

## AMINO ACID COMPOSITION OF SPRING BARLEY GENOTYPES WITH DIFFERENT PROTEIN CONTENT

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The aim of this study was to evaluate amino acid composition in spring barley genotypes from the collection and spring barley breeding material characterized with different crude protein content. The field trials were established at the State Stende Cereal Breeding Institute from 2006 to 2008. For eleven spring barley genotypes with different crude protein content (111.4-167.5 g kg<sup>-1</sup>), lysine content in grain dry matter and in protein were investigated. Content of amino acids (g kg<sup>-1</sup>) for parental varieties, covered and hulless lines (5 covered, 8 hulless) from hybrid combination 04-09 (Justina/L 302) were evaluated. Crude protein content for barley lines varied from 118.0-161.0 g kg<sup>-1</sup>. The relative content of amino acids in dry matter (g kg<sup>-1</sup>) and in the protein (g per 100 g of protein) was calculated. The analyzed genotypes were divided into groups, where each of them differed significantly (p<0.05) regarding the average crude protein content. It was observed that lysine content in grain dry matter increased when crude protein content increased. Statistically significant differences in the average lysine content in grain dry matter (4.31 and 4.83 g kg<sup>-1</sup> respectively) were not found between genotypes with moderate (133.0 g kg<sup>-1</sup>) and heightened crude protein content (162.8 g kg<sup>-1</sup>). The proportion of lysine in protein decreased with increasing crude protein content in grain. Analyzing protein quality it was found out that spring barley genotypes with average crude protein content had more balanced protein - the ratio of essential and non-essential AA in the protein was significantly (p<0.05) higher for this group. The highest lysine content in dry matter and the highest lysine proportion in protein as well as more balanced protein in respect of the composition of essential and nonessential amino acids was found in barley genotypes with moderate grain crude protein content (120-140 g kg<sup>-1</sup>).

Key words: spring barley, amino acids, crude protein, quality

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### INTRODUCTION

Genetically diverse breeding material is an important prerequisite for successful barley breeding work for different final uses of spring barley (*Hordeum vulgare* L.). Therefore deep investigation of grain quality for different barley

genotypes (two-row, six-row, covered and hulless) is necessary in the agrometeorological conditions of Latvia. Barley is one of the main sources of protein for the nutrition of humans and livestock. Grain protein concentration and composition of amino acids are major determinants of grain nutritional value (Shewry

& Halford 2002).

Lysin is the first limiting amino acid in cereals for humans and monogastric animals and therefore increased lysine content in protein results in improved nutritional quality of barley grains. Nonessential amino acids, especially glutamic acid, aspartic acid and proline practically are of no use to monogastric animals that promote subsequent environmental pollution in high production conditions therefore as much as possible lower content of non-essential amino acids are more desirable (Lange et al. 2006). Protein quality of barley has been improved by increasing its lysine content genetically. The high-lysine genotypes have heightened lysine content in the storage protein and at the same time the levels of non-essential amino acids are considerably reduced (Jood & Singh 2001). Nutritional studies have confirmed the improvement in nutritional quality of the high-lysine barley selections (Eggum et al. 1995, Jorgensen et al. 1997).

Hulless barley is a comparatively new crop for Latvian breeding program. Up to now in Latvia by realizing barley breeding program for hulless and covered barley lines with increased protein content were developed (Belicka 1998). Perspective hulless lines selected from crossings between hulled high yielding and hulless high protein parents had heightened lysine content (Belicka 1999).

The aim of this study was to evaluate amino acid composition in spring barley genotypes characterized with different crude protein content.

## MATERIAL AND METHODS

The field trials were established at the State Stende Cereal Breeding Institute from 2006 to 2008. The soil at the site was sod-podzolic Albeluvisol (Eutric), the humus content – 26 mg kg<sup>-1</sup>, the soil pH KCl – 5.9-6.3, the content of phosphorus P<sub>2</sub>O<sub>5</sub> available for plants – 168-182 mg kg<sup>-1</sup>, and potassium K<sub>2</sub>O – 250-334 mg

