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EFFECT OF VARIOUS METHODS OF WEED CONTROL AND NITROGEN FERTILISATION ON BIOLOGICAL VALUE OF WINTER TRITICALE GRAIN PROTEIN

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Abstract. In the years 2004–2005, a field experiment with cultivation of winter triticale, cv. Woltario, was conducted at the Teaching and Experimental Station situated in Tomaszkowo near Olsztyn, belonging to the University of Warmia and Mazury in Olsztyn. The aim of the experiment was to determine the effect of various methods of weed control (harrowing, herbicide Mustang 306 SE (florasulam – 6.25 $g \cdot dm^{-3}$ + 2,4-D – 452 $g \cdot dm^{-3}$, and harrowing + herbicide Mustang 306 SE) and nitrogen application, total of 120 kg $N \cdot ha^{-1}$ (60+60, 60+25+35, $60+25_{foliar}$+ 35) on the biological value of grain protein. The protein quality assessment was based on 18 amino acids, according to the standard PN-EN ISO 13903:2006. Tryptophan was determined according to the standard PN-R-64820:1977, the limiting amino acid index (CS) – by the Mitchell and Block method, and essential amino acid index EAAI by the Oser method. The methods of weed control and nitrogen application did not affect the exogenous amino acid content. Harrowing, herbicide application as well as harrowing plus herbicide application only reduced the total endogenous amino acid content, while nitrogen application – regardless of its manner – raised it. From the application methods only harrowing slightly reduced the EAA index, as did the application of nitrogen. The amino acid which limited the nutritional value of triticale grain was isoleucine, followed by methionine and lysine.

Key words: winter triticale, harrowing, herbicide, nitrogen, amino acids, biological value of protein

INTRODUCTION

Protein, the structural elements of which are amino acids, is a fundamental element in animal feeding. Of particular importance are exogenous amino acids that are not produced in animal organisms. The absence or deficit of even one of them in the fodder causes a reduction in the availability of the other ones and inhibits protein synthesis, hence they are often referred to as amino acids limiting the nutritive value of protein (of the first, second or third order). At present, deficit of some of them (lysine, methionine, treonine and tryptophane) can be supplemented through the addition to fodders of suitable preparation containing synthetic amino acids, most frequently produced by way of microbiological fermentation of molasses and sugar, among other things [Buraczewski 2001, Myer et al. 1996]. No less important and a natural way of supplementing such deficits is the use of fodders rich in plant proteins, including triticale [Myer et al. 1996, Stankiewicz et al. 2003]. Hence the need for studies on the determination of the content as well as the biological value (indices CS and EAAI) of proteins of cereal grain, including grain of winter triticale as a fodder cereal, and for determination of the effect of agrotechnical measures on their quality. Under the effect of certain agrotechnical measures the chemical composition of plants may be subject to a variety of changes that, in extreme cases, may lead to deterioration of their nutritive value [Brzozowska 2003, Brzozowski 1997, Klimont 2007, Kwiatkowski and Kubecki 2006, Makarska 1990], which is a signal for the need of skilful care of crop plant canopy during vegetation.

The objective of the study was determination of the effect of various methods of weed control and nitrogen application on the content and biological value of proteins of grain of winter triticale cv. Woltario.

MATERIAL AND METHODS

In the years 2004–2005, a field experiment with cultivation of winter triticale cv. Woltario was conducted at the Experimental Station situated in Tomaszkowo (53°59′ N, 19°84′ E), near Olsztyn, belonging to the University of Warmia and Mazury in Olsztyn. The experiment was set up with the method of split random blocks, in four replications, on a typical grey-brown podzolic soil developed from medium loam, turning sandy in the surface horizon. Every year triticale was grown on a stand after winter triticale, the previous crop for which was also winter triticale, followed by leguminous plants.

The first factor of the experiment was weed control methods: 1. Without – control object, 2. Harrowing in the tillering stage [GS 23–24 in the scale of Zadoks et al. 1974], 3. Mustang 306 SE (florasulam – 6.25 g·dm^{-3} + 2,4-D in the form of 2-ethylhexyl ester – 452 g·dm^{-3}) – GS 27–28; 4. Harrowing + Mustang 306 SE (GS 23–24 and 27–28, respectively) – acc. to Zadoks et al. [1974].

