purpose of dynamic load simulation the load on the wind turbine has been simulated within the range 0-3500 W (Fig. 3). Graphic results at a constant wind speed show that $\lambda = 1.6 \pm 0.5$, which brings about $C_p = 0.4$. The achieved generator angular speed after the process stabilisation is 9 rad·s^{-1}. The significant instant for the control method is the results for the P_e, which show that $P_e = 2800 \pm 200$ W (Fig. 8). The electric power deviation around the average value is of major importance. The deviation shows that this calculation cannot be used as the direct control method of the wind turbine because of the stability in the generator. With the additional transfer process the control should be improved to stabilise the P_e (Fig. 8).

Conclusions

1. Simulation results show that the load has a profound influence on the wind turbine performance; it can either improve or worsen the turbine performance. The only method of load control that proved to be correct for the wind turbine is the dynamic load control; however, further and more profound research of the discrete control method will make it possible to achieve very good results, which may be useful for rural development.
2. Wind turbine loading with one rated power load of the PMSG shows bad results for the influence of the dynamic parameters where λ is -55% below the required value $\lambda = 1.6 \pm 0.2$. At $\lambda = 0.7$ the power coefficient of the wind turbine $C_p = 0.18$, which is approximately 55% below the rated $C_p = 0.4$. The wind turbine angular speed, reduced on the generator shaft is $\omega_g = 3.5$ rad·s^{-1}. This represents 20 % of the rated angular speed of PMSG (Fig.7)
3. Wind turbine loading with one rated power load of the PMSG shows the load influence on the dynamic parameters, where λ constitutes 81% of the rated $\lambda = 1.6 \pm 0.2$. At $\lambda = 1.3$ the power coefficient of the wind turbine $C_p = 0.37$, which is approximately 92% of the rated $C_p = 0.4$.
4. Wind turbine load control with the help of the dynamic load of the PMSG shows good results on the achieved dynamic parameters where λ and C_p are both at the rated value $\lambda = 1.6 \pm 0.2$, $C_p = 0.4$.

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SOLAR AIR HEATING COLLECTOR ENERGETIC EFFICIENCY

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Abstract
The aim of investigations was to compare different absorber material efficiency to examine collector tracking the Sun and stationary collector efficiency as well as the efficiency of insulated and non-insulated collectors. The 0.1x0.5x1.0 meter long experimental solar collectors were constructed for investigations and different types of absorber materials were made. The manifold length, the Sun radiation effect on the degree of air heating was analyzed. ASHARE used standard 93-2003 is determined for insulated and non-insulated solar collectors and absorber effectiveness. We determined the influence of the Sun radiation on the air heating degree for those types of absorbers. The experimental data were measured and recorded in the electronic equipment REG (electronic metering and recording equipment of temperature, radiation and lighting). The collector covered material was a polystyrol plate and different absorbers. We compared insulated and non-insulated collectors to prove that the insulated collector is more effective. The insulated collector was made of the collector surfaces faced with cellular plastic two cm plates. Our task was to calculate the air heating solar collector efficiency.
The collector tracking the Sun is approximately 30% more efficient than the same type of stationary collectors in operation time for 6 hours. Insulation increases solar panel efficiency especially in windy weather. The insulated collector demonstrates almost two times higher efficiency than the non-insulated collectors (up to 93%) with absorbent material steal tinplate on top.
Key words: solar energy, collector, absorber, air heating.

Introduction

Almost all energy on the Earth comes from the Sun. If we did not have the Sun, the Earth would be a cold, lifeless world. Plants grow due to the solar energy, the wind, and even fossil fuel is just the energy stored from the Sun.

The Sun, as an alternative energy source is more and more widely used in national economics. The greatest advantage of solar energy as compared with other forms of energy is that it is clean and can be supplied without environmental pollution. Over the past century, fossil fuels provided most of our energy, because it was much cheaper and more convenient than the energy from alternative energy sources. The limited reserves of fossil fuels cause a situation in which the price of fuels will accelerate as the reserves are decreased.

Today, solar heating is becoming more important than ever before. Natural gas and oil, which are used to heat our homes and water, are limited. As reserves of gas and oil shrink, these fuels become more expensive. If more people began using solar heating systems, fossil fuels such as oil and gas would become less expensive and last longer. Burning natural gas and oil in our heating systems also causes air pollution. Thus, the more people use solar energy to heat the air and water in their homes, the cleaner our environment would be (El Paso, 2011).

Solar energy is used to heat and cool buildings (both actively and passively), dry products, heat water for domestic and industry use, heat swimming pools, generate electricity, for chemistry applications and many more operations (Kalogirou, 2009).

The efficiency of a solar collector depending on the collector covered materials (polyvinylchloride film, cell polycarbonate PC, translucent roofing slate) absorber (black colored wood, steel-thin plate etc.), with different air velocities in the collector was investigated (Aboltins et al., 2009a; Aboltins et al., 2009b; Aboltins et al., 2010). The main efficiency parameter of a solar collector is the air heating degree, and we chose it as a criterion of efficiency.

There is no need for heat ventilation at the expense of the economy, because the system provides fresh air circulation, while the walls of the building do not overheat in summer, as the system acts as a coolant. In many countries, there is an increasing interest in solar wall panel use. Solar wall panel use is vastly discussed in works on Italian climate (Stazi et al., 2008).

The application of solar energy is completely dependent on solar radiation. An intrinsic difficulty in using solar energy is given by the wide variation in the solar radiation intensity. The availability of solar radiation depends not only on the location, but also on the season. Extreme differences are between summer and winter time, as well as on daily basis.

In general, solar air heaters are flat-plate collectors (FPCs), consisting of an absorber, a transparent cover, and backward insulation. The performance of solar air heaters is mainly influenced by meteorological parameters (direct and diffuse radiation, ambient temperature and wind speed), design parameters (type of collector, collector materials) and flow parameters (air flow rate, mode of flow). The principal requirement of these designs is a large contact area between the absorbing surface and air (Kalogirou, 2009).

The aim of this study was to compare different absorber materials' efficiency, to examine collector tracking the Sun and stationary collector efficiency as well as the efficiency of insulated and non-insulated collectors.

The main tasks of the research were:

1. to make 0.1x0.5x1.0 meter long experimental solar collectors for investigations;
2. to make different types of absorber materials;
3. to analyze the manifold length the Sun radiation effect on the degree of air heating;
4. to use ASHARE used standard 93-2003 to determine the insulated and non-insulated solar collector and absorber effectiveness.

Materials and Methods

Solar collector efficiency is influenced by multiple factors such as surface area, heat gain, heat loss through convection and conduction, and the conversion factor. Each collector type is ideally suited for certain types of external conditions.

The aim of our investigations was to compare different absorber material uses and distinguish its usability in the Sun air heating collectors for air heating in typical weather conditions in Latvia.

Investigation was related to the stationary flat-plate collectors situated on the wall in southward direction and also to collector tracking the Sun. In order to prove that the insulated collector is more effective, insulated and non-insulated collectors, were compared. Heat always tries to move from a hotter object to a cooler one. Insulation prevents or slows down the movement of heat. The insulated collector was made of the collector surfaces faced with cellular plastic two cm plates.

The 0.1x0.5x1.0 meter long experimental solar collectors were constructed for absorber materials properties research. Different materials were used as absorbers: steel tinplate with cylinders of black coloured cans (Fig.1), black coloured steel tinplate (Fig.2). The air velocity in the experiments in the collector was v=0.9 m s^{-1}. In the collector, we used a fan with power 100 $m^3 h^{-1}$. The aim of research was to investigate the air heating solar collector possibilities in order to use them in room heating.

Three equal sizes FPC were compared: collectors of insulated and un-insulated surfaces with absorber steel-tinplate as a covering material and a classic collector with the polystyrene plate as covering material and absorber tinplate in the middle of the collector. The experiments were made in September, 2010 under different weather conditions and different atmospheric air temperatures. To assess different absorber's influence, comparative research was done in similar weather conditions. The collector tracking the Sun in the experiment is shown in Figure 2.