kg<sup>-1</sup>, the pre-crop – potatoes. Before cultivation a complex mineral fertilizer was applied to the soil: N-60, P<sub>2</sub>O<sub>5</sub>-35, K<sub>2</sub>O – 50, S – 42 kg ha<sup>-1</sup>. Eleven spring barley genotypes from collection were chosen for this study: two-row, covered – ‘Ansis’, ‘Linga’, Idumeja’ (Latvia), ‘Lisymax’ (Denmark), ‘Primus II’ (Sweden), ‘Landsorte Aus Tirol’ (Austria); six-row, covered – ‘Druvis’ (Latvia); two-row, hulless – ‘L 302’ (Latvia), ‘KM 2084’ (Czech Republic), ‘Merlin’ (USA), ‘Wanubet’ (Japan). The lysine content in dry matter (g kg<sup>-1</sup>) was evaluated by the LVS EN ISO 13903:2005 method. The relative lysine content in the protein (g per 100 g of protein) was calculated.

Fifteen genotypes from spring barley breeding material – parental varieties (‘Justina’, ‘L 302’), covered and hulless lines (5 covered, 8 hulless) from hybrid combination 04-09 (Justina / L 302) included in the study. Content of amino acids in the dry matter (g kg<sup>-1</sup>) were evaluated by Ultra Performance Liquid Chromatography (UPLC). In the analysis results focuses on the most important essential amino acids – lysine (Lys), methionine (Met), cystine (Cys), threonine (Thr), tryptophan (Trp), isoleucine (Ile), leucine (Leu), valine (Val), and those nonessential amino acids – glutamic acid (Glu) and proline (Pro), that in the barley protein compose the largest percentage. The crude protein (CP) content (g kg<sup>-1</sup>) was evaluated by Kjeldahl method (LVS 277) (a conversion factor of 6.25). The relative content of amino acids in the protein (g per 100 g of protein) was calculated.

To group genotypes according to crude protein content the cluster analysis Wards method was used (the clusters or groups based on a minimal variance inside the groups were created). Two samples t-test assuming unequal variance was used for comparison of means of crude protein and amino acids between groups. Trait mean values in each comparison between groups with different labels on superscript are significantly different at the p<0.05. The data were analyzed using correlation analysis.

## RESULTS

The analyzed genotypes were divided into groups, where each of them differed significantly ( $p < 0.05$ ) regarding the average crude protein content (Table 1). It was observed that lysine content in grain dry matter increased when crude protein content increased. Significant ( $p < 0.01$ ) positive correlation between these two parameters ( $r = 0.934 > r_{0.01} = 0.798$ ,  $n = 9$ ) was found. Statistically significant differences in the average lysine content in grain dry matter (4.31 and 4.83 g kg<sup>-1</sup> respectively) were not found between genotypes with moderate (133.0 g kg<sup>-1</sup>) and heightened crude protein content (162.8 g kg<sup>-1</sup>). The proportion of lysine in protein decreased with increasing crude protein content in grain; significant negative correlation was found between these two parameters ( $r = -0.775 > r_{0.05} = 0.666$ ,  $n = 9$ ). Lysine content in protein (3.34 g per 100 g of protein) was significantly higher in genotypes with lower crude protein content (<120 g kg<sup>-1</sup>) if compared to genotypes with heightened crude protein content in grain (>155 g kg<sup>-1</sup>) - 2.97 g per 100 g of protein.

Lysine proportion in protein was not significantly different if genotypes with moderate crude protein content (133 g kg<sup>-1</sup>; lysine content 3.25 g per 100 g protein) and genotypes with low crude protein content (115.7 g kg<sup>-1</sup>; lysine content 3.34 g per 100 g of protein) were compared; it was significantly ( $p < 0.05$ ) higher than lysine content for genotypes with heightened crude protein content (lysine content 2.97 g per 100 g protein). For evaluation protein quality of spring barley genotypes the hybridization combination 04-09 (Justina / L 302) was selected, where the parents had the greatest difference between the crude protein content (40 g). The analysis of results focuses on eight essential amino acids (lysine, methionine, cystine, threonine, tryptophan, isoleucine, leucine, valine) and those nonessential amino acids (glutamic acid, proline) that in the barley protein contains the largest percentage.

