BIOETHANOL OUTCOME FROM WINTER RYE, TRITICALE AND WHEAT DEPENDING ON N-FERTILIZER RATE

Liena Poiša[1], Aleksandrs Adamovičs[1], Veneranda Stramkale[2]
[1] Latvia University of Agriculture
[2]Agricultural Science Centre of Latgale, Latvia
lienapoisa@inbox.lv

Abstract. Grain after enzymatic treatment, which is a starch-containing raw material, is used for ethanol production. Bioethanol production in Latvia began in 2006. Extraction of biofuels is a clean process, because the byproduct is used in various sectors of the economy. The bioethanol in Latvia was derived primarily from winter wheat, winter rye, and winter triticale. The objective of the research is to determine the different nitrogen fertiliser rates required for winter cereal crop yields and bioethanol outcome. The trials were carried out from 2005 to 2008 in Agricultural Science Centre of Latgale (Latvia). The method (ethanol outcome) is based on fermentation of saccharified cereal samples by yeast *Saccharomyces cerevisiae* followed by the calculation of ethanol outcome and speed of fermentation. The highest starch content was in winter wheat and winter triticale grain, but the lowest - in winter rye grains. A close negative correlation ($p<0.05$) was found for winter triticale and winter wheat between the ethanol outcome and thousand grain weight. Production of bioethanol from rye starch content is used with full utilisation of grain. The winter wheat has the largest ethanol outcome from one hectare.
Key words: bioethanol, N fertilizer rate, winter wheat, winter rye, winter triticale.

Introduction

Bioethanol is ethanol which is produced through fermentation of the biomass for use as a fuel. The grain after enzymatic treatment is starch-containing raw material which is used for bioethanol production (Enerģētisko..., 2007; Kalniņš, 2009). As bioethanol can be obtained from grain, it counts as a renewable fuel. One litre of ethanol replaced about 0.65-0.75 litre of gasoline (Kalniņš, 2009) or the energy value of the coefficient for bioethanol - 32% less than fossil petrol (Kalniņš, 2006).

Cars in Latvia were powered by biogasoline before the Second World War. The fuel from the gasoline and ethanol was called the latol. According to A. Kalniņš (Kalniņš, 2009) 70% of gasoline and 30% of ethanol were in the biogasoline in winter-time, and 50% of gasoline and 50% of ethanol were in summer-time.

The use of grain is increasing the production of bioethanol in the world (Булаткин, 2009; Kalniņš, 2009). Bioethanol can also be obtained from low quality grain (Технология..., 1981; Enerģētisko..., 2007). According to the International Grain Council (GC) estimates, in the financial year 2007/08, around 96 million t of grain were used for ethanol production or by 32% more than in the previous year (Kalniņš, 2009). The more ethanol is produced, the lower its cost (Биоэтанол, 2006). This is due to the improvement of the growing and processing technologies. The area for grain cultivation varies Latvia, and winter wheat is grown. In 2010 32 thousand tonne bioethanol productions need 26 thousand ha cereal aria (Benfelde, 2005).

The fermenting grains are used for bioethanol production in Latvia. The main by-product of distilling dregs is liquid of distillation residue, which is used for cattle forage, or as substrata for biogas production (Технология..., 1981; Enerģētisko..., 2007; Kalniņš, 2009). In Sweden, from 2.65 kg of wheat the following products can be produced: one litre of ethanol E100, 0.85 kg of protein for cattle feed, and 0.7 kg of carbon dioxide in carbonated beverages and food cooling (Kalniņš, 2009).

Ethanol production in Latvia began in 2006 (Latvijas..., 2010). The data show that 48% in 2006 of ethanol production in Latvia was exported, but in 2007 and 2008 the export reached already 99%. It means that the production of ethanol is not consumed domestically.

Bioethanol can be obtained from various plants: mainly from corn in the USA, from sugar cane in Brazil, and from grain of wheat, triticale, and rye in Latvia (Grosvalds and Alksnis, 2009; Kalniņš, 2009). Sunlight is also an important and cheap source of energy that plants use for photosynthesis. It is important to increase the yield of plants. Nitrogen is one of the most important elements for crop yield and quality (Jermušs and Vigovskis, 2002).