In the experiments, the collector's cover material was polystyrol plate. This material has gained immense popularity due to such properties as safety, mechanical crashworthiness, translucence and high UV radiation stability. At the same time the covered material - polystyrol plate reduced the sun radiation by 12-15%. The solar radiation measuring instrument

Figure 1. View of stationary solar collector with cylinders of black coloured cans situated on steel-tinplate absorber.

Figure 2. The collector tracking the Sun comparative research in the experiment.

was the pyranometer which is used to measure total radiation.

The experimental data are recorded by means of an electronic metering and recording equipment of temperature, radiation and lighting REG (Baltic Instruments, 2004). It is equipped with 16 temperature transducers and metering sensors of solar radiation and lighting. The reading time of the data was one minute. The recorded data are stored in the REG memory and in case of need they are transferred to a computer for archiving for further processing. The information is stored in the form of a table. If it is needed, it is depicted as a graph.

Results and Discussion

Research was aimed at tracking the air temperature increase in the outlet of the collector. We would like to examine the degree of the Sun radiation influence on the air heating level of a stacionary collector. The radiation and air temperature dependence trend is shown in Figure 3 (a collector with absorber steel tinplate with cylinders of black coloured cans). Large data dispersion (R^2=0.5722) indicates that the horizontal irradiance angle of the Sun to the collector surface influences temperature increase.

The situation when an absorber (a steel-tinplate) is placed at the top of the collector was researched. To compare insulated and non-insulated collectors with a steel-tinplate as a covering material, it was observed that in the same weather conditions the insulated collector air warms up 3 degrees higher (at radiation 800 W m^{-2}) than the collector with non-insulated surfaces (Fig. 4.).

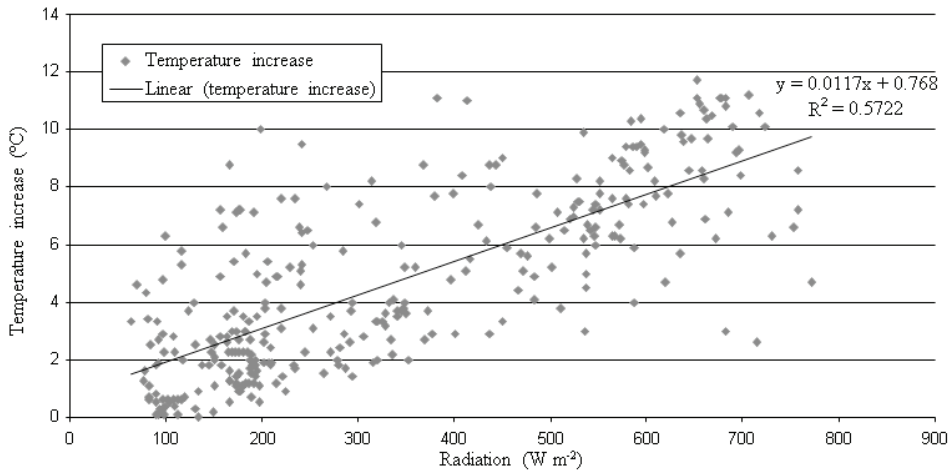

Figure 3. Temperature increase in outlet of collector (absorber steel-tinplate with cylinders of black coloured cans) compared with sun radiation.

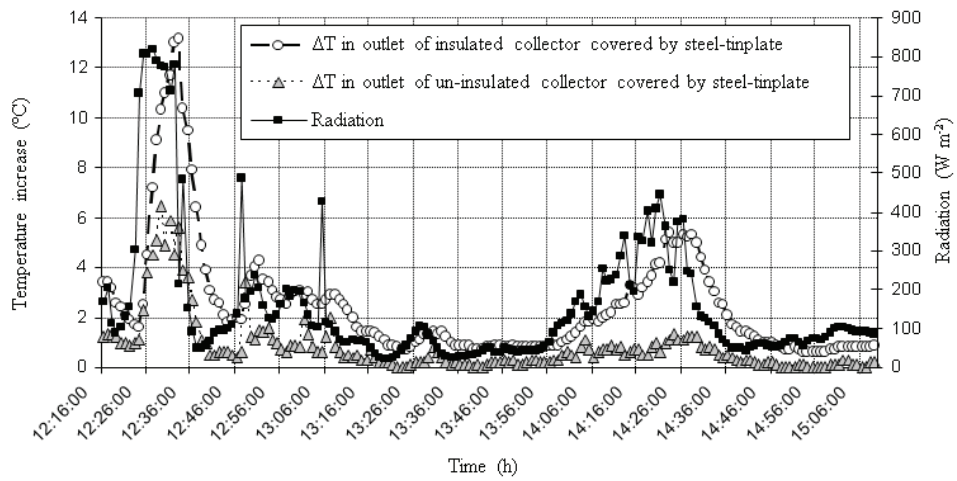

Figure 4. Temperature difference in outlet of collector with steel-tinplate covering material for insulated and un-insulated surfaces compared with sun radiation.

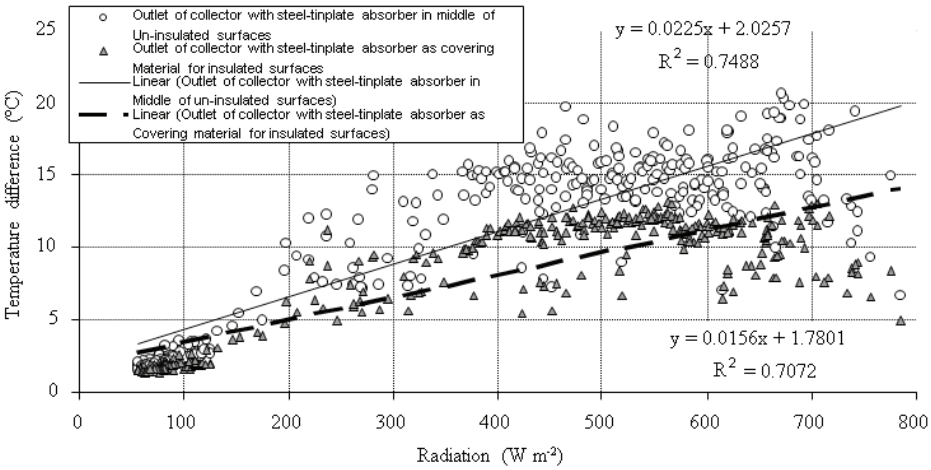

Figure 5. Temperature difference in the outlet of collector (with absorber tinplate at middle of collector body and with steel-tinplate absorber as a covering material) compared with the solar radiation.

The graph shows that the solar radiation changes significantly affecting passing air temperature. This effect does not happen instantly, but with a delay of 3-5 minutes. It should be noted that the non-insulated collector‘s efficiency is strongly influenced by the wind speed, which cools the surface of the collector body.

As it can be seen (Figure 5), if the sun radiation is low, constitutive air heating is not visible, whereas by increasing the Sun radiation, the air heating level is growing, and it can be noticed that the collector with absorber steel-tinplate at the middle of the collector body is more efficient than the collector with steel-tinplate absorber as covering material. The air heating level is not closely dependent on ambient temperature. Furthermore, it is influenced by solar radiation and insulation. If the collector is covered with the steel tinplate, this collector’s efficiency is highly influenced by environmental conditions, especially wind and ambient air temperature. These conditions reduce the absorber temperature. In the classic collectors these effects are much smaller.