Covered and hulless barley genotypes from the hybrid combination 04-09 (Justina/L 302) were divided into two groups (group 1 - with the grain crude protein content <140 g kg<sup>-1</sup> and group 2 with the grain crude protein content >140 g kg<sup>-1</sup>) for the comparison of amino acids'

Table 1. Lysine content in the dry matter of grain (g kg<sup>-1</sup>) and in the protein (g 100 g protein) for spring barley genotypes with different crude protein content (mean, 2006-2007)

Genotype	Crude protein, g kg <sup>-1</sup>	Lysine content, g kg <sup>-1</sup>	Lysine content, g 100 g protein
Group 1			
Lysimax	111.4	3.90	3.50
Ansis	116.3	3.85	3.32
Druvis	119.2	3.80	3.19
Mean	115.7 <sup>c1</sup>	3.82 <sup>b</sup>	3.34 <sup>a</sup>
Group 2			
Linga	127.9	4.20	3.28
Merlin	129.5	4.20	3.25
KM 2084	132.6	4.15	3.13
Wanubet	136.7	4.55	3.33
Idumeja	138.3	4.45	3.25
Mean	133.0 <sup>b</sup>	4.31 <sup>a</sup>	3.25 <sup>a</sup>
Group 3			
Primus II	155.9	4.40	2.82
Landsorte Aus Tirol	165.0	5.00	3.05
L 302	167.5	5.10	3.05
Mean	162.8 <sup>a</sup>	4.83 <sup>a</sup>	2.97 <sup>b</sup>

(AA) content on the grain dry matter (g kg<sup>-1</sup>) basis and in the protein (g 100 g protein) of barley with different crude protein content. The average crude protein content in both groups was significantly ( $p < 0.05$ ) different (129.2 and 156.7 g kg<sup>-1</sup> respectively) (Table 2).

Analyzing the amino acid content in the dry matter for parents of crossing the high-protein hulless line 'L 302' had higher in both essential and nonessential amino acids content compared to the covered barley variety 'Justina'. Lysine content in grain for this line exceeded the covered barley variety 'Justina' by 1.0 g kg<sup>-1</sup>, threonine - 1.5 g kg<sup>-1</sup>, leucine - 3.2 g kg<sup>-1</sup>, isoleucine - 1.1 g kg<sup>-1</sup>, glutamic acid - by 15 g kg<sup>-1</sup>, proline content - 8.8 g kg<sup>-1</sup>. According to both the essential and nonessential amino acids

content the covered and hulless lines in the investigated cross combination falls in-between the two parent varieties.

For low crude protein parent variety 'Justina' the sum of nonessential amino acids was 33.7 g kg<sup>-1</sup>, but for high protein hulless barley genotype 'L 302' - it was 57.5 g kg<sup>-1</sup>. It means that the higher crude protein content the higher both the content of essential and nonessential amino acids in the grain dry matter.

As indicated the correlation coefficients between the protein and individual amino acids than increasing crude protein content in the grain dry matter significantly increased also content of essential amino acids such as cystine ( $r = 0.734 > r_{0.01} = 0.684$ ), leucine ( $r = 0.688$ ),

Table 2. Content of essential and non-essential aminoacids in the dry matter for spring barley genotypes from hybridization combination 04-09 (Justina/L 302), g kg<sup>-1</sup>, 2008

