# PROTEIN, LYSINE AND METHIONINE CONTENT IN THE GRAIN OF TRITICALE GROWN UNDER ORGANIC SYSTEM

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#### Abstract

The present study was conducted to determine the effect of variety, organic fertilizer and predecessor on protein, lysine and methionine contents, and their variation in triticale grains under biological cultivation. During the 2014-2017 period, a three factors experiment was managed on field certified for organic farming in Field Crops Institute -Chirpan, Bulgaria. Three varieties, three doses of organic fertilizer and two predecessors (sunflower and durum wheat) were tested. Based on the data obtained, it was found that the variety has a significant influence on the parameters studied. The highest significant values of the chemical parameters were observed when applying organic fertilizer at a dose of 1,750.0 kg/ha. After a predecessor of durum wheat, the value of lysine was higher and statistically significant compared to predecessor of sunflower. Similar coefficients of variation of the studied parameters were found, which not depended of a variety, a predecessor and an applied organic fertilizer, varying between 4.38% and 5.35% for protein, 4.1% and 5.33% for lysine and 4.31% and 5.22% for methionine.

Key words: lysine, methionine, organic farming, protein, triticale.

### INTRODUCTION

The quality of the grain is determined by the content of organic compounds, minerals, vitamins, antioxidants and more. The protein is one of the most important chemical component of the grain, which largely determines its nutritional value as a food crop and feed. In cereals, the content of lysine as a proteinogenic essential amino acid largely determines the biological value of proteins. Triticale has a high nutritional value, contains considerable quantity of protein, fiber, vitamins and minerals (Kruma et al., 2018) and is rich in essential amino acids (Fernandes-Figares et al., 2000). The most important nutritional characteristics of triticale grains are starch, as well as the quantity and quality of the protein (Edel Leon et al., 2008).

The interest in triticale as a source of feed is determine by a higher protein concentration and a better amino acid balance than other forage crops (Myer et al., 2004). On the other hand, the triticale has low gluten content, effective viscoelasticity and the prepared bread has low quality compared to wheat bread (Doxastakis et al., 2002). Myer et al. (2004) have summarized data from various authors for the period 1990-2002 and have found that the protein and lysine levels in triticale grains are higher than those in maize and wheat. Recent studies have showed that the protein content of triticale grains is close to wheat protein (11.4-14.0%) but has a higher amount of lysine (0.33-0.71%) (Fraú et al., 2016). According to Glamočlija et al. (2018), the average total protein content of triticale grains varies from 10.2% to 15.6% of the dry matter. These protein and lysine advantages are major factors for the development of triticale as a commercial crop (Gebre-Mariam and Larter, 1979) and determine its use primarily as a source of feed. According to Glamočlija et al. (2018), the direction for triticale use depends from the characteristics of the variety, namely higher

protein varieties can be use as concentrated feed for poultry, ruminants and non-ruminants, and varieties with high biomass can be use for grazing, silage or hay.

The protein quality is determined by the proportions of essential amino acids, which cannot be synthesized by animals and humans and must be provided through a diet. The lysine and methionine (sulfur-containing amino acid) are the first two essential amino acids, of great importance composing diet for farm animals, as they participate in a numbers of biochemical processes in the animal cell (Bouyeh, 2012). In this regard, although winter cereal grain is poor of lysine, it is an optimal source of sulfur amino acids (Alijošius et al., 2016).

When triticale is used in feed ration, it is more important to consider lysine content than protein (Angelova and Angelov, 1981). The lysine and methionine contents are low in plants, i.e., their levels are insufficiently to guarantee optimal growth for animals and humans (Galili and Amir, 2013). Consequently, the composing of fodder rations entirely from organically grown crops is complicated.

The organic farming is gain in popularity as an alternative to conventional farming systems (Benaragama, 2016), and should be understood as highly specialized farming. Along with the search for technological solutions to increase productivity for cereals, there is a requirement for grain quality. The triticale is a suitable crop for cultivation under biological system due to its stable yield, tolerance to unfavorable conditions, resistance to diseases and high competitiveness against weeds (Kronberga et al., 2013).