The efficiency of the solar collector, as defined in ASHRAE Standard 93-2003 was determined. The efficiency of the solar collector can be calculated by the following equation (Morson, 2007):

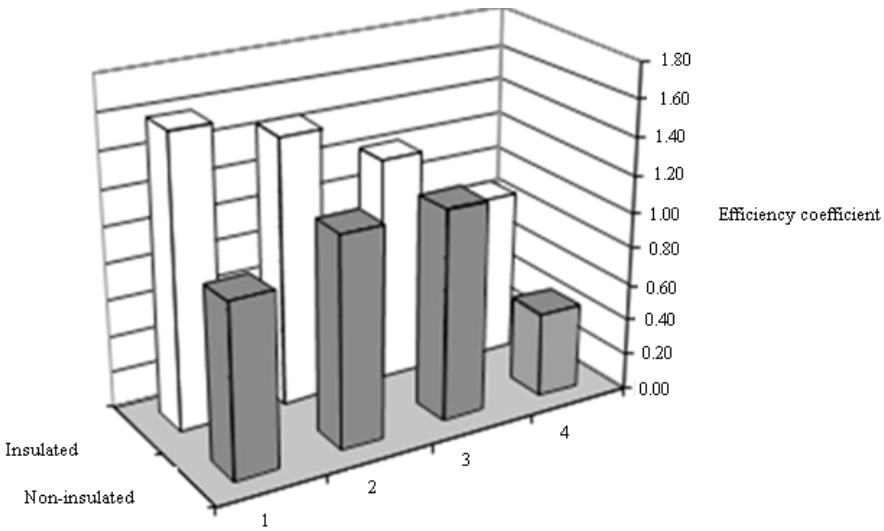

Figure 6. Efficiency diagram of insulated and non-insulated collectors:
1 – Black coloured cans on a steal tinplate; 2 – Bended steal tinplate in a centre;
3 – Steal tinplate in the middle; 4 – Steal tinplate on top.

$$\eta = \frac{m \cdot c_p \cdot (T_{fo} - T_{fi})}{S \cdot I_T} \quad (1)$$

where η = efficiency coefficient of solar radiation converted into heat;
m = mass flow rate of air, kg·s^{-1};
c_p = specific heat, J·kg °C^{-1};
S = area of solar collector, m^2;
T_{fo} = collector outlet working air temperature, °C;
T_{fi} = collector inlet working air temperature, °C;
I_T = global solar irradiance incident upon the aperture plane of collector, W m^{-2}.

Insulated and non-insulated collectors with the same absorbent material were compared. The results are shown in the diagram (Fig. 6). This graph clearly shows that the insulated collector is more effective than the non-insulated collector.

Conclusions

1. The most effective absorber is a steel-tinplate positioned in the middle of the collector for stationary and Sun-tracking collectors.
2. The collector tracking the Sun is approximately 30% more efficient than the same type of stationary collectors in operation time for 6 hours.
3. Collectors with insulated and non-insulated surfaces and with a steel-tinplate absorber as covering material heated ambient air by 10-12 and 5-6 degrees correspondingly (at solar radiation level 800 W m^{-2}). This difference between the two types of collectors assures the great importance of insulating the collector body.
4. Insulation shows great efficiency in windy weather conditions. Insulated collectors are almost two times more efficient than non-insulated collectors (up to 93%) with steal tinplate as an absorbent material on top.

Acknowledgements

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EXPERIMENTAL INVESTIGATION OF FUEL CONVERSION ADAPTER USING BIOETHANOL AND GASOLINE BLENDS

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Abstract
The paper contains description of the working principles and evaluation of the operational parameters of the commercially available fuel conversion adapter, intended to adapt gasoline fuelled spark ignition (SI) engine for use of high ethanol content blended fuel, known as E85. Commercially available gasoline and E85 fuel were used as test fuels. Production automobile, equipped with 1.8 litre 4 cylinder SI port fuel injection (PFI) engine was tested on the roll-type eddy-current chassis dynamometer in wide open throttle (WOT) constant speed mode. High precision fuel consumption measurement system AVL KMA Mobile was used. Engine operating parameters, used for evaluation of the efficiency of the fuel conversion adapter was engine torque (T), engine brake power (P_b), air/fuel equivalence ratio (λ), specific fuel consumption (SFC) and engine thermal efficiency (η_t). Analysis of engine operational parameters showed successful operation of fuel conversion adapter with E85 fuel, resulting in increase of engine peak torque by 4.4%, increase of energy efficiency in whole tested engine speed range up to 6.1% but increase of specific fuel consumption by approximately 22%, when compared with the gasoline use.
Key words: Fuel conversion adapter, ethanol, biofuel, spark ignition engine.

Introduction

In ongoing search for sustainable human mobility, biofuels are rising particular interest. Biofuels are considered to have less severe influence on climate change. Use of biofuels reduces dependence on fossil oil supply and rises energy supply security (Usner and Mueller-Langer, 2009). According to the directive 2009/28/EC of the European Parliament and of the Council, target of 10% for energy from renewable sources in transport must be reached in the year 2020 by all Member States (Directive 2009/28/EC). The recently approved European directive 'On the promotion of the use of energy from renewable sources' 2009/28/CE and the stringent environmental regulations have favored the use of bio-fuels in all the energy sectors and especially in transport. In the European Community, fuel quality is regulated by different fuel standards. The EN228 normative establishes the specifications for gasoline fuels. Ethanol for blending with gasoline must meet the EN15376 standard (Armas et al., 2012). Current implementation of EN228 standard in Latvia defines the use of 5% ethanol in gasoline blend as standard fuel for automobiles with spark ignition (SI) engines. Modern automobiles are built to operate with such fuel blend. Another standardized fuel blend, colloquially known as E85, contains 85% of anhydrous ethanol and 15% of gasoline. Requirements and testing of E85 are established in standard CWA 15293. Using ethanol as a fuel additive to unleaded gasoline causes an improvement in engine performance and exhaust emissions. Ethanol addition results in an improvement in brake power, brake thermal efficiency, volumetric efficiency and fuel consumption; however, the brake specific fuel consumption and equivalence air-fuel ratio decrease because of lower calorific value of the ethanol. Using an ethanol-gasoline blend leads to a significant reduction in exhaust emissions of carbon monoxide (CO) and hydrocarbons (HC) for all engine speeds (Agarwal, 2006). Major automobile producers offer automobiles, compatible with E85 or even hydrous ethanol. Availability of such automobiles, known as FFV or Flexible-fuel vehicles, depends on market and marketing decision (Pirs and Malnicenko, 2010). Part of the existing automobile fleet can be converted from use of gasoline to E85. Application of E85 fuel for powering the automobile built for use of gasoline requires evaluation of material compatibility (Baena et al., 2012). Lower vapour pressure and the lower combustion heating values of ethanol leads to requirements for a higher fuel injection quantity, and results in higher fuel consumption. The use of ethanol is associated with cold start problems (Jiang et al., 2009). To overcome these difficulties, conversion adapters exist in the market. There is lack of research results on the conversion adapter design, requirements and testing. This study focuses on detailed analysis of the typical fuel conversion adapter, offered in the European market. The aim of the study is to evaluate performance of fuel conversion adapter, its ability to ensure safe operation of petrol engine operating on E85 fuel. Engine power, torque, air/fuel equivalence ratio, specific fuel consumption and engine thermal efficiency will be measured and compared using petrol and E85 fuel, and with and without fuel conversion adapter.

Materials and Methods

Engine operating parameters, used for evaluation of the efficiency of the fuel conversion adapter are

engine brake power (P_b), air/fuel equivalence ratio (λ), specific fuel consumption (*SFC*) and engine thermal efficiency (η_t).

Commercial gasoline of standard EN228, identified as A95 and gasoline-ethanol blend of standard CWA 15293, identified as E85 were selected for this study. Properties of the test fuels are presented in Table 1. Technically both fuels are gasoline/ethanol blends. Pure gasoline is not commercially available for consumers in Latvia. Fuels were purchased in Statoil fuel stations in Latvia. Properties of test fuels were obtained from the certificates provided by fuel supplier. Lower heating value of the test fuels was estimated from basic values available in Biomass Energy Data Book, 2011.

Table 1

Properties of test fuels

Property	A95	E85
Density, kg m^3 at 15°C	740.0	786.9
Research octane number RON	95.4	106.5
Motor octane number MON	85.9	91.1
Ethanol content, volume %	4.8	84.5
Lover heating value Q_{LV}, MJ kg^{-1}	44.0	28.9
Air/ fuel ratio	14.7	9.8

Testing was performed on production vehicle Volkswagen Passat with a port fuel injection (PFI) spark ignition (SI) engine. Technical characteristics of the automobile are presented in Table 2.