The objective of the research was to determine the influence of different nitrogen fertiliser rates on the production of winter cereal yields and bioethanol outcome.

Materials and Methods

Winter rye (*Secale cereale* L.), winter wheat (*Triticum aestivum* L.), and winter triticale (*Triticosecale* Wittm) were cultivated for the investigation in 2004/2005, 2006/2007 and 2007/2008. Winter cereals did not survived in winter of 2005/2006. The used winter rye varieties were ‘Kaupo’ (Latvia), ‘Amilo’ (Poland), and ‘Walett’ (Poland), winter wheat varieties – ‘Stava’ (Sweden), ‘Harnesk’ (Sweden), ‘Bjorke’ (Sweden), and ‘SW Maxi’ (Germany), winter triticale – ‘Lamberto’ (Poland), and ‘Falmoro’ (Sweden). The influence of these varieties was not analysed in the research. The field trials were conducted on sod-podzolic sandy loam soil: organic matter content – 27 g kg^{-1} (Tyurin’s method), pH 6.7, P – 67.64 mg kg^{-1} of soil, and K – 76.37 mg kg^{-1} of soil (DL method). The soil parameters were fit for winter cereal cultivation. The pre-crop was bare fallow.

The winter cereals in the trial were sown on 18 September 2004, 14 September 2006, and 14 September 2007. The seeding rate was 450 germinate able seeds per m^2. The field experiment was carried out using a randomised block design. The area of a trial plot was 20 m^2 (2 m × 10 m), 4 replicates. N fertiliser variants were N_{30}, N_{60}, N_{90}, N_{60+30}, and N_{90+30} for winter rye: N_{60}, N_{90}, N_{120}, N_{90+30}, and N_{90+60} for winter triticale: and N_{60}, N_{60+30}, N_{90+30}, N_{90+60}, and $N_{90+60+30}$ for winter wheat. The herbicides and fungicides were used at the plant growth stage (GS) 26-32. The growth regulator was used at GS 32-49. N fertiliser (ammonium nitrate) was applied at GS 20-29 - in spring after renewal of the vegetation growth. 2nd nitrogen fertilizer rate was applied at GS 30-32, 3rd rate – at GS 51-53.

Protein content, starch content, and volume weight of winter grains were determined with *Infratec1241*. 1000 grain weight was determined by standard method LV ST ZM 43-95. Quality and harvest traits were calculated at 100% purity and 14% moisture level.

Ethanol fermentation was carried out with different parameters of evaluation for the winter species. The method is based on fermentation of a saccharified wheat sample by yeast *Saccharomyces cerevisiae* followed by the calculation of ethanol output and speed of fermentation (Технология..., 1981; Enerģētisko..., 2007; Vigants et al., 2008). The ethanol outcome for the 2008 harvest was determined in the Laboratory of Institute of Microbiology and Biotechnology of the University of Latvia.

Work procedure:

1) Inoculum's preparation. The used *Saccharomyces cerevisae* strain was maintained on an agar medium. The inoculum for fermentation was prepared by transferring the yeast strains from the agar on the liquid malt medium and by cultivating for 24 hours at +30 °C (Vigants et al., 2008). Liquid malt medium for inoculums propagation: sucrose, 100.0 g L^{-1}, $(NH_4)_2SO_4$ 1.6 g L^{-1}, KH_2PO_4 2.5 g L^{-1}, $MgSO_4$ x $7H_2O$ 1.0 g L^{-1}, yeast extract 5.0 g L^{-1}, malt extract 50.0 g L^{-1}. The volume was made up with distilled water to 1 L, the initial pH of the medium was 5.35-4.55.

2) Saccharification. The sample of 20 g of ground cereals and 80 g of water was taken and mixed. Liquefaction of the sample was done by 54 µL of α-amylase preparation *Talzyme AL90* (JPBiotechnology) for 40 min at 90 °C. Then pH was adjusted to 5 and the mixture was cooled to 60 °C. Saccharification was done by 400 µL of glucoamylase *Talzyme GL60* (JPBiotechnology) for 40 min at 60 °C. Liquefaction and saccharification were performed by constant stirring of the sample.