The second factor was the method of foliar application of nitrogen (120 kg N·ha^{-1}): a) control object – without nitrogen; b) nitrogen applied at 60 kg N·ha^{-1} after restart of vegetation – ammonium nitrate, 60 kg N·ha^{-1} in full shooting phase – granulated urea; c) 60 kg N·ha^{-1} after restart of vegetation – ammonium nitrate, 25 kg N·ha^{-1} at the beginning of shooting stage and 35 kg N·ha^{-1} at the end of shooting stage – granulated urea; d) as in treatment "c", but the second part of nitrogen (25 kg N·ha^{-1}) applied by means of foliar application of urea at concentration of 18.1% (8.33% N). Moreover, triticale was fertilised with phosphorus (30.5 kg P·ha^{-1}) and potassium (83 kg K·ha^{-1}). The surface area of a single experimental plot was 16 m^2 (8 m × 2 m). The herbicide Mustang 306 SE (florasulam – 6.25 g·dm^{-3} + 2,4-D in the form of 2-ethylohexyl ester – 452 g·dm^{-3}) was applied at the dose of 0.5 dm^3·ha^{-1}.

Spraying was performed using a knapsack sprayer, in recommended weather conditions, before evening, applying 300 dm^3 of the working fluid per 1 ha.

Analyses were performed for triticale grain from two years of the study – 2004 and 2005. Quality traits of protein were determined on the basis of 18 amino acids, in accordance with the standard PN-EN ISO 13903:2006, with the method of ion-exchange chromatography, using the automatic analyser Biochrom 20 plus [AOAC, 1990]. Tryptophane was determined acc. to the standard PN-R-64820:1977. The determinations were made using a spectro-photometer, at wavelength of 590 nm. The index of limiting amino acid - CS (Chemical Score) – was determined with the method of Mitchell and Block, and the integrated index of essential amino acids EAAI (Essential Amino-Acids Index) – with the method of Oser [after Kunachowicz et al. 1977]. The amino acid composition of hen egg was adopted as the standard, according to FAO/WHO [after Kunachowicz et al. 1977]. The calculations of CS and EAAI were made taking into consideration the following amino acids: lysine, methionine, cystine, treonine, tryptophane, isoleucine, leucine, phenylalanine, tyrosine and valine.

RESULTS AND DISCUSSION

The growth and development of winter triticale cv. Woltario were strongly affected by weather conditions, and rainfall in particular (Tab.1). In the first season of the experiment, in the

Table 1. Air temperatures and rainfall in the vegetation period of winter triticale in 2004–2005 according to Meteorological Station in Tomaszkowo

Months	Air temperature (ºC)			Rainfall (mm)		
	average of many years	mean of month		sum of many years	sum of month	
	1961–2000	2004	2005	1961–2000	2004	2005
IX	12.5	12.9	12.4	59.0	32.2	22.6
X	7.8	4.7	8.4	43.4	88.6	52.3
XI	2.7	4.8	2.5	47.7	45.5	29.8
XII	–1.3	1.6	2.3	36.2	48.0	43.4
I	–2.9	–7.5	2.3	22.8	29.8	43.0
II	–2.4	1.0	–5.0	20.4	51.9	27.1
III	1.2	0.1	–3.6	26.8	33.7	38.6
IV	6.9	6.4	7.5	36.1	46.5	10.9
V	12.7	12.4	11.6	51.9	79.3	33.7
VI	15.9	15.1	13.9	79.3	111.6	47.6
VII	17.7	16.9	19.7	73.8	76.1	93.6
VIII	17.2	19.8	16.3	67.1	99.1	33.1
Mean or sum	7.3	7.4	7.4	564.5	742.3	475.7
Mean or sum (IV–VII)	14.1	14.1	13.8	308.2	412.6	218.9

Table 2. Weed control efficacy, expressed by loss of air dry matter of weeds, in grain milk stage of winter triticale (%)

Treatments	Years		Mean 2004–2005
	2004	2005	
Methods of weed control			
Harrowing	38.0	31.2	34.6
Herbicide	74.1	78.3	76.2
Harrowing + herbicide	81.8	84.4	83.1
$LSD_{(0.05)}$	4.8	5.8	3.4
Nitrogen application method ($kg\ N \cdot ha^{-1}$)			
Without nitrogen	61.5	58.9	60.2
70 + 65	65.9	65.6	65.8
70+25+40	66.3	67.5	66.9
70+25*+40	64.6	66.6	65.6
$LSD_{(0.05)}$	n.s.	n.s.	n.s.