Table 2

Technical characteristics of the test automobile

Model	Volkswagen Passat
Identification number	WVWZZZ3BZWE103686
Date of production	27.07.1997
Engine	Type ADR, 4-cylinder 20-valve
Compression ratio	10.3
Displacement volume	1781 cm^3
Bore	81.0 mm
Stroke	86.4 mm
Engine control system	Bosch Motronic M3.8.2
Gearbox	Type DHZ, 5-gear manual

Automobile was additionally equipped with fuel conversion adapter RMG-Rapsol B5. Installation was performed according to the manufacturer instructions. Conversion module was electrically connected to engine control unit (ECU) and fuel injectors, interrupting existing connections, and connected to chassis ground and power feed. Additionally, a conversion module was connected to the vehicle original oxygen sensor and throttle position sensor. Module uses its own coolant temperature sensor. The diagram of connection is shown in Fig. 1.

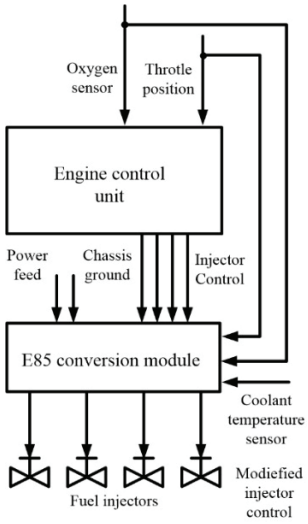

Figure 1. Connections of conversion module.

Testing was performed on roll-type eddy-current chassis dynamometer Mustang MD1750. Dynamometer applies specific load and measures torque on roll shaft. By knowing roll speed and engine speed of the tested vehicle, relative engine torque (*T*) can be calculated. Engine brake power (P_b) in kW is the product of engine torque (*T*) in Nm and rotational speed (ω) in rad s^{-1}:

$$P_b = T \cdot \omega. \quad (1)$$

Air/fuel equivalence ratio, λ, is relationship between actual and stoichiometric air/fuel ratio. At stoichiometric ratio all fuel and oxygen present in combustion chamber theoretically can react completely. Air/fuel equivalence can found using following equation:

$$\lambda = \frac{(A/F)}{(A/F)_S} = \frac{(\dot{m}_a/\dot{m}_f)}{(\dot{m}_a/\dot{m}_f)_S}; \quad (2)$$

where $\dot{m}_a$ and $\dot{m}_f$ are engine intake air flow rate and fuel flow rate, respectively (Costa et al., 2010).

Specific fuel consumption, *SFC*, in kg kW h^{-1}, as defined by Costa et al., 2010, is the fuel amount consumed per unit of power produced, and can be found by following equation:

$$SFC = \frac{\dot{m}_f}{P_b}. \quad (3)$$

The engine thermal efficiency η_t is a measure in percentage (%) of the fuel conversion efficiency, given by the relationship between the energy available at the engine output and the fuel energy content (Costa et al., 2010; Melo et al., 2012):

$$\eta_t = \frac{P_b}{\dot{m}_f \cdot Q_{LV}} = \frac{P_b \cdot 3.6}{FC \cdot Q_{LV}}; \quad (4)$$

where Q_{LV} is fuel lower heating value in MJ kg^{-1} and FC is fuel consumption in kg h^{-1}.

Air temperature in test room was 19 °C. Dynamometer control unit was used to calculate engine brake power and to register air/fuel ratio and exhaust gas temperature. Air-fuel ratio was measured using Bosch LSU 4.2 wideband oxygen sensor, connected to LM-1 Digital meter. Exhaust gas temperature at manifold exit was measured using K-type thermocouple. No corrections relative to the mechanical losses were applied to engine torque and power measurements. Measurements of fuel consumption were performed, using AVL KMA Mobile system. Fuel consumption was measured in volume domain. Fuel was supplied from external tank, cooled to 15 °C. When the fuel type was changed, fuel system was flushed three times to avoid influence of previously used fuel. Fuel flow was maintained by AVL KMA Mobile system internal fuel pump. Testing on chassis dynamometer was performed in constant speed mode. Using dynamometer control software, specific fixed roll speed steps were set, corresponding to the engine rotation speed from 1000 to 5500 rotations per minute (min^{-1}), by increasing step 500 min^{-1}. Each step lasted 20 seconds. Gearbox was set in 4th gear. Engine was tested in wide open throttle mode, limiting rotational speed at each measurement point by chassis dynamometer. The Motronic ECU works according to the proprietary algorithms, some of which are constantly adapting injection and ignition map values to maintain certain engine output characteristics. To avoid effect of ECU fuel trim adaptation, which can alter repeated measurements in unpredicted way, ECU adaptations were reset before each test drive. Value of ECU adaptation was monitored using Bosch KTS 570 diagnostic tool. Test results were excluded, if value of the ECU fuel trim adaptation exceeded 0%. Ignition advance correction was performed automatically, according to the ECU strategy, and recorded using Bosch KTS 570 diagnostic tool. Testing was performed with the vehicle set in four different configurations. Tests performed with the vehicle in production setup was identified A95 and E85, depending on the used fuel. Tests with the vehicle, equipped with fuel conversion adapter, were identified A95B and E85B. Each test was repeated 5 times and average values used as results. Data in results section is presented with error bars of 95% confidence intervals. Layout and principal connections of the testing equipment are shown in Fig. 2.

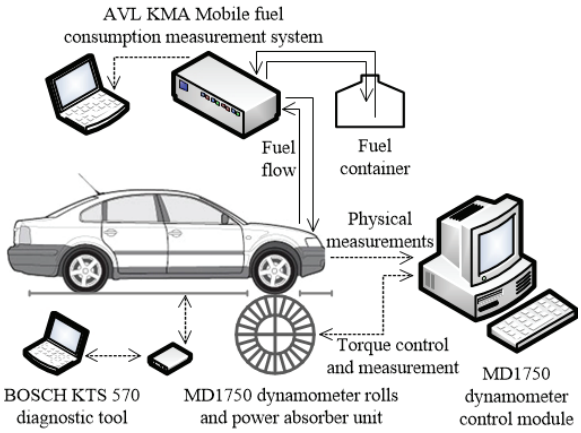

Figure 2. Test equipment setup.

Results and Discussion

Fuel conversion adapter RMG Rapsol B5 works by extending fuel injector control impulse which is supplied by ECU. ECU injector control circuit is isolated from the fuel injector solenoid coil. According to the information supplied by the producer of the fuel conversion adapter, amount of the correction of the injector opening impulse depends on engine temperature, throttle valve position and most of all, air/fuel ratio supplied by the oxygen sensor. Original and modified control impulse is shown in Fig. 3.

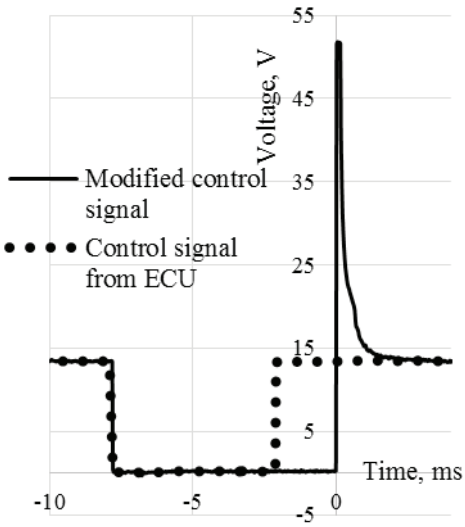

Figure 3. Injector control impulse.