In Bulgaria a detailed investigation about the chemical qualities of triticale grain prodused under biological technology was missing so far. Considering the advantages and importance of triticale, the present study was conducted to determine the influence of the variety, organic fertilizer and predecessors on protein, lysine and methionine content, and their variation in the triticale grain under biological breeding system.

## MATERIALS AND METHODS

During the period 2014-2017 experiment was managed in field certified for organic farming of Field Crops Institute, Bulgaria (42°11'58"N, 25°19'27"E). The experiment was based on a block method, with a perpendicular arrangement of the degrees of the tested factors in four replicates. The area of the crop plot was

18 m<sup>2</sup> with a seed rate of 550 g s/m<sup>2</sup>. The following factors and levels were tested: factor A - variety (Colorit, Boomerang and Respect), factor B - organic fertilizer Lumbrical (applicated in a rate of 0, 1,400.0 kg/ha and 1,750.0 kg/ha) and factor C - Predecessor (sunflower and durum wheat). The organic fertilizer was applied manually before the last pre-sowing treatment of the soil, after which the area was cultivated. Lumbrical is a product of the processing of manure and other organic wastes from red Californian worms (Lumbricus rubellus and Eisenia foetida). It is applicated for organic farming under EU Regulation 889/2008. According to the FAO classification systems (FAO), the soil of the experimental field of the Field Crops Institute is Pellic Vertisols (Vp.). The contents of protein, lysine and methionine were monitored. The protein content (g/kg DM) was determined as the total nitrogen content by the Kjeldahl method in duplicate (Bremner, 1965), and was calculated using nitrogen conversion coefficient 6.25. The contents of lysine and methionine (g/100 g crude protein) in a protein were determined by Degussa (2001) regression equations. In order to establish statistically significant influences of the studied factors the software BIOSTAT (Penchev et al., 1989-1991) was applied on the data from the three years. Statistica 13 was used to determine the coefficients of variation. Acording to Table 1, compared to the average of multi-year period 1928-2013 (2009.7°C), the sum of temperatures during the harvest years 2014/15 and 2015/16 were higher, respectively 2,264.1°C and 2,530.4°C. In the third year of the study, the temperature during the growing season was lower (1,843.5°C) than the 86-year period. With regard to precipitation 2014/15 was very humid (578.1 mm), 183.2 mm more than the average for a period 1928-2013. The total amount of precipitation for 2015/16 (323.4 mm) was lower compared to a multi-year period (395.0 mm), and for 2016/17 (375.2 mm) was about the climatically average 1928-2013.

Period	Months									
	XI	XII	Ι	II	III	IV	V	VI	1	
Temperature sums ( $\Sigma^{\circ}C$ )										
1928-13	215.9	61.1	-6.2	49.4	188.9	357.9	511.5	630.7	2,009.7	
2014/15	227.2	138.0	74.9	96.0	192.7	340.4	586.0	608.9	2,264.1	
2015/16	299.3	115.1	-8.7	233.6	273.5	439.8	498.0	679.8	2,530.4	
2016/17	201.7	26.2	-160.7	-46.5	289.2	355.7	513.7	664.2	1,843.5	
				Rainfall	(mm)					
1928-13	47.3	54.0	44.3	37.7	37.0	45.2	64.1	65.4	395.0	
2014/15	36.9	142.3	50.3	61.7	134.9	15.1	58.8	78.1	578.1	
±	-10.4	+88.3	+6.1	+24.0	+97.9	-30.1	-5.3	+12.7	+183.2	
2015/16	50.2	1.3	73.9	28.3	53.1	26.6	75.0	15.0	323.4	
±	+2.9	-52.7	+29.6	-9.4	+16.1	-18.6	+10.9	-50.4	-71.6	
2016/17	47.7	5.9	80.1	23.8	51.3	22.6	59.5	84.3	375.2	
±	+0.4	-136.4	+35.8	-13.9	+14.3	-22.6	-4.6	+6.2	-19.8	

Table 1. Meteorological conditions during triticale vegetation

#### **RESULTS AND DISCUSSIONS**

The averages date for the period showed differences in the values of the studied parameters, both after the two predecessors and the varieties (Figure 1). A linear increase of values depending on the dose of organic fertilizer was observed due to the higher quantity of macronutrients imported with organic fertilizer. The content of protein, lysine and methionine was highest in grain at Colorit variety after the two predecessor, followed by Boomerang and Respect varieties.