Geno- types	CP <sup>1</sup>	Essential amino acids, g kg <sup>-1</sup>									Nonessential amino acids, g kg <sup>-1</sup>		
		Thr	Cys	Lys	Met	Val	Ile	Leu	Trp	Sum	Glu	Pro	Sum
Group 1 (crude protein < 140 g kg <sup>-1</sup> )													
Justina♂	119.0	3.1	1.2	3.3	1.0	3.5	2.5	6.1	0.4	<b>20.9</b>	23.1	10.6	<b>33.7</b>
L-558	118.0	3.5	1.1	3.6	0.7	3.6	2.5	6.8	0.4	<b>22.2</b>	26.8	13.0	<b>39.8</b>
L-567	124.0	4.0	1.5	4.2	0.9	4.4	3.1	7.9	0.5	<b>26.6</b>	29.0	13.6	<b>42.6</b>
L-565	126.0	2.8	1.1	2.9	0.9	3.2	2.3	5.7	0.4	<b>19.3</b>	22.8	10.9	<b>33.8</b>
L-568	127.0	3.7	1.4	4.0	1.2	4.1	2.9	7.4	0.5	<b>25.2</b>	28.7	13.7	<b>42.4</b>
L-559	139.0	4.3	1.3	4.6	1.0	4.8	3.5	8.6	0.5	<b>28.6</b>	33.0	16.2	<b>49.2</b>
L-562	135.0	2.9	1.6	3.3	1.3	2.9	2.0	6.2	0.4	<b>20.4</b>	23.8	12.1	<b>35.8</b>
L-571	137.0	3.9	1.4	3.9	0.9	4.5	3.3	8.0	0.5	<b>26.5</b>	31.9	15.4	<b>47.3</b>
L-557	138.0	3.3	1.3	3.3	1.0	3.6	2.6	6.9	0.4	<b>22.3</b>	28.7	14.1	<b>42.8</b>
<b>Mean</b>	<b>129.2<sup>b2</sup></b>	3.5	1.3	3.7	1.0	3.8	2.7	7.1	0.4	<b>23.6<sup>b</sup></b>	27.5	13.3	<b>40.8<sup>b</sup></b>
Group 2 (crude protein > 140 g kg <sup>-1</sup> )													
L-556	150.0	4.1	1.5	4.1	1.0	4.4	3.2	8.0	0.5	<b>26.6</b>	32.2	15.5	<b>47.7</b>
L-555	154.0	3.4	1.4	3.4	1.0	3.8	2.6	7.1	0.4	<b>23.2</b>	29.5	14.4	<b>43.9</b>
L-570	156.0	3.9	1.6	3.9	1.5	4.1	3.0	8.4	0.5	<b>27.1</b>	35.1	17.8	<b>52.9</b>
L-569	160.0	4.4	1.7	4.4	1.3	4.7	3.4	9.2	0.6	<b>29.6</b>	36.4	18.3	<b>54.8</b>
L-564	161.0	4.0	1.4	4.1	1.1	4.3	3.0	8.1	0.5	<b>26.5</b>	32.7	16.5	<b>49.2</b>
L 302♀	159.0	4.6	1.6	4.3	1.2	4.9	3.6	9.3	0.6	<b>30.1</b>	38.1	19.4	<b>57.5</b>
<b>Mean</b>	<b>156.7<sup>a</sup></b>	4.1	1.5	4.0	1.2	4.4	3.1	8.4	0.5	<sup>27.2a</sup> <b>27.2a</b>	34.0	17.0	<b>51.0<sup>a</sup></b>

methionine ( $r=0.611 > r_{0.05}=0.553$ ), tryptophan ( $r=0.666$ ) content (Table 3).

In this barley population did not find the significant correlation between protein and the main limiting amino acid lysine content in the

dry matter. In addition, increasing of crude protein content in the dry matter, significantly ( $p < 0.01$ ) increased nonessential amino acids - glutamic acid and proline content (correlation coefficients 0.796 and 0.836 respectively).

Table 3. Coefficients of correlation (r) between crude protein and amino acids in the dry matter, g kg<sup>-1</sup>, 2008 (n=13,  $r_{0.05}=0.553$ ;  $r_{0.01}=0.684$ )

Amino acid	r	Amino acid	r
Cysteine	0.734**	Methionine	0.611*
Glutamic acid	0.796**	Proline	0.836**
Isoleucine	0.488	Threonine	0.590*
Leucine	0.688**	Tryptophan	0.666*
Lysine	0.448	Valine	0.496

\*, \*\* coefficient of correlation significant with 95 and 99% probability respectively.

Table 4. Content of essential and non-essential amino acids in the protein for spring barley genotypes from hybridization combination 04-09 (Justina/L 302), g 100 g protein, 2008