3) Fermentation. After saccharification the sample was cooled to 30 °C. They 2 mL of *Saccharomyces cerevisae* inoculum were added, and batch fermentation in the flasks was carried out at 30 °C for 1-2 days. The flask weight changes were measured during the fermentation. When the weight became constant (which indicates the end of fermentation), a sample was taken and ethanol concentration was determined by gas chromatography.

4) Ethanol from the theoretical result was calculated from the starch data (Технология..., 1981):

$$c_{glycose}(\%, w/w) = \frac{c_{starch}(\%, w/w) \cdot 180.16}{162.16} \quad , (1)$$

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH + 118 \text{ kJ}$$

(Технология..., 1981; Enerģētisko..., 2007)

$$c_{eth.theor}(\%, w/w) = \frac{c_{glycose}(\%, w/w) \cdot M_{glycose}}{K \cdot 2 \cdot M_{ethanol}} \quad , (2)$$

where K – the dilution factor, which in our case it is 5 (fermentation medium: 20% of corn, 80% of water); *M* – molar mass for ethanol and glucose.

$$c_{et(\% \ from \ theoretical)} = \frac{(c_{eth.theor}(\%, w/w) - c_{ethanol}(\%, w/w)) \cdot 100\%}{c_{eth.theor}(\%, w/w)} \quad , (3)$$

Ethanol outcome from grain calculated formula No. 4.

$$(g_{ethanol} \text{ on } g_{grain}) = c_{eth.} (\%, w/w) / C_{grain} (g/100 g). \quad (4)$$

5) Determination of ethanol. Ethanol content was determined by gas chromatography *CHROM 4*, the thermostat temperature 80 ºC, the evaporator temperature of 200 ºC, with a flame-ionization detector. Content: A 1.2 m length x 3.0 mm (inner diameter) filled column, filling: Inerton AW-HMDS + 5% PEG, carrier gas: helium (Vigants et al., 2008).

Mathematical data processing analysis was used from ANOVA.

The weather conditions during the trial years were different.

The dry and cool weather delayed winter vegetation to the 3rd ten-day period of April in 2005. In, June the weather conditions were favourable for the growth and development of winter cereals. Also in July the weather contributed to a normal maturation of winter cereals. Excessive moisture content of grain during the ripening period negatively affected the quality of the grain.

In 2007, the winter cereals endured in the winter relatively well. In May, the sufficient rainfall positively affected plant growth and development. In June the rainfall was 69% of the long-term average, but in July it adversely affected the plant development and grains began to germinate in the ears.

The rainfall and the air temperature of the stage of cereal growing in tufts (GS 20-29) were more than the long-term average in the vegetation period of 2007/2008.

In the third ten-day period of May 2008, the air temperature was close to the possibility long- term average, but there was no rain. The rainfall was half of the long-term average. In winter cereals, the stalks of the lower leaves became sallow. In July the average daily temperature corresponded to the long-term average. The amount of precipitation in the 1st and 3rd ten-day period of July was only 40% of the long-term average, but in the second ten-day period it was 173% of the long-term average. In the first ten-day period of August, the daily temperature was 15.8 °C, close to the long-term average, but the rainfall was 86% of the long-term average.

Results and Discussion

The influence of N fertilizer rates for food grains on the bioethanol outcome is analyzed in this paper.

Nitrogen fertiliser rates N_{60} kg ha^{-1} and N_{90+30} kg ha^{-1} which were compared, were the same for all winter cereal species. The highest average yield per all N treatments was obtained from winter wheat (Table 1). The highest yield was obtained when applying nitrogen rates N_{90+30} kg ha^{-1} for winter rye, N_{90+60} kg ha^{-1} for winter triticale, and $N_{90+60+30}$ kg ha^{-1} for winter wheat. The total nutrient demand for wheat is greater than for other cereals (Jermušs and Vigovskis, 2002).