B) predecessor durum wheat

Figure 1. Protein, lyzine and methionine content 2014/2017 period

A similar coefficients of variation were found for the three indicators within two predecessors (Table 2). After a predecessor of durum wheat varied between 5.22% and 5.33%, and after sunflower between 4.71% and 4.96%.

A) predecessor sunflower

Rodehutscorda et al. (2016) have reported similar values of the variation coefficients for triticale. The coefficients found in their study were 5.23%, 4.66% and 3.30%, respectively for crude protein, lysine and methionine. The

higher variation coefficients after durum wheat are consequence of higher averages values of crude protein, and subsequently of lysine and methionine (Tables 4 and 5). The variation coefficients of the indicators under fertilization were between 4.81% and 5.07%. The results obtained show stability of the studied indicators for all varieties under different agroecological conditions during 2014-2017 period. Base on the date from three years, the analysis of variance showed no significant influence of the interaction between the studied factors on the content of lysine, protein and methionine (Table 3).

	Colorit	Boomerang	Respect	Predecessor sunflower	Predecessor durum wheat	Fertilizer Lumbrical
	1					
Mean	134.9	128.8	126.9	129.5	130.9	131.4
Min	125.2	119.5	118.7	118.7	118.7	120.8
Max	145.6	139.4	136.5	145.6	143.0	145.6
CV%	4.56	4.54	4.38	4.96	5.35	5.07
Error	1.45	1.46	1.31	1.23	1.34	1.11
			Lizyne (g/100 g	g CP)		
Mean	3.03	2.89	2.85	2.91	2.94	2.97
Min	2.82	2.69	2.68	2.68	2.68	2.72
Max	3.25	3.12	3.06	3.25	3.25	3.25
CV%	4.1	4.33	4.18	4.71	5.33	4.81
Error	0.03	0.02	0.02	0.026	0.029	0.02
			Methionine (g/10	0 g CP)		
Mean	1.87	1.79	1.76	1.80	1.82	1.82
Min	1.74	1.66	1.65	1.65	1.65	1.68
Max	2.02	1.93	1.89	2.02	1.98	2.02
CV%	4.5	4.46	4.31	4.85	5.22	4.96
Error	0.01	0.01	0.01	0.01	0.01	0.015

Table 2. Crude protein, lysine and methionine coefficients of variation

Table 3. Effect of cultivars, biofertilizing, predecessors and their interaction on protein, lysine and methionine content of triticale grain

Source of	df	Protein		Ly	vsine	Methionine		
variation		η	MS	η	MS	η	MS	
А	2	26.03	309.0**	26.05	0.14**	25.20	0.05**	
В	2	9.90	117.5 ns	9.95	0.05 ns	10.10	0.02 ns	
С	1	1.15	27.31 ns	1.22	0.01 ns	1.05	0.004 ns	
A×B	4	0.35	2.06 ns	0.33	0.0009 ns	0.31	0.0003 ns	
A×C	2	0.84	9.91 ns	0.81	0.004 <sup>ns</sup>	0.66	0.004 <sup>ns</sup>	
B×C	2	0.38	4.53 <sup>ns</sup>	0.37	0.002 ns	0.36	0.0008 ns	
A×B×C	4	0.46	2.70 <sup>ns</sup>	0.46	0.001 <sup>ns</sup>	0.34	3.76 <sup>ns</sup>	
Error	36	60.9	40.17	60.82	0.02	61.99	0.008	

A great, significant effect on the three indicators had the variety, respectively 26.03%, 26.05% and 25.20% of the total variance.