Genotypes	Essential amino acids, g kg <sup>-1</sup>									Nonessential amino acids, g kg <sup>-1</sup>			EA/NA ratio <sup>1</sup>
	Thr <sup>1</sup>	Cys	Lys	Met	Val	Ile	Leu	Trp	Sum	Glu	Pro	Sum	
Group 1 (crude protein < 140 g kg <sup>-1</sup> )													
Justina ♂	2.6	1.0	2.7	0.8	2.9	2.1	5.1	0.3	<b>17.6</b>	8.9	19.4	<b>28.3</b>	0.62
L-558	3.0	0.9	3.0	0.6	3.1	2.1	5.8	0.4	<b>18.8</b>	11.0	22.7	<b>33.7</b>	0.56
L-567	3.2	1.2	3.4	0.8	3.6	2.5	6.4	0.4	<b>21.5</b>	11.0	23.4	<b>34.4</b>	0.63
L-565	2.2	0.9	2.3	0.7	2.5	1.8	4.5	0.3	<b>15.3</b>	8.7	18.1	<b>26.8</b>	0.57
L-568	2.9	1.1	3.1	1.0	3.2	2.3	5.8	0.4	<b>19.9</b>	10.8	22.6	<b>33.4</b>	0.60
L-559	3.1	0.9	3.3	0.7	3.5	2.5	6.2	0.4	<b>20.6</b>	11.6	23.8	<b>35.4</b>	0.58
L-562	2.2	1.2	2.4	0.9	2.2	1.5	4.6	0.3	<b>15.1</b>	8.9	17.6	<b>26.5</b>	0.57
L-571	2.9	1.0	2.9	0.6	3.3	2.4	5.9	0.4	<b>19.3</b>	11.2	23.3	<b>34.5</b>	0.56
L-557	2.4	0.9	2.4	0.7	2.6	1.9	5.0	0.3	<b>16.2</b>	10.2	20.8	<b>31.0</b>	0.52
<b>Mean</b>	2.7	1.0	2.8	0.8	3.0	2.1	5.5	0.4	<b>18.0<sup>a</sup></b>	10.3	21.3	<b>31.5<sup>a</sup></b>	<b>0.57<sup>a</sup></b>
Group 2 (crude protein > 140 g kg <sup>-1</sup> )													
L-556	2.7	1.0	2.7	0.6	2.9	2.1	5.3	0.3	<b>17.7</b>	10.4	21.4	<b>31.8</b>	0.56
L-555	2.2	0.9	2.2	0.7	2.5	1.7	4.6	0.3	<b>15.1</b>	9.3	19.2	<b>28.5</b>	0.53
L-570	2.5	1.0	2.5	1.0	2.6	1.9	5.4	0.3	<b>17.4</b>	11.4	22.5	<b>33.9</b>	0.51
L-569	2.7	1.1	2.7	0.8	2.9	2.1	5.7	0.4	<b>18.5</b>	11.4	22.8	<b>34.2</b>	0.54
L-564	2.5	0.9	2.5	0.7	2.7	1.8	5.1	0.3	<b>16.5</b>	10.2	20.3	<b>30.6</b>	0.54
L 302 ♀	2.9	1.0	2.7	0.8	3.1	2.3	5.8	0.4	<b>18.9</b>	12.2	24.0	<b>36.2</b>	0.52
<b>Mean</b>	2.6	1.0	2.6	0.8	2.8	2.0	5.3	0.3	<b>17.2<sup>a</sup></b>	10.8	21.7	<b>32.5<sup>a</sup></b>	<b>0.53<sup>b</sup></b>

<sup>1</sup>EA – sum of essential amino acids, NA – sum of non-essential amino acids.

Although the analysis of the results showed that the average content of essential and non-essential amino acids in grain dry matter ( $\text{g kg}^{-1}$ ) was significantly higher for genotypes with heightened crude protein content however, the protein quality of these two groups of genotypes was equivalent, which was approved by a non-significant ( $p > 0.05$ ) difference in essential and non-essential AA contents in protein ( $\text{g 100 g protein}$ ) between the genotypes of the 1st and the 2nd groups (Table 4). The highest content of essential AA in protein was found in lines 'L-567' ( $21.5 \text{ g 100 g protein}$ ), 'L-559' ( $20.6 \text{ g 100 g protein}$ ) and 'L-568' ( $19.9 \text{ g 100 g protein}$ ) which exceeded that in both the parent varieties. Crude protein content in these lines ranged from 124 to  $139 \text{ g kg}^{-1}$ .