* – foliar application
n.s. – non significant differences

vegetation period of triticale (September-July) the sum of precipitations was greater by ca. 30%, and in the second by ca. 30% lower, respectively, compared to the multi-year sum. In 2004, in the period of June–July, the amount of precipitations was greater by as much as 43% than in the analogous period of 2005. The different conditions of vegetation in the years of the study affected not only the level but also the quality of triticale grain yields. Strong dependence of protein content in triticale grain on climatic conditions has been indicated by numerous authors [Brzozowska 2003, Brzozowski 1997, Gil 1996, Makarska 1997, Pisulewska 1995, Stankiewicz 1998]. In the study by Gil [1996], increased level of precipitations and low air temperature in the period from the setting of kernels to full ripeness and harvest had an unfavourable effect on the technological value of triticale grain. Moreover, that author demonstrated that winter triticale grown in the northern and eastern regions of Poland had a lower protein content (average of 11.0%) than that grown in the west and south of the country (13.5%).

In the years of the study under analysis (2004–2005) the efficacy of weed control in winter triticale was varied with relation to the weed control method applied (Tab. 2). The greatest reduction of weed infestation was obtained in the treatment with mechanical and chemical weed

Table 3. Amino acid composition of grain protein of winter triticale, $g \cdot 100\ g^{-1}$ protein

Amino acid	Years		$LSD_{(0.05)}$
	2004	2005	
Ile	3.61	3.50	0.11
Leu	6.92	6.75	n.s.
Lys	3.86	4.04	0.16
Met	1.86	1.84	n.s.
Cys-Cys	2.81	2.77	n.s.
Phe	4.12	4.63	0.09
Tyr	3.01	3.05	n.s.
Thr	3.36	3.36	n.s.
Trp	1.11	0.98	n.s.
Val	4.91	4.94	n.s.
Sum	35.57	35.86	n.s.
His	2.49	2.64	0.14
Arg	5.57	5.75	0.16
Pro	8.32	8.57	n.s.
Asp	6.59	6.77	n.s.
Ala	4.23	4.15	n.s.
Glu	20.20	22.35	1.28
Ser	4.48	4.84	0.16
Gly	4.36	4.32	n.s.
Sum	56.24	59.39	3.15

n.s. – non significant differences

control (84.3 and 81.8% in the years, respectively), while the lowest was observed in the treatment with single harrowing (38.0 and 31.2 %). The method of nitrogen application did not differentiate the efficacy of weed control in winter triticale.

In the years of the study, the content of exogenous amino acids in the grain protein oscillated around a constant level (average of 35.57–35.86 g·100 g^{-1} of protein) (Tab. 3). Nonetheless, in the generally dry second year of the study, but with plentiful rainfalls in the period of ripening, in July, the content of lysine and phenylalanine was significantly higher than in the first – wet – year of the experiment. The content of isoleucine, on the other hand, was significantly lower. In the case of endogenous amino acids, in the second year of the study their total content was 59.39 g·100 g^{-1} of protein, and was significantly higher compared to the first year, by 3.15 g, i.e. by 5.6%, which was also reflected in the total amino acid content (95.25 versus 91.81 g·100 g^{-1} of protein). In the second year of the study, compared to the first, among the endogenous amino acids there were significantly higher levels of histidine, arginine, glutamine and serine. The dependence of amino acid content in cereal grain on weather conditions has been pointed out by, among others, Makarska [1997], Starczewski et al. [2000] and Stankiewicz et al. [2005] who reported that increased levels of precipitation were conducive to the accumulation of amino acids in the grain. During the period under analysis, in the second year classified generally as a dry one, in the stage of triticale ripening – in July – the level of precipitations was ca. 23% higher than in the first year and 27% higher than the multi-year sum, which was favourable for amino acid biosynthesis. Similar relations have been reported also by Makarska [1997], Stankiewicz et al. [2005] and Klimont [2007].

The methods of weed control had no effect of the total content of exogenous amino acids in winter triticale grain (Tab.4.), which is in line with the results o studies by Makarska [1997] and Brzozowska et al. [1997] in the case of winter wheat, and by Stankiewicz et al. [2005] for spring triticale. Analysis of the content of particular exogenous amino acids shows that the methods of weed control, irrespective of their application, caused a tendency towards lowering the content of leucine and lysine in triticale grain protein, and in the case of harrowing – also of valine. At the same time, the application of herbicide alone caused a increase in the level of tyrosine and phenylalanine, and when complemented with harrowing – also phenylalanine. The plant care treatments applied to triticale, not causing any significant changes in the content of essential amino acids, indirectly did not differentiate the value of the integrated index of essential amino acids (EAAI), as was the case in the study by Sienkiewicz et al. [2005]. Nonetheless, harrowing applied to that cereal caused a slight deterioration in the value of that index – by 0.9.