Since ethanol is obtained from starch, the starch content should be as large as possible. The highest starch content in our trials was determined in winter triticale grain (Table 1), but the lowest – in winter rye. Our results correspond to the results of other investigations (Шаршунов et al., 2009). Also other studies confirme that in Latvia the production of bioethanol is the most advantageous from wheat (Bonāts and Sīviņš, 1978; Enerģētisko..., 2007; Grosvalds and Alksnis, 2009). The time of nitrogen fertiliser application affects also the starch content (Bonāts and Sīviņš, 1978). Significant fluctuations in the starch content over the years (Table 1) could be explained by the meteorological conditions during the growing season. The relatively high rainfall and relatively low temperature during the growing season increase the starch content in grains (Bonāts and Sīviņš, 1978). In our research the cultivation year and N fertilizer rates had significant influence on the winter cereal yield and starch content. The same conclusions have been made also by other researchers (Gaile and Kopmanis, 2002).

Table 1

Quality indicators for winter cereals

Nitrogen fertiliser rates*, kg ha^{-1}	Winter rye			Winter triticale			Winter wheat		
	2005*	2007*	2008*	2005*	2007*	2008*	2005*	2007*	2008*
Harvest yield, t ha^{-1}									
N30	5.47	6.83	7.33	-	-	-	-	-	-
N60	6.09	7.02	7.52	9.07	6.87	8.41	8.06	7.54	10.03
N90	6.66	7.68	8.10	9.23	7.20	8.73	-	-	-
N120	-	-	-	9.58	750	8,86	-	-	-
N60+N30	7.01	8.05	8.55	-	-	-	8.77	8.01	10.59
N90+N30	7.42	8.84	9.05	9.70	7.44	9.02	9.27	8.52	10.75
N90+N60	-	-	-	9.91	8.02	9.40	9.85	8.98	11.17
N90+N60+N30	-	-	-	-	-	-	10.25	9.34	11.38
Average	6.5	7.7	8.1	9.5	7.4	8.9	9.2	8.5	10.8
$LSD_{0.05A}$	0.04			0.03			0.03		
$LSD_{0.05C}$	0.05			0.04			0.03		
$LSD_{0.05ABC}$	0.12			0.09			0.12		
Starch, g kg^{-1}									
N30	613	619	643	-	-	-	-	-	-
N60	616	629	639	673	673	712	672	575	697
N90	609	621	634	676	675	707	-	-	-
N120	-	-	-	665	670	706	-	-	-
N60+N30	612	618	633	-	-	-	674	574	691
N90+N30	611	617	625	676	666	703	667	575	693
N90+N60	-	-	-	670	668	705	662	573	690
N90+N60+N30	-	-	-	-	-	-	656	569	686
Average	612	619	635	672	670	706	666	573	691
$LSD_{0.05A}$	19			11			7		
$LSD_{0.05C}$	24			14			9		
$LSD_{0.05ABC}$	58			34			31		

* Factor A – year; Factor B – variety; Factor C – fertilizer rate

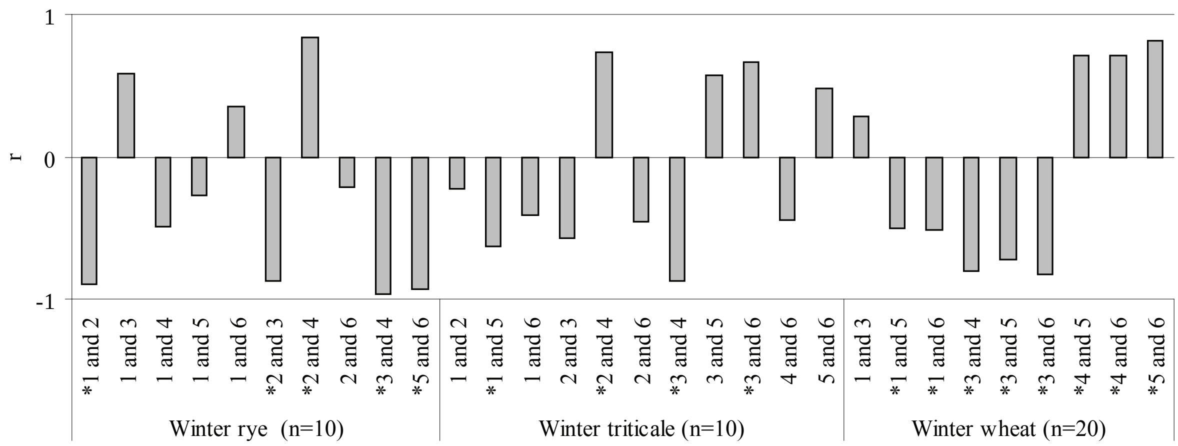

Figure 1. The interaction between average yield and average quality indices of winter cereal in 2008: * ($p<0.05$), 1 – g by ethanol g^{-1} of grain, 2 – yield, 3 – starch, 4 – protein content, 5 – 1000 grain weight, 6 – volume weight.