Alaru et al. (2003) also have found that the protein level in triticale grain to a great extent dependent of a variety.

Table 4. Protein content (g/kg DM	)
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А			В				С		
	Colorit	134.9	0 kg/ha		127.7	5	Sunflower	129.5	
Be	oomerang	128.9 ns	1,400.0 kg/ha		130.2 ns	Durum wheat		130.9 ns	
Respect		126.9 <sup>ns</sup>	1,750.0 kg/ha		132.8*	-		-	
_	5%	4.3		5%	4.3	_	5%	3.5	
GD	1%	5.7	GD	1%	5.7	] <del>[</del> ]	1%	4.7	
	0.1%	7.6		0.1%	7.6	-	0.1%	6.2	

Various values of protein in triticale grains under biological breeding system have been cited within the scientific literature. Kronberga (2008) has reported protein content between 10.4 and 13.2%, Straumite et al. (2017) within 9.10 and 11.14 g/100 g<sup>-1</sup>, and Mikulioniene and Balezentiene (2009) - 8.2%.

On Table 4 the results of ANOVA are presented for independently action of the factors on the protein content in the grain. In the study was established particularity of the varieties for indicator studied. Similar results for the influence of variety have reported by Gulmezoglu et al. (2010).

The data showed statistically no significant values, with lower crude protein content for Boomerang (128.9 g/kg) and Respect (126.9 g/kg) varieties, compared to a control variety Colorit (134.9 g/kg). These results are higher than obtained by Wlcek and Zollitsch (2003), which have establiched a protein value 101.0 g/kg of dry matter under biological system of triticale growing. The highest crude protein

content was obtained in the grain (132.8 g/kg) when was applied 1,750.0 kg/ha Lumbrial, and the difference was statistically confirmed compared to a control option-Lumbrial 0 kg/ha. The value for Lumbrial 1,400.0 kg/ha (130.2 g/kg) was not statistically confirmed. Buhedma et al. (2016) also have reported an increase in protein content (%) in triticale grains when applying organic fertilizer, with no significance statistical values. The control values of their study ranged within 12.33 and 12.23%, and from test variants were between 12.59 and 12.79%. Although the protein content in the grain after a predecessor of durum wheat was 1.4 g/kg higher than after sunflower. the difference was no significant. Unlike our research Dimitrova-Doneva (2010) has reported for significant influence of predecessor on the crude protein content for triticale grain.

The content of lysine followed the trends of protein content, regarding the independent influence of the test factors (Table 5).

Table 5. Lysine content (g/100 g CP)

А			В				С		
	Colorit	3.029	0 kg/ha 2.874		2.874	Sunflower		2.913	
Bo	oomerang	2.900 ns	1,400.0 kg/ha		$2.928^{*}$	Du	rum wheat	2.944*	
Respect		2.858 ns	1,750.0 kg/ha		$2.984^{*}$	-		-	
-	5%	0.009	-	5%	0.009		5%	0.008	
GD	1%	0.123	ß	1%	0.123	GD	1%	0.101	
	0.1%	0.162	-	0.1%	0.162	•	0.1%	0.133	

The highest lysine content was obtained for Colorit variety (3.029 g/100 g CP). Wlcek and Zollitsch (2003) have reported a similar content of lysine in the protein in triticale grains (3.1 g/100 g CP), grown under organic farming system. The values of lysine for Boomerang (2.900 g/100 g CP) and Respect (2.858 g/100 g CP) varieties were lower and statistically no significant. Concerning the fertilizer factor the both tested doses of 1,400.0 and 1,750.0 kg/ha had significant statistical effect on the indicator studied. When the fertilizer rates were increased, the lysine content also increased -2.92 g/100 g CP and 2.984 g/100 g CP.

The content of lysine after a predecessor of durum wheat was higher compared to value after predecessor sunflower and statistically significant.