In the case of endogenous amino acids, the application of harrowing, herbicide, as well as harrowing and herbicide for triticale caused a notable tendency towards a decrease in their total content, on average by more than 2 g·100 g^{-1} of protein (57.32 and 59.33 g·100 g^{-1}) compared to triticale with no plant care measures applied. Analysing the content of individual amino acids in grain protein it was observed that the weed control measures caused only a slight decrease in the levels of arginine, prolamine, aspargine and glutamine.

Nitrogen fertilisation, irrespective of the method of its application, caused a tendency towards a lowering in the sum of exogenous amino acids, on average by 0.43 g·100 g^{-1} of protein (Tab. 5). Analysis of the content of individual amino acids shows that foliar application of a part of the dose of nitrogen had an unfavourable effect on protein quality, significantly decreasing the content of methionine, from 1.88 to 1.79 g·100 g^{-1}. Moreover, nitrogen fertilisation was conducive to a slight increase in the concentration of endogenous amino acids in grain protein (by 2.76 g·100 g^{-1}), especially glutamic acid. Analysis of the integrated index of essential amino acids (EAA) shows that nitrogen fertilisation, irrespective of the method of its application, caused

Table 4. Amino acid composition of grain protein of winter triticale, depending on the method of weed control, g·100 g^{-1} protein (mean of 2004–2005)

Amino acid	Methods of weed control				$LSD_{(0.05)}$
	without (control object)	harrowing	herbicide	harrowing + herbicide	
Exogenous amino acids					
Ile	3.56	3.56	3.54	3.55	n.s.
Leu	7.01	6.81	6.81	6.72	n.s.
Lys	4.09	3.84	3.90	3.97	n.s.
Met	1.87	1.81	1.87	1.86	n.s.
Cys-Cys	2.79	2.74	2.82	2.81	n.s.
Phe	4.32	4.33	4.42	4.42	n.s.
Tyr	2.99	2.91	3.23	3.00	n.s.
Thr	3.38	3.32	3.35	3.40	n.s.
Trp	1.00	1.05	1.07	1.06	n.s.
Val	4.94	4.81	4.94	5.00	n.s.
Sum	35.95	35.18	35.95	35.79	n.s.
Endogenous amino acids					
His	2.61	2.54	2.50	2.61	n.s.
Arg	5.75	5.65	5.56	5.69	n.s.
Pro	9.02	8.41	8.27	8.07	n.s.
Asp	6.86	6.60	6.67	6.60	n.s.
Ala	4.29	4.13	4.20	4.14	n.s.
Glu	21.57	21.25	21.17	21.12	n.s.
Ser	4.73	4.55	4.68	4.69	n.s.
Gly	4.50	4.25	4.37	4.25	n.s.
Sum	59.33	57.38	57.42	57.17	n.s.
EAAI	69.0	68.1	69.6	69.2	n.s.

n.s. – non significant differences

a tendency towards deterioration of the index value (on average from 71.3 to 70.0). The calculated ratio of essential amino acids to total content of amino acids in all experimental treatments (with plant care and fertilisation) oscillated at the level of 0.38–0.39.

Analysis of values of CS index shows that the first amino acid to reduce the nutritive value of protein of winter triticale grain from all the treatments (with plant care and fertilisation) was isoleucine, followed methionine and lysine (Tab. 6). Also in the study by Makarska [1997] the

Table 5. Amino acid composition of grain protein of winter triticale, depending on the method of nitrogen application, $g \cdot 100\ g^{-1}$ protein (mean of 2004–2005)

Amino acid	Nitrogen application method ($kg\ N \cdot ha^{-1}$)				$LSD_{(0.05)}$
	without nitrogen	60 + 60	60+25+35	60+25* + 35	
Exogenous amino acids					
Ile	3.59	3.54	3.42	3.56	n.s.
Leu	6.89	6.80	6.74	6.87	n.s.
Lys	4.04	3.96	3.96	4.04	n.s.
Met	1.88	1.85	1.85	1.79	0.05
Cys-Cys	2.87	2.85	2.89	2.78	n.s.
Phe	4.35	4.48	4.42	4.41	n.s.
Tyr	3.02	2.95	2.90	2.91	n.s.
Thr	3.41	3.37	3.37	3.38	n.s.
Trp	1.12	1.06	1.05	1.03	n.s.
Val	5.03	5.02	4.95	5.10	0.08
Sum	36.20	35.88	35.55	35.87	n.s.
Endogenous amino acids					
His	2.59	2.66	2.62	2.70	n.s.
Arg	5.75	5.70	5.72	5.74	n.s.
Pro	8.13	8.70	8.50	8.37	n.s.
Asp	6.73	6.59	6.67	6.76	n.s.
Ala	4.20	4.13	4.17	4.19	n.s.
Glu	19.80	22.05	22.46	22.10	1.47
Ser	4.63	4.67	4.61	4.70	n.s.
Gly	4.36	4.27	4.33	4.43	n.s.
Sum	56.19	58.77	59.08	58.99	n.s.
EAAI	71.3	70.4	69.8	69.7	n.s.