For bioethanol production, raw material composition characteristics are very important. After starch the amount of protein is the next important component for bioethanol production. Proteins are alcohol yeast nutrients, which increase the quality and outcome of alcohol (Шаршунов et al., 2009). Correlation analysis (r) showed that there was a strong ($p<0.05$) negative correlation between starch and protein content in grain (Fig. 1) for winter cereals (Linina and Ruza, 2005; Ruzgas and Plycevaitene, 2005). A close ($p<0.05$) negative correlation was found for winter triticale and winter wheat between the ethanol and 1000 grain weight, because ethanol is produced from grains with a higher 1000 grain weight. A weak ($p>0.05$) positive correlation was found between ethanol and starch content in grain.

The starch content in winter grain is different, therefore the theoretical outcome of ethanol is also different. This explains the high level of the theoretical ethanol rye yield (Fig. 2), which, in its turn, that is explained by the differences between rye and wheat grains.

Our test results showed (Fig. 3) that for production of one tonne of bioethanol on average 3.2 tonne of rye, 3.1 tonne of triticale, and 3.2 tonne of wheat are required. It can be said that production of one tonne of bioethanol requires approximately 3.7 tonne of grain or 1 m^3 of bioethanol needed about 2.8 tonne of grain (Kalniņš, 2009). Areas of winter wheat and winter triticale will be smaller if we take one tonne of bioethanol (Биоэтанол, 2006).

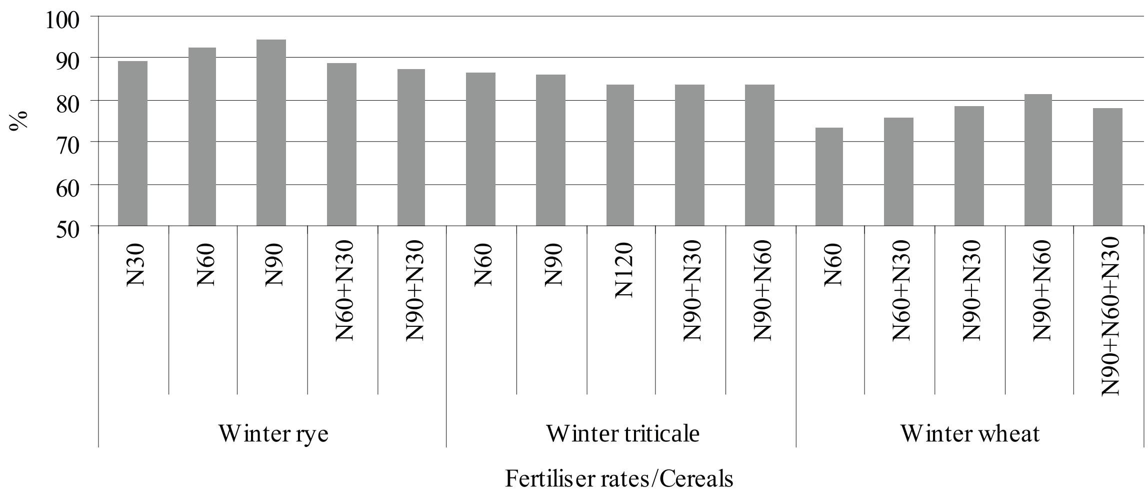

Figure 2. Average ethanol outcome from the grain harvested in 2008, % of theoretical (calculated from real starch content data).