Air/fuel equivalence ratio is presented in Fig. 4. In chosen test conditions, with the wide open throttle, ECU operates in an open loop mode. In the open loop

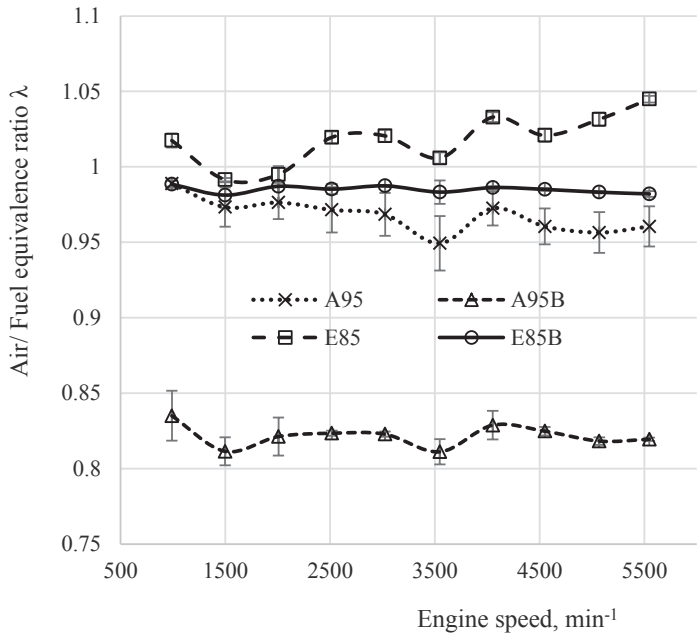

Figure 4. Fuel and conversion adapter influence on air/fuel equivalence ratio.

mode, feedback from the oxygen sensor is not used and air/fuel mixture is being prepared according to the pre-set map. That explains lean mixture in E85 test conditions, when unmodified fuel system works on E85 fuel. As the pre-set map was prepared for standard fuel (gasoline), but the stoichiometric ratio of the E85 is different, mixture is incorrect. Such operation conditions can lead to high temperature in combustion chamber and following engine damage. In test conditions A95B, when gasoline is used with an active fuel conversion adapter in place, air/fuel mixture is too rich. The reason for that can be long reaction time to fuel change or technical shortcomings of the particular conversion adapter. Tests performed in conditions A95 and E85B return satisfactory results of air/fuel mixture preparation during operation with the wide open throttle.

Diagrams of the engine torque and brake power are presented in Fig. 5. At engine speed range from 1500 to 3500 min^{-1} gasoline/ethanol blend in conditions E85B produced higher torque compared to the gasoline in conditions A95. At a low engine speed, around 1000 min^{-1}, the use of gasoline or gasoline/ethanol blend produced approximately the same torque. According to Costa et al., 2010, higher heating value of the gasoline is responsible for higher torque at low engine

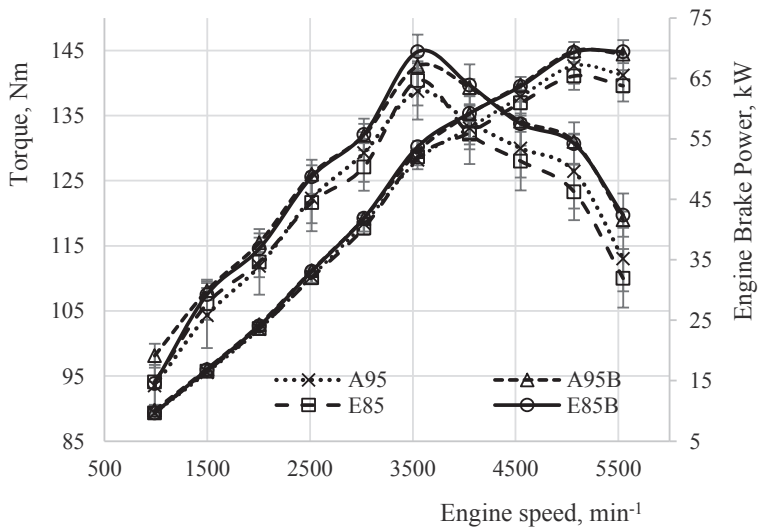

Figure 5. Fuel and conversion adapter influence on engine power and torque.

speeds. For higher engine speeds, faster flame velocity and higher resistance to detonation gives advantage to gasoline/ethanol blend E85 to produce higher torque, compared to gasoline (Costa et al., 2010; Jiang et al., 2009; Shifter et al., 2011). According to Szybist et al., 2010, a charge cooling effect is another factor which increases engine power. Latent heat of vaporization is 0.85 MJ kg^{-1} for E85 fuel and 0.35 MJ kg^{-1} for gasoline (Aleiferis et al., 2010). The charge cooling effect is created when fuel is sprayed into the intake air charge. During vaporization of the fuel, the air is cooled, reducing the specific volume of the intake charge. Since the latent heat of vaporization is significantly higher for E85 fuel, comparing to the gasoline, E85 is more effective at cooling the intake charge than gasoline.

Use of E85 fuel and conversion adapter produces maximal torque 144.8 Nm at 3547 min^{-1}, an increase

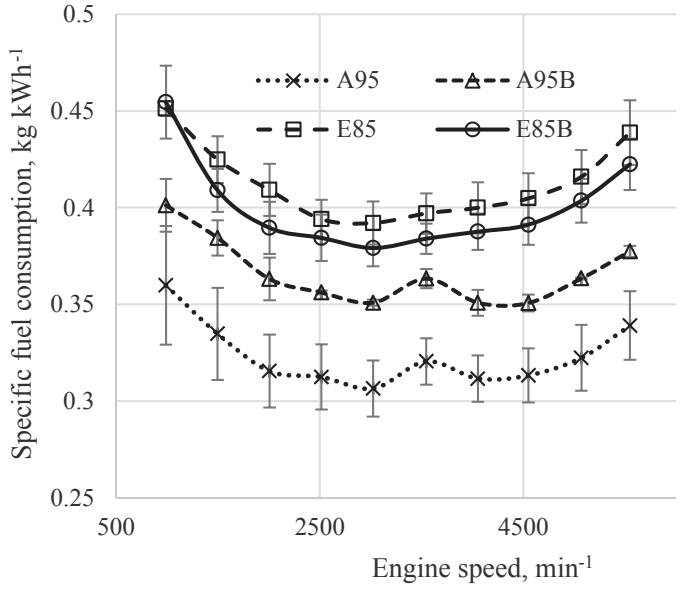

Figure 6. Fuel and conversion adapter influence on specific fuel consumption.

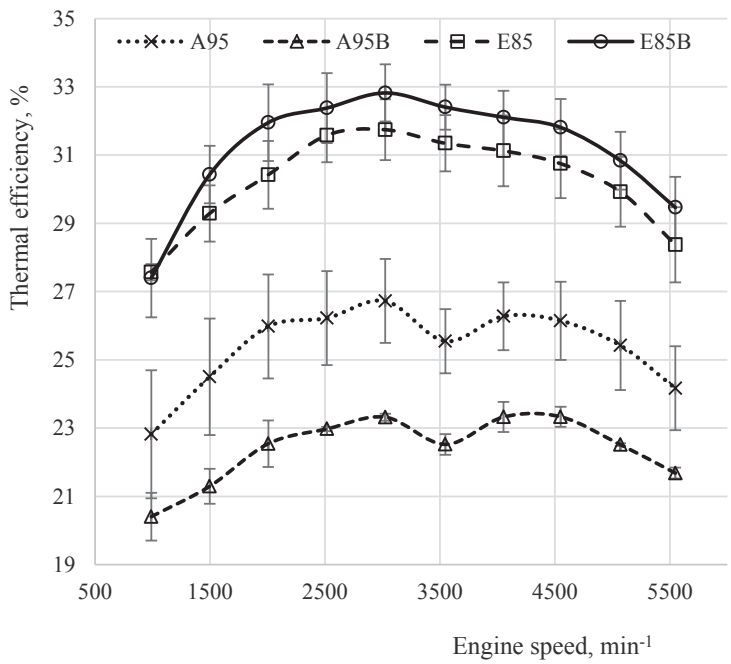

Figure 7. Fuel and conversion adapter influence on thermal efficiency.

by 4.4% compared to gasoline A95 and maximal power 69.3 kW at 5000 min^{-1}, an increase by 3.3% compared to gasoline.

Specific fuel consumption of E85 fuel, shown in Fig. 6, reached lower value 0.38 kg kWh^{-1} at 3000 min^{-1}. It was 22.6% increase for producing equivalent power, compared with the consumption of the gasoline. An increase can be explained with lower heating value of E85 fuel caused by higher content of the ethanol as shown in Table 1.

The engine thermal efficiency, calculated according to Eq. 4 is shown in Fig. 7. The lowest thermal efficiency and highest specific fuel consumption were observed when the engine equipped with conversion adapter was operated using gasoline. The engine thermal efficiency for E85 fuel was increased in all tested engine speed range Maximal thermal efficiency using E85B test setup reached 32.8% at 3000 min^{-1}, from 26.7% for gasoline in A95 test. Contributing factors of increased thermal efficiency of E85 compared to gasoline are the same that were mentioned earlier at engine power analysis - faster flame velocity, higher resistance to detonation and charge cooling effect. Based on the test data analysis, it can be stated that difference in engine operating parameters depending on used fuel - E85 or gasoline is in agreement with Agarwal, 2006; Costa et al., 2012 and Jiang et al., 2007. Consequently, it can be concluded that the conversion adapter RMG-Rapsol B5 provides normal operation of the engine using E85 fuel.