* – foliar application
n.s. – non significant differences

first amino acid that limited the fodder value of winter triticale was isoleucine. Other authors point to lysine as the primary amino acid that limits the biological value of winter triticale grain protein [Wróbel and Budzyński 1994, Stankiewicz 1998], and in the case of spring triticale – also valine [Stankiewicz et al. 2005].

Table 6. Effect of the method of weed control and nitrogen application on the essential amino acid index CS (mean of 2004–2005)

Treatments	Amino acid		
	Ile	Met	Lys
Methods of weed control			
Without protection	53.9	60.3	63.9
Harrowing	53.9	58.4	60.0
Herbicide	53.6	60.3	60.9
Harrowing + herbicide	53.8	60.0	62.0
Mean	53.8	59.8	61.7
$LSD_{(0,05)}$	n.s.		
Nitrogen application method (kg $N \cdot ha^{-1}$)			
Without nitrogen	54.4	60.7	63.1
60+60	53.6	59.7	61.9
60+25+35	51.8	59.7	61.9
60+25*+35	53.9	57.7	63.1
Mean	53.4	59.5	62.5
$LSD_{(0.05)}$	n.s.		

1/ – limiting amino acid – the isoleucine and the next in order of
* – foliar application
n.s. – non significant differences

CONCLUSIONS

1. Meteorological conditions during plant vegetation had no effect on the total content of exogenous amino acids in the grain protein. Nevertheless, in the second (dry) year, though with plentiful rainfall in July, in the phase of ripening of the plants, the content of certain exogenous amino acids (lysine and phenylalanine) was significantly higher than in the first – wet – year of the study.
2. Plant care treatments and nitrogen fertilisation had no effect on the total content of exogenous amino acids in the protein, but foliar application of a part of the dose of nitrogen resulted in a significant decrease in the level of methionine.
3. The experimental factors applied had no effect on the value of the integrated index of essential amino acids (EAAI).
4. The amino acid that limited the nutritive value of winter triticale grain protein was isoleucine, followed by methionine and lysine.

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WPŁYW RÓŻNYCH METOD ZWALCZANIA CHWASTÓW I NAWOŻENIA AZOTEM NA WARTOŚĆ BIOLOGICZNĄ BIAŁKA ZIARNA PSZENŻYTA OZIMEGO

Synopis. W latach 2004–2005 w Zakładzie Dydaktyczno-Doświadczalnym w Tomaszkowie k/Olsztyna, należącym do UWM w Olsztynie, prowadzono doświadczenie polowe z uprawą pszenżyta ozimego odmiany Woltario. Celem badań było określenie wpływu różnych metod zwalczania chwastów: bronowanie, herbicyd Mustang 306 SE (florasulam – 6,25 $g \cdot dm^{-3}$ + 2,4-D – 452 $g \cdot dm^{-3}$ oraz bronowanie + herbicyd Mustang 306 SE) i nawożenia azotem (łącznie 120 kg $N \cdot ha^{-1}$: 60+60, 60+25+35, $60+25_{dolistnie}$+ 35) na wartość biologiczną białka ziarna. Cechy jakościowe białka określono na podstawie 18 aminokwasów, według Normy PN-EN ISO 13903:2006. Tryptofan oznaczono według Normy PN-R-64820:1977, wskaźnik aminokwasu ograniczającego CS metodą Mitchella i Blocka, a zintegrowany index aminokwasów egzogennych EAAI – metodą Osera.

Stosowane zabiegi odchwaszczania oraz nawożenia azotem nie miały wpływu na zawartość aminokwasów egzogennych w białku ziarna. Zastosowanie bronowania, herbicydu oraz bronowania i herbicydu w pszenżycie wpływało jedynie na zmniejszenie sumy aminokwasów endogennych, a nawożenie azotem, niezależnie od sposobu wykonania, na jej wzrost. Samo zaś bronowanie skutkowało pogorszeniem nieco wartości wskaźnika EAA, podobnie jak nawożenie azotem. Aminokwasem ograniczającym wartość odżywczą białka ziarna pszenżyta jarego była izoleucyna, a następnymi w kolejności metionina i lizyna

Słowa kluczowe: pszenżyto ozime, bronowanie, herbicyd, azot, aminokwasy, biologiczna wartość białka