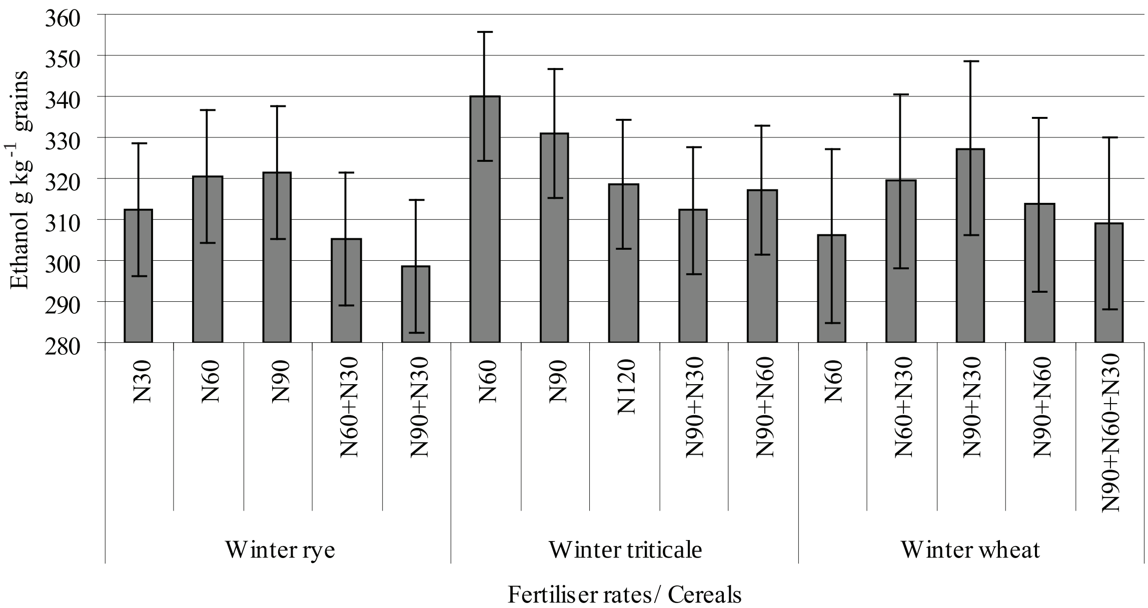

Figure 3. Average ethanol outcome, g kg^{-1} of grain in the laboratory from the 2008 harvest.

The proportion for the factors influencing on the ethanol produced in the laboratory g g^{-1} grain from the 2008 harvest follows ($p<0.05$) was the following: nitrogen fertiliser rate for winter rye 36% and winter triticale 16%, interaction between variety and nitrogen fertiliser rate for winter triticale 21%. For winter wheat all these factors were not important ($p>0.05$).

Although rye starch content in bioethanol has the highest value (Fig. 2), the results of ethanol per hectare of wheat were higher (Fig. 4). In other investigations, one litre (0.7 kg) of ethanol production required 2.57 kg of wheat, 2.73 kg of rye, and 2.67 kg of triticale (Kalniņš, 2006).

One of the most important indicators is the ethanol outcome from one hectare. Comparing nitrogen fertiliser rates N_{60} kg ha^{-1} and N_{90+30} kg ha^{-1}, it can be seen, that winter wheat was the most responsive to nitrogen application (Fig. 4).