Conclusions

1. Fuel conversion adapter RMG-Rapsol B5 generates fuel injector control impulse based on ECU injection timing and ECU sensor readings.
2. Operation of the unmodified gasoline SI engine with E85 fuel in wide open throttle mode will lead to a lean air/fuel mixture and is not recommended, as the engine damage may take place.
3. Fuel conversion adapter RMG-Rapsol B5 provides adequate air/fuel mixture when E85 fuel is used, but fails to adapt when engine works on petrol.
4. The use of E85 fuel and conversion adapter give an increase of engine peak torque by 4.4% compared to gasoline, producing 144.8 Nm at 3500 min^{-1}, and maximal power 69.3 kW at 5000 min^{-1}, an increase by 3.3% compared to gasoline.
5. Specific fuel consumption is 20-23% higher, when E85 fuel with a conversion adapter is used, comparing with the gasoline use in the unmodified engine.
6. The thermal efficiency is increased in all tested engine speed range, when the engine is operated with E85 fuel instead of gasoline.
7. The peak thermal efficiency of the engine working with a conversion adapter and E85 fuel reaches 32.8% at 3000 min^{-1}, resulting in 6.1% increase, compared to the gasoline use.
8. The fuel conversion adapter RMG-Rapsol B5 provides basic optimization of air/fuel preparation system for using gasoline/bioethanol blend E85 in production PFI SI engine designed for the gasoline use.

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INNOVATIVE FIBREBOARD FROM WET-PRESERVED HEMP

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Abstract
The growing popularity of wooden panels renders this market segment increasingly competitive. The article describes a new type of fibre boards e.g. the furniture production, developed in cooperation with ATB (Leibniz-Institute for Agricultural Engineering Potsdam-Bornim) by using a new method of raw material preparation and specific production technologies of ATB. The main raw material is preserved hemp (*Cannabis sativa*) stalks. The samples are made of raw materials with different wet-preservation time and varying types of binder. For the first time there is used main raw material with short time wet-preservation. Samples that are 8 mm thick correspond to a medium-density fibreboard and that are 16mm thick correspond to a low-density fibreboard, fitting in its mechanical properties to standard BS EN622. On ATB's experimental processing line 1,200x800x8 mm and 1,200x800x16 mm size board samples are produced and the tests are performed to determine such parameters as bending strength, thickness swelling and thermal conductivity according to EU standard methods.
Key words: Hemp, Fibreboard, Urea-formaldehyde, Phenol-formaldehyde, wet-preservation.

Introduction

Natural plants are completely recyclable, widely available, and regularly renewable, comparatively cheap, with a sufficiently good level of physical and mechanical properties with a low density and friendly to environment (Faruk et al., 2012). Their natural ability of decomposition solves ecological problems, comparably low costs and good qualities induce economic interests (Kirilovs et al., 2011). Research and practice have shown that alongside with natural fibres used in textiles, they can also be used successfully as reinforcements of composites, compounds of building materials, as heat and sound insulation materials, and in many other applications (Pecenka et al., 2009). Fibreboards as well as three-dimensional pressed parts can be produced for the application in construction and furniture industry (Radosavljevic et al., 2009). Hemp fibreboard can be seen as an alternative to such boards that are made from processed wood fibres and resins. MDF (Medium Density Fibreboard) is a cellulose composite that is processed comparable to the strength found in trees (Crowley, 2001). Therefore, it is not necessary to use over 60 years old trees to make houses and furniture that lasts less. Instead of wood, hemp that takes only about 100 to 150 days to grow, can ensure the same house and furniture that lasts as long (Crowley, 2001). At the usual harvest date in September, European weather conditions are often harmful to harvest good quality hemp straw. The harvest of hemp by chopping method followed by anaerobic storage is favourable for the farmer, because the typical weather risk can be avoided. The following actions are the same as for ensiling of fodder (Amaducci et al., 2010).

Materials and Methods

According to ATB developed technology harvested and chopped whole hemp plants (seeds, leaves, fibres, shives) are wet preserved under anaerobic conditions (Idler et al., 2011). Raw material that is stored for 14 days to maximum 12 months is used to manufacture the boards; Phenolformaldehyde resins (PF) – Prefere 16J536 and Urea-formaldehyde copolymer in water (UF) - Hexion LL4547 in amount 10 g kg^{-1} of mixture dry mass are used as the binders. The plant raw material processing as well as the subsequent procedures were conducted at on experimental production line with 330 kg h^{-1} capacity, that is developed and tested in Leibniz-Institute for Agricultural Engineering Potsdam-Born (ATB) (Figure 1). To ensure optimal moisture the preserved material is mixed with dry hemp straw and processed with an extruder and in a second step with a disc mill. Next the material is passed to the hot air dryer (150 °C). Dry material is divided into 20 kg units that are placed in the mixer, where it is mixed with glue and passed to the three chamber dissipation machine where with airflow system on conveyer belt fleece is formed and passed to the double belt pre-press. The resulting fleece (6.5 kg m^{-2}) is pressed in the heated press to 180 degrees with holding time 283 seconds fewer than 100 bar pressure (Table 1). Pressing resulted in the board with dimensions 1,200x800x8 mm and 1,200 x 800 x 16 mm which were cut according to testing standards.

All products subjected to commutation are characterized by means of the average as well as the variation particle size. Sieving as the simplest and most widely used methods for particle size analysis determines the separation of fine material from coarse

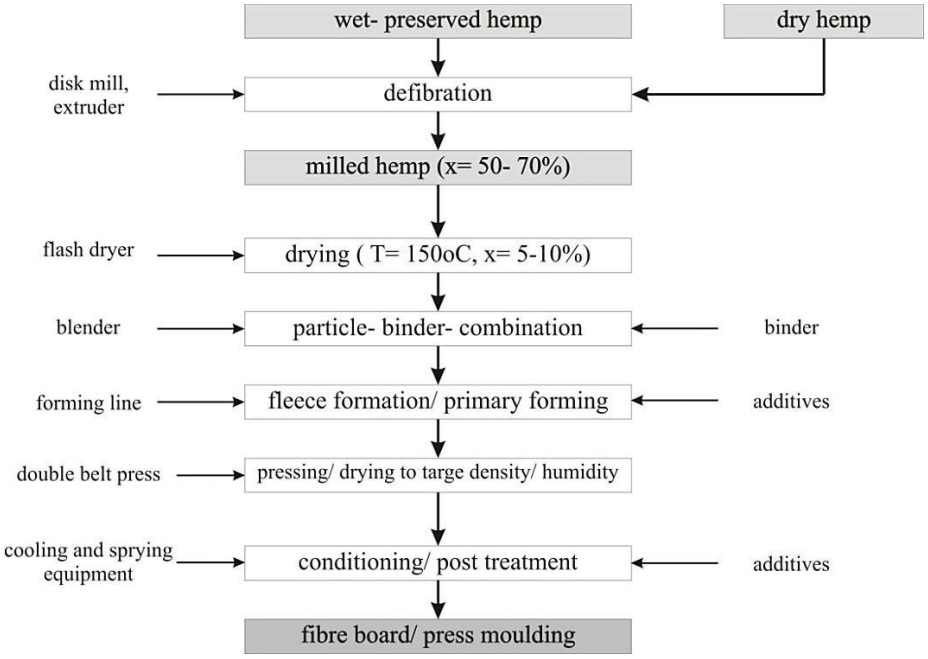

Figure 1. ATB Technological line for wet-preserved hemp processing.