Concerning the methionine content (Table 6) the values were no significantly, both in the tested varieties and after a predecessor of durum wheat. A significant effect on increasing the methionine content was observed when applying 1,750.0 kg/ha Lumbrial - 1.848 g/100 g CP.

Table 6. Methionine content (g/100 g CP)

А			Б			С		
C	olorit	1.874	0 kg/ha		1.778	Sunflower		1.803
Boomerang		1.795 <sup>ns</sup>	1,400.0 kg/ha		1.811 <sup>ns</sup>	Durum wheat		1.822 ns
Respect		1.768 ns	1,750.0 kg/ha		1.848*	-		
	5%	0.059	-	5%	0.059	-	5%	0.048
GD	1%	0.079	d5	1%	0.079	GD	1%	0.064
	0.1%	0.104	Ũ	0.1%	0.104	Ũ	0.1%	0.085

### CONCLUSIONS

The obtained results can be summarized as follows: The variety has a significant effect on the protein, lysine and methionine content; When applying organic fertilizer at a rate of 1,750.0 kg/ha, the highest and statistically significant values of the indicators tested were established: After a predecessor of durum wheat, the protein, lysine and methionine content was higher than after sunflower, but was significant for lysine: A similar coefficients of variation of the investigated parameters were found. which are not dependent of the variety, the predecessor and the applied organic fertilizer.

### REFERENCES

- Alaru, M., Laur, Ü., Jaama, E. (2003). Influence of nitrogen and weather conditions on the grain quality of winter triticale. *Agronomy Research*, 1, 3–10.
- Alijošius, S., G. Juozas Švirmickas, S. Bliznikas, R. Gružauskas, V. Šašytė, A. Racevičiūtė-Stupelienė, V. Kliševičiūtė, A. Daukšienė (2016). Grain chemical composition of different varieties of winter cereals. *Zemdirbyste–Agriculture*, 103(3), 273–280. DOI 10.13080/za.2016.103.035.
- Angelova, L., Angelov, A. (1981). Triticale as pig feed. Sat. Production, storage and use of plant proteins in nimal husbandry. Proceedings paper. 26–27.02. Varna.
- Benaragama, D. (2016). Long-term weed dynamics and crop yields under organic and conventional cropping systems in the Canadian prairies. *Field Crops Research Res.* 196, 357–367. http://doi.org/10.1016/j.fcr.2016.07.010.
- Bouyeh, M. (2012). Effect of excess lysine and methionine on immune system and performance of broilers. *Annals of Biological Research*, 7, 3218–3224.
- Bremner, J.M., Mulvaney, C. A. (1965). *Total nitrogen*. Methods of soil analysis. Part 2. Agron. Monogr., vol. 9. ASA, Madison, WI, 599–610.
- Degussa, A.G. (2001). The amino acid composition of feedstuffs, 5th completely revised ed. (AminoDat); Degussa AG, Feed Additives Division: Hanau, Germany.
- Dimitrova-Doneva, M. (2010). Chemical composition and energy value of triticale depending on predecessor and nitrogen fertilization. *Field Crops Studies*, 6(3), 451–456.