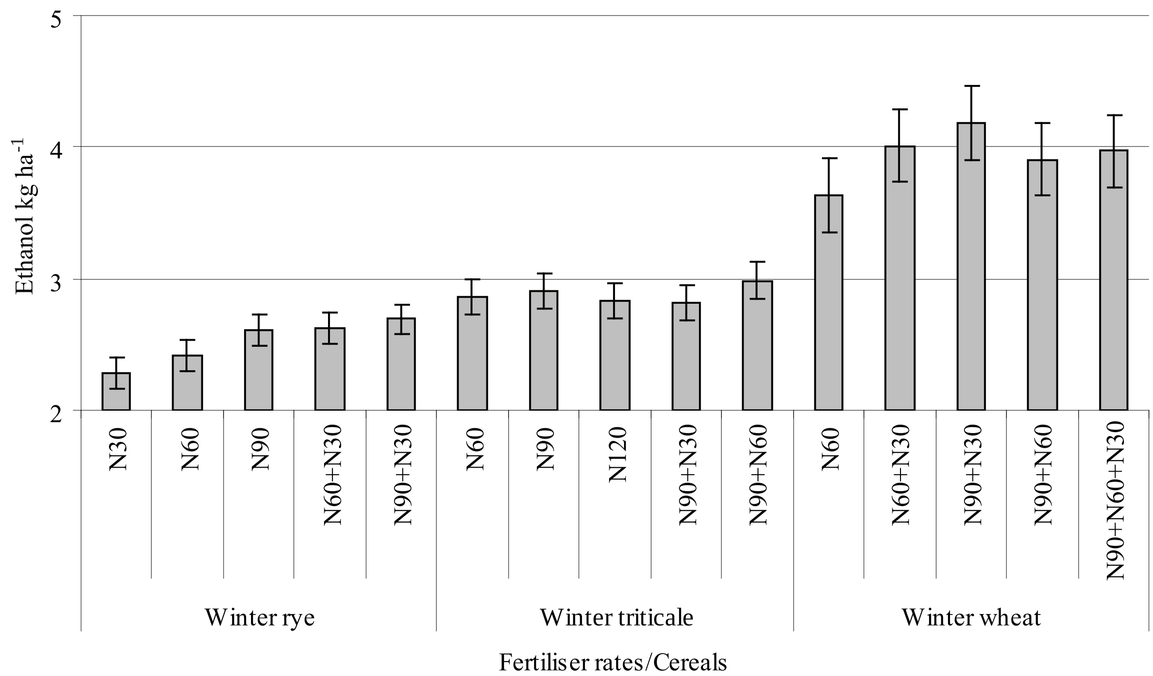

Figure 4. Ethanol outcome from one hectare in 2008, kg ha^{-1}.

In assessing the various factors influencing the ethanol outcome from one hectare, it was found that nitrogen fertiliser rate had a significant ($p<0.05$) influence on the ethanol outcome from winter rye (75.0%) and winter wheat (22.6%), but significant ($p<0.05$) variety and N fertiliser rate interaction effect was found only for winter triticale (19.8%).

Now in Latvia growed till cereal varieties with great protein and gluten content. They are used for food production. Besides the food grains have a higher purchase price. Therefore it is important to find the optimal N fertilizer rates for obtaining large yields as well as grain with a high starch content.

Conclusions

1. Nitrogen fertiliser rates N_{60} kg ha^{-1} and N_{90+30} kg ha^{-1} which were compared were the same for all winter cereal species. The highest yield was obtained from winter wheat.
2. The highest starch content was found in winter triticale grain, and the lowest – in winter rye grain.
3. Correlation analysis showed that there was a strong ($p<0.05$) negative correlation between starch content and protein content in winter cereal grain. A close ($p<0.05$) negative correlation was for winter triticale and winter wheat between the ethanol and 1000 grain weight.
4. The production of one tonne of bioethanol required 3.2 tonne of rye, 3.1 tonne of triticale, and 3.2 tonne of wheat. In order to obtain one tonne of bioethanol, winter wheat and winter triticale needed the smallest areas.
5. The proportion of factors ($p<0.05$) influencing the ethanol produced in the laboratory g g^{-1} of grain, from the 2008 harvest was the following: nitrogen fertiliser rate for winter rye - 36%, and for winter triticale - 16%, interaction between variety and nitrogen fertiliser rate for winter triticale – 21%. For winter wheat all these factors were not important ($p>0.05$).
6. In assessing the various factors influencing the ethanol outcome from one hectare, it was found that nitrogen fertiliser rate had a significant ($p<0.05$) influence on the ethanol outcome from winter rye (75.0%) and winter wheat (22.6%).

Acknowledgements

Thanks to Vītols Fund and the LAB-AN (Latvian Agronomic Society – Foreign Department) for granting me (L. Poiša) a bursary. The authors thank the Latvian Council of Science for providing funding for the research. Our gratitude also to the senior researcher A. Vīgants from the Institute of Microbiology and Biotechnology of University of Latvia for assistance in determining the ethanol outcome. This publication has been prepared within the framework of the ESF Project „Attraction of human resources to the research of the renewable energy sources", Contract No. 2009/0225/1DP/1.1.1.2.0/09/APIA/VIAA/129.