Table 1

Technological parameters

Properties	14 days preserved hemp + dry hemp, FHD	12 months preserved hemp + dry hemp, PHD
Board density, kg m^{-3}	790	770
Adhesion ratio, %	10	10
Pressing time, sec	283	283
Pressing pressure, bar	100	100
Pressing temperature, °C	180	180

material (Turner et al., 2009). Sieving is carried out by stacking sieves in ascending order of aperture size and placing the sample on the top sieve. The stack is vibrated for a fixed time (8 minutes) and the residual weight on each screen is determined for each sample. Results are reported in the form of a cumulative percentage of passing sizes. Board samples were prepared corresponding to the matrix of full factor experiment plan 2^2 , where as independent variables were thickness of the board (x_1) and wet-preservation period (x_2) and as response factors were bending strength, tensile strength perpendicular to surface and thermal conductivity of samples.

The thickness swelling and water absorption tests after immersion in water were carried out according to EN 317 (LVS, 2000). Pre-weighed-measured specimens (25 from each board type) were immersed in water for 24 hours at 20 °C. After 15, 30, 45, 60 min and then 2, 3, 4, 5, 24 hours each soaking, the specimens were wiped of excess of water, measured for thickness and weight. The thickness swelling and water absorption was determined on the basis of initial dry measurements. Bending strength test of board material samples (25 from each board type) were executed on universal testing device Zwick/Roell Z010 (maximum strength 100 KN) using EN310 (LVS, 2001) testing standard. Thermal conductivity of the board samples was determined using the thermal conductivity measuring instrument FOX600 of the company Laser Comp according to the standard ISO 8301(LVS, 1991).

Results and Discussion

Gravimetric analysis of milled fibre material

The seven screens used for sieving enabled the determination of the distribution of the particle masses according to the dimensions of the mesh aperture (Fig. 2). The largest specific weight is for

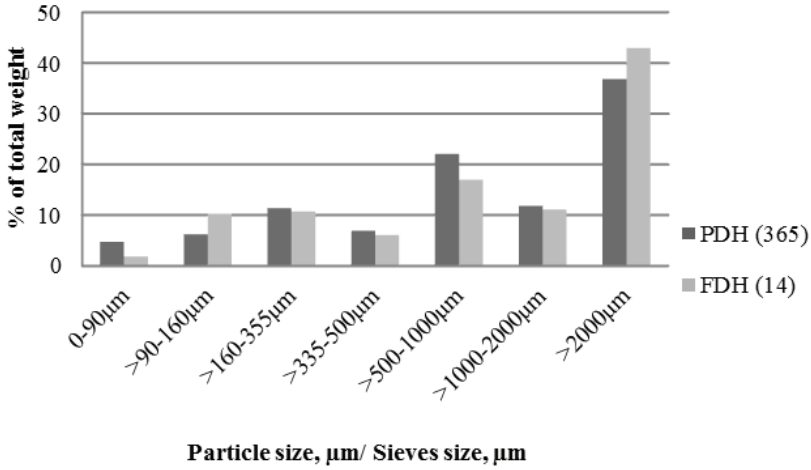

Figure 2. Distribution of particle masses.

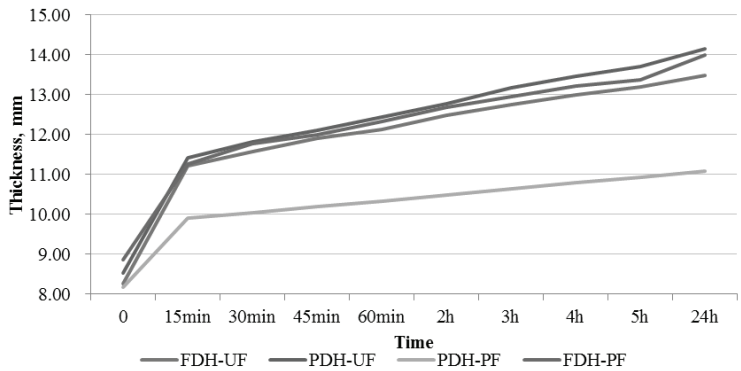

Figure 3. Changes of material samples in thickness.

fractions with the dimensions exceeding 2,000 µm, for the PDH (mixture of 365 days wet preserved and dry hemp) material they are 37% of the total amount, but for the PDH material – 43%. Having mixed the material preserved for twelve months with dry hemp, there are 17% less big particles and 60% more small particles obtained. For the FDH (mixture of 14 days wet preserved and dry hemp) material the number of these fractions is 16% higher compared to the PDH material. A fraction with the particle dimensions within the range from 500 to 1,000 µm of the preserved component (PDH) for 12 months exceeds the corresponding ratio of the preserved component (FDH) for 14 days for 30%. Dust, amounting to a total of ~12% consists of fractions with the dimensions of 0-90 µm and 90-160 µm. An optimum content of fibre – shive components for board production is obtained if the material preserved for two weeks is mixed with the dry fibre material, resulting in more large particles.

Board material swelling

Changes in thickness of the board samples made of the preserved hemp with PF adhesive are on average 16% less than with other samples (Fig. 3). It is justified by the fact that in the PDH variant from the hemp fibre – mix moisture exudes under the influence of lactic acids in more extended fermentation process. Mixing it with the PF adhesive, the wet-preservation effect provides better stability against the impact of moisture that is strengthened by the PF adhesive. More extended wet-preservation effect in the boards with the UF adhesive is weakly expressed – on average the FDH-UF has 3% less water absorption than the PDH-UF. The most rapid changes in the thickness of samples can be observed during the first 15 minutes.

Bending Strength

The mathematical models have the form of an incomplete second degree polynomial, that describe the results adequately depending on the thickness of the board (x_1) and wet-preservation period (x_2), calculated from the values of bending strength and thermal conductivity obtained in the testing process (Table 2).

Table 2

Mathematical models of boards of hemp fibre – shive mix

Properties	Mathematical Model
Y_{L1}- bending strength of the PF adhesive boards, MPa;	Y_{L1}=6.81-5.78*x_1-1.16*x_2+0.92*x_1*x_2
Y_{L2}- bending strength of the UF adhesive boards, MPa;	Y_{L2}=4.47-3.76*x_1-0.18*x_2+0.11*x_1*x_2
Y_{L3}- thermal conductivity, W mK^{-1};	Y_{L4}= 0.097-0.022*x_1- 0.003*x_2 + 0.004*x1*x_2

Boards of 8 mm thickness with the density of 740 – 790 kg m^{-3} and boards of 16 mm thickness with the density of 360 – 419 kg m^{-3} were used in the tests. The coefficients of linear members of equations are the same ranking numbers, that proving that the bending resistance reduces both when the thickness of a board and the wet-preservation period are increased (it is indicated by the negative signs of coefficients with x_1 and x_2). Since the coefficient of the interaction effect is also important, the position of the corresponding echo surface in the space of coordinates and its configuration is rather complicated.

The wet-preservation period has more effect on the bending resistance on PF adhesive high density samples whereas impact on low density samples bending resistance within the scope of the experiment is low as clearly seen from the equation Y_{L1} and corresponding response surface (Fig.4): when the wet-preservation period is increased from 14 to 365 days, the bending strength of an 8 mm thick board increases from 10.51 to 14.66 MPa, that is by 39%. The bending strength of a 16 mm board increases from 0.79 to 1.27 MPa that is by 61%, although the absolute increase of strength 0.48 MPa is almost by order lower. Thus, when designing high density boards in order to increase the bending resistance, it is recommended to choose the fibre – shive mix preserved for extended period of time.

It can be seen from the equation Y_{L2} (Tab. 2) and the response surface (Fig. 5), that changes of wet-preservation period in the range from 14 to 365 days leads to the bending strength increase of an 8 mm thick board from 7.95 to 8.52 MPa, that is by 7%. For a 16 mm thick board the bending strength increases from 0.64 to 0.78 MPa respectively. Within the limits of the experiment, if the thickness changes within the range from 8 mm to 16 mm, the bending strength of the board samples increased from 0.78 to 8.52 MPa. Thus it can be concluded that the bending strength of the 8 mm thick UF adhesive boards with long period preserved raw materials is better within the limits of this experiment. Generally speaking, the bending strength is by 33% less for material with the UF adhesive than it is with the PF adhesive (- 41% PDH (365), -24% FDH (14)).