Analyzing protein quality (the ratio of essential and non-essential AA in protein) it was found out that genotypes from group 1 (crude protein content  $< 140 \text{ g kg}^{-1}$ ) had more balanced protein. The quality was approved by the ratio of essential and non-essential AA in protein which was significantly ( $p < 0.05$ ) higher for this group. The overall conclusion is that increasing the grain crude protein content increases the content of both, essential and non-essential AA in grain dry matter as well. But if the ratio of essential and non-essential AA in protein ( $\text{g 100 g protein}$ ) was evaluated, barley genotypes with a moderate grain crude protein content - from 119 to  $138 \text{ g kg}^{-1}$  showed the best results. The ratio of the essential AA and non-essential AA in protein was worse for genotypes with heightened crude protein content ( $> 140 \text{ g kg}^{-1}$ ).

## DISCUSSION

For characterization of barley quality not only data on grain protein content are useful but also its quality is important (Shewry 2007). Protein and amino acids play also an important role in physiological processes of ruminants, especially young animals, which have high demands for protein. When essential amino acids and lysine content in particular are increased, fewer supplements are needed for animal nutrition (V. Gabert et al. 1995). Protein quality of barley

is characterized by essential amino acids, especially lysine ratio of protein which is the main limiting amino acid for nonruminant animals (Eggum et al. 1995, Gabert et al. 1995, Jood & Singh 2001).

Results of this research indicated that the proportion of lysine in the protein decreased with increasing crude protein content in grain. This is primarily due to an increase in hordein, the principal storage protein type that contains high percentages of the non-essential amino acids and low percentage of the essential amino acids (Shewry & Halford 2002). It means from point of view of the protein quality the barley genotypes corresponds to requirements of malting barley characterized with higher feed value compare with genotypes with heightened grain protein content. Similar results have been obtained in other studies where genotypes with low crude protein content ( $95\text{-}109 \text{ g kg}^{-1}$ ) lysine content in the protein was on average  $4.2 \text{ g per 100 g}$  of protein, but genotypes with high protein content grains ( $178\text{-}181 \text{ g kg}^{-1}$ ) lysine accounted for only  $2.9 \text{ g per 100 g}$  of protein. There was found a negative correlation ( $r = -0.89$ ,  $n = 16$ ) between the crude protein content of grains and the proportion of lysine in the protein (Bhatty & Rosnagel 1981). Also C. Newman concluded that an increase in crude protein content decreases percentage of essential amino acids in the protein. Significant differences in amino acid composition were found between barley varieties. These differences were correlated to varying grain protein content, to a greater extent than to other cultivar properties. Varieties with high grain protein content generally had less favorable amino acid composition than did cultivars with low protein content (Assveen 2009). It is therefore difficult to combine in one genotype at the same time increased content of essential amino acids and decreased amount of nonessential amino acids.

Here was visible the benefit of high-lysine genotypes for which according to the literature found increased essential and low content of nonessential amino acids (Jood & Singh, 2001). High-lysine genotypes have a relatively

higher proportion of lysine in the protein than normal genotypes (Gabert et al. 1995, Jood & Singh 2001, Jorgensen et al. 1998). For high-lysine spring barley variety 'Lysimax' lysine content in the protein was significantly higher (4.6 g per 100 g of protein), compared with the normal variety 'Sultan' (3.3 g per 100 g of protein), where the crude protein content of the varieties were 126 and 131 g kg<sup>-1</sup> respectively (V.Gabert et al. 1995). Also in our study high-lysine genotype 'Lysimax' on average of two years showed a relatively higher lysine content in the dry matter (3.90 g kg<sup>-1</sup>) compare with other varieties from the first group ('Ansis' and 'Druvis'). The results confirmed the benefits of variety 'Lysimax' as it provided the highest proportion of lysine in the protein - 3.50 g per 100 g of protein if compared to the other analyzed genotypes.

## CONCLUSIONS

The proportion of lysine in the protein decreased with increasing crude protein content in the grain. Analyzing protein quality it was found out that spring barley genotypes with average crude protein content had more balanced protein - the ratio of essential and non-essential AA in the protein was significantly ( $p < 0.05$ ) higher. The highest lysine content in dry matter and the highest lysine proportion in protein as well as more balanced protein in respect of the composition of essential and nonessential amino acids was found in barley genotypes with moderate grain crude protein content (120-140 g kg<sup>-1</sup>). The results confirmed the benefits of high-lysine variety as it provided the highest proportion of lysine in the protein if compared to the other analyzed genotypes.

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