References

1. *Enerģētisko augu audzēšana un izmantošana* (Energy crops cultivation and usage) (2007) Adamovičs A., Agapovs J., Aršanica A. u. c. Valsts SIA „Vides projekti", Rīga, 190. lpp. (in Latvian).
2. Benfelde S. (2005) Biodegviela – ceļš uz tīrāku nākotni (Biofuels – the way to the cleaner future). Available at: http://www.videsvestis.lv/content.asp?ID=83&what=24, 21 June 2010. (in Latvian).
3. Bonāts I., Sīviņš O. (1978) *Graudu kvalitātes uzlabošanas problēmas* (Grain quality problems) Liesma, Rīga, 105. lpp. (in Latvian).
4. Gaile Z., Kopmanis J. (2002) Pētījums par ziemas kviešu graudu ražību un kvalitāti atkarībā no slapekļa papildmēslojuma veida un normas (1999.-2001.) (Investigations on performance and quality of winter wheat grain yield using different kinds and rates of nitrogen top-dressing (1999-2001)). *Proceedings in Agronomy*, 4, lpp. 74-78. (in Latvian).
5. Grosvalds I., Alksnis U. (2009) Alkoholiskie dzērienu, cietes un cukura ražošana Latvijā (1918-1944) (Production of alcoholic beverage, starch and sugar in Latvia (1918-1944)) Available at: http://www.rtu.lv/component/option.com_docman.doc_view/gid.2162/lang/lv/, 24 February 2010. (in Latvian).
6. Jermušs A., Vigovskis J. (2002) Vasaras kviešu raža un slāpekļa mēslojuma izmantošanās atšķirīgos augšanas apstākļos (Spring wheat yield and utilization of nitrogen in different conditions of growing). *Proceedings in Agronomy,* 4, lpp. 69-73. (in Latvian).
7. Kalniņš A. (2009) *Biodegvielas* (Biofuels). Latvijas biodegvielu asociācija, Rīga, 140. lpp. (in Latvian).
8. Kalniņš A. (2006) *Par bioetanola pielietošanas iespēju paplašināšanu transportā* (About the possibility of extending the use of bioethanol for transport). Latvijas biodegvielu asociācija, Rīga, 128. lpp. (in Latvian).
9. Latvijas Republikas Centrālā statistikas pārvalde, Lauksaimniecība (Central statistical administration of Latvia, Agricultural). Available at: http://data.csb.gov.lv/Dialog/Saveshow.asp, 25 January 2010. (in Latvian).
10. Linina A., Ruza A. (2005) Variety – by – enviroment interactions for winter wheat quality traits. *Latvian Journal of Agronomy*, 8, pp. 122-126.
11. Ruzgas V., Plycevaitene V. (2005) Activity of α-amylase in rye grain and its relationship with other traits. *Latvian Journal of Agronomy*, 8, pp. 162-165.

12. Vigants A., Lukjanenko J., Upite D., Kaminska E., Bekers M. (2008) Jerusalem artichoke based substrates as raw material for ethanol production by Z.mobilis and S.cerevisiae. *Proceedings of 16th European Biomass Conference & Exibition, Valencia, Spain 2-6 June, 2008*, pp. 1610-1612.
13. Биоэтанол (Bioethanol) (2006) Available at: http://www.zavkom.com, 17 February 2010. (in Russian).
14. Булаткин Г. (2009) Оценка эффективности производства нетрадиционных энергоносителей из растительного сырья (Evaluating the effectiveness of non-traditional energoholders from plant material). *Агро XXI*, 7-9, с. 6-8. (in Russian).
15. *Технология спирта* (Alcohol technology) (1981) Легкая и пищевая промышленность, Москва, 416 с. (in Russian).
16. Шаршунов В., Василенко З., Цед Е. (2009) Использование новых зерновых культур – важнейший резерв повышения эффективности производства высококачественного спирта (The use of new crops - the major reserve of more efficient production of high quality alcohol). *Весцi Нацыянальнай Акадэмii навук Беларусі,* 2, с. 104-112. (in Russian).