As it is seen from the graph of Figure 6, the average bending strength of all experimental samples are higher than for hemp shive board, but only PDH-PF sample (365 days preserved hemp, PF adhesive) shows average bending strength value higher (22%) than wood chip board (Figure 6). Bending strength of samples with short wet-preservation time (14 days) are lower than for samples with a long wet-preservation time (365 days) for both adhesive types with higher impact on PF adhesive samples bending strength. The 8PDH-PF (8 mm thick PDH board samples, PF adhesive) has the highest bending strength. Veneering them with 0.7 mm cut veneer of ash-tree from both sides and binding the veneering with the board with 120 g m^{-2} of polyvinyl acetate D3 (PVA) adhesive,

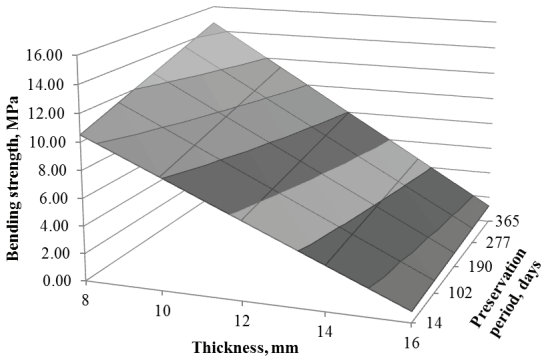

Figure 4. Response surface of bending strength of the PF adhesive boards.

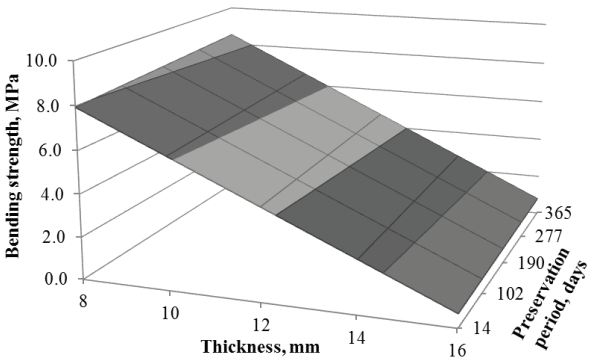

Figure 5. Response surface of bending strength of the UF adhesive boards.

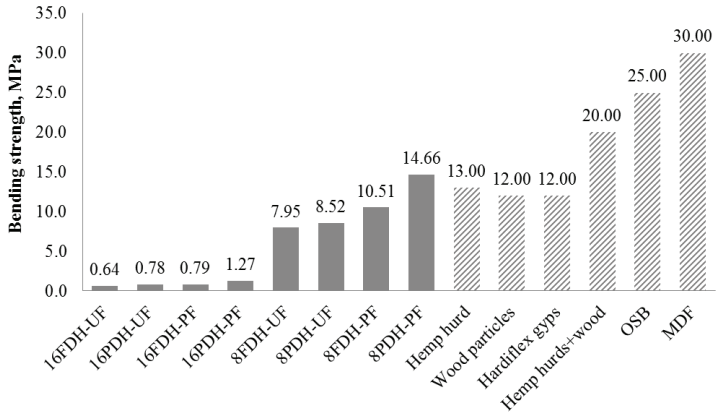

Figure 6. Comparison of experimental hemp and other board bending strength.

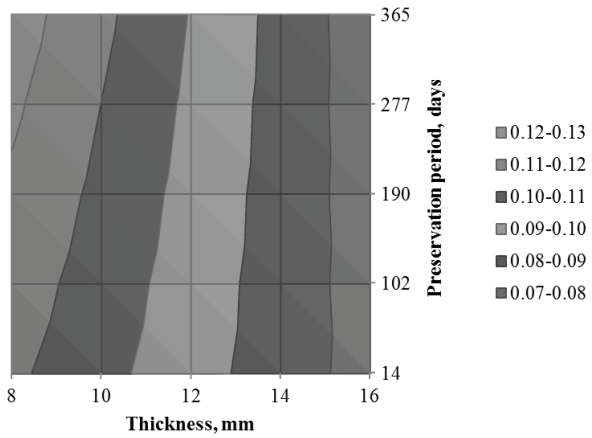

Figure 7. Response surface of thermal conductivity of 8 mm and 16 mm board.

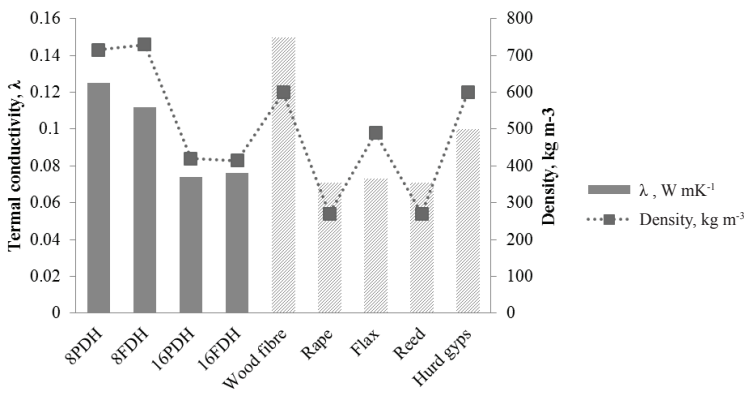

Figure 8. Thermal conductivity of experimental and reference boards.

the bending strength of the board increases by 52%, reaching the average value of 30.7 MPa that is enough to use the board as a construction material.

Thermal conductivity

As it is seen in Table 2 (Equation Y_{L3} and response surface, Fig. 7), thermal conductivity of the board samples changed within the range from λ= 0.074 to λ= 0.125 W mK^{-1}. The smallest thermal conductivity is for 16 mm samples with the wet-preservation period of hemp components from 278 to 365 days. Thermal conductivity within the range between λ=0.08-0.09 W/m·K can be also provided by 14-16 mm thick samples with shorter wet-preservation period. When the wet-preservation period increases, the thermal conductivity coefficient of the 8 mm thick board

increases within the range from λ=0.112 to λ=0.125 W mK^{-1}, that is by 12%. The thermal conductivity coefficient of a 16 mm board decreases within the range from λ=0.076 to λ=0.074 W mK^{-1}, that is by 3%. When the thickness decreases, the thermal conductivity coefficient increases from λ=0.076 to λ=0.112 W/m·K, if the hemp fibre – shive mix has been in the wet-preservation mode for 14 days, and from λ=0.074 to λ=0.125 W/m·K, if the hemp fibre – shive mix has been in the wet-preservation mode for 365 days.

Compared to the wood-fibre board, both the 16 mm experimental board with the hemp fibre – shive mix preserved for 14 days, and the board with the hemp fibre – shive mix preserved for 365 days, the thermal conductivity and density are much lower (Fig. 8), whereas their thermal conductivity can be compared with the respective indicators of rape, flax and reed boards; at the same time the density of boards with rape and reed hemp fibre – shive mix is lower, but for the board filled with flax it is higher than the densities of the previously mentioned experimental boards of 16 mm thickness. The thermal conductivity coefficients of the 8 mm thick boards are lower than those of the wood-fibre board; however their densities do not exceed the density of the wood-fibre boards to be taken into account.

Conclusions

1. The use of chopped and wet preserved hemp for the production of boards permits usage of the whole stem, including its leaves and seeds. It makes obtaining of the raw material independent of the weather conditions, the material storable in a compact way, reduces changeability of its properties, shortens the processing cycles significantly, reduces their power-intensity and simplify the technological processes, at the same time create necessity to develop new types of products, test their properties, and determines their application areas. The investigations to combine hemp fibre – shive mix types with different binders show that higher quality of board could be reached by combination of PF glue with one year preserved hemp compared to UF glue and fresh material (two weeks preserved).
2. As shown in the Table 2 bending strength swelling thickness with UF bond boards are much lower for the samples where hemp component is aged for 14 days compared with the same aging time of samples with PF resin. The same trend is observed at the holding time 365 days. The results with the PF resin are higher.
3. The mechanical properties of hemp fibre – shive boards are still not satisfying enough to use them as constructive materials, but they could be ideally used as separating panels between the working places, wall boarding, furniture details and door panels.
4. Several things have to be improved in order to the construction material. Mechanical properties of proposed hemp boards could be improved if the dry hemp chips partly replaced by wood chips and density should be increased; the material can be used as core where the outside panels are for example, husked veneer.

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