- Doxastakis, G., Zafiriadis, I., Irakli, M., Marlani, H., Tananaki, C. (2002) Lupin, soya and triticale addition to wheat flour doughs and their effect on rheological properties. *Food Chemistry*, 77(2), 219–227.
- Edel Leon, A., Perez, G.T., Ribotta, P.D. (2008). *Triticale flour*. Food 2 (1) Global Science Books, 19–24.
- FAO http://www.fao.org/soils-portal/soil-survey/soilmaps-and-databases/faounesco-soil-map-of-theworld/en/
- Fernandez-Figares, I., J. Marinetto, C. Royo, M. Ramos, L.F. Garcia del Moral (2000). Amino–acid composition and protein and carbohydrate accumulation in the grain of triticale grown under terminal water stress simulated by a senescing agent. *Journal of Cereal Science*, *32*, 249–258.
- Fraú, A., Goùæbiewska, K., Goùæbiewski, D., Mañkowski, D.R., Boros, D., Szecówka, P. (2016).
- Variability in the chemical composition of triticale grain, flour and bread. J. Cereal. Sci., 71, 66–72.
- Galili, G., Amir, R. (2013). Fortifying plants with the essential amino acids lysine and methionine to improve nutritional quality. *Plant Biotechnol.* J., 211–222. doi: 10.1111/pbi.12025.
- Gebre-Mariam, H., Larter, E.N. (1979). Effect of plant density on yield, yield components and quality in triticale and glenlea wheat. *Canadian Journal of Plant Science*, *59*, 679–683.
- Glamočlija, N., Starčević, M., Ćirić, J., Šefer, D., Glišić, M., Baltić, M.Ž., Marković, R., Spasić, M., Glamočlija, Đ. (2018). The importance of triticale in animal nutrition. *Veterinary Journal of Republic of Srpska*, XVIII(1), 84–94, DOI: 10.7251/Vetjen1801073G
- Gulmezoglu, N., Alpu, O., Ozer, E. (2010). Comparative performance of triticale and wheat grains by using path analysis. *Bulgarian Journal* of Agricultural Science, 16(4), 443–453.
- Kronberga, A. (2008). Selection criteria in triticale breeding for organic farming. Agronomijas Vēstis. Latvian Journal of Agronomy, LLU(11), 89–94.
- Kronberga, A., Legzdiòa, L., Strazdiòa, V., Vicupe, Z. (2013). Comparison of selection results in organic and conventional environments for winter triticale. *Proceedings of the Latvian Academy of Sciences. Section B*, 67(3), 268–271. DOI: 10.2478/prolas-2013–0047.
- Kruma, Z., Straumite, E., Kince, T., Klava, D., Abelniece, K., Balgalve, A. (2018). Influence of technological parameters on chemical composition of triticale flakes. *Agronomy Research*, *16*(S2), 1417–1424. https://doi.org/10.15159/AR.18.109.
- Mikulioniene, S., Balezentiene, L. (2009). Responses of cereals grain quality on organical and

conventional farming. *Agronomy Research*, 7 (Special issue II), 677–683.

- Myer, R., Lozano del Río, A.J. (2004). *Triticale as animal feed*. Food and agriculture organization of the United nations Rome, 2004. ISBN 92-5-105182-8, pp. 49–51.
- Penchev, L., Bankov, E., Koev, A. (1989–1991). BIOSTAT. Statistical software Biostat© version 1.0. Dobrich.
- Rodehutscorda, M., Rückerta, C., Peter Maurerb, H., Schenkelc, H., Schipprackd, W., Knudsene, K.E.B., Schollenbergera, M., Lauxa, M., Eklunda, M., Siegerta, W., Mosenthina, R. (2016). Variation in chemical composition and physical characteristics of cereal grains from diferent genotypes. *Animal nutrition*, 70(2), 87–107 http://dx.doi.org/ 10.1080/1745039X.2015.1133111.
- Salem Issa Buhedma, A., Farag Mohamed, F., Hamuda Saleh, S. (2016). Effect of biofertilizer and mineral nitrogen levels on yield and yield components of triticale. *J. Agric. Res.* Kafr El-Sheikh Univ., 42(4), 480–493.
- Straumite, E., Tomsone, L., Kruma, Z., Gramatina, I., Kronberga, A., Galoburda, R., Sturite, I. (2017). Nutritional quality of triticale (× *Triticosecale*) grown under different cropping systems. *Proceedings of the Latvian Academy of Sciences*. *Section B*, 71(6), 481–485. DOI: 10.1515/prolas-2017–0083.
- Wlcek, S., Zollitsch, W. (2003). Rohprotein-und aminosäuregehalte von winterweizen und triticale aus biologischem anbau. In B. Freyer, Ökologischer Landbau der Zukunft. BOKU, Wien, 269–272.