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EFFECT OF MEAT AND BONE MEAL AND EFFECTIVE MICROORGANISMS ON CONTENT AND COMPOSITION OF PROTEIN IN CROPS PART I. SPRING WHEAT*

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Abstract. The purpose of this paper was determination of the effect of meat and bone meal applied without and with effective microorganisms compared with manure fertilization, mineral fertilizers or lack of fertilization on grain true protein content and composition of spring wheat cv. Nawra and Tybalt. The five-year studies (2005-2009) with crop rotation were conducted in Bałcyny at the Experimental and Plant Production Station near Ostróda. It was observed, that the applied fertilization with NPK as well as with manure and meat and bone meal in doses from 1.0 to 2.5 t·ha^{-1} affected the significant increase in protein content in grain of the studied spring wheat cv. Tybalt in 2009. Under the effect of the applied meat and bone meal, mainly the gliadin and glutenin content in spring wheat grain of both cultivars increased. The most beneficial influence on protein composition (high percentage participation of constitutional and storage proteins in relation to the total protein content) had fertilization with 1.5 t·ha^{-1} dose of meat and bone meal.

Key words: EM-1, Nawra, storage proteins, structural proteins, quality of wheat grain, Tybalt

INTRODUCTION

The problem of the environmentally-safe organic waste utilization is still up-to-date despite numerous research studies and despite making recommendations based on their results. The amount of these wastes still increases. First of all, their weight within particular groups changes, depending on the direction and rate of economic development as well as on social changes. Contemporary scientific literature indicates many different possibilities of utilizing waste substances [Dechnik and Skowrońska 2000]. In Poland, apart from the studies of Stępień [2011] as well as of Nogalska and

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Czapla [2009], there are practically no new scientific papers on applying meat and bone meal in agriculture. Initiation of the research presented in the paper is motivated by indicated difficulties when managing post-slaughter waste products, caused by the elimination of them as animal nutrition products. In recent years, annual production of animal meal in Poland has been approximately 150,000 tons. A similar problem with the disposal of meat and bone meal also have other European countries [Cummins *et al.* 2006, Deydier *et al.* 2007]. European Union allows, according to the legislation of member countries, to utilize by-products processed into meat and bone meals and to apply them as soil adjuvants.

Protein content is a quality trait of spring wheat grain, depending on, among others, fertilization. Fertilization, mainly with nitrogen, usually increases the amount of protein, but also changes proportions of proteins with slight changes of the amino acid composition [Johansson *et al.* 2001].

The aim of the paper was determination of the effect of meat and bone meal applied without or with effective microorganisms, compared with manure fertilization, mineral fertilizers or lack of fertilization, on the content and composition of protein in spring wheat grain. In the context of set out research aims, null hypothesis (H_o) was verified, assuming lack of significant differences between levels of the studied factor. In case of rejecting the null hypothesis (H_o), an alternative hypothesis (H_A) was assumed on the occurrence of significant differences between fertilization methods in the formation of protein fractions in spring wheat grain.

MATERIAL AND METHODS

Research results come from a long-term field experiment, set up in the randomized blocks design in 4 replications. The experiment was carried out in the years 2005-2009 at the Experimental and Plant Production Station in Bałcyny near Ostróda (53°36’ N; 19°51’ E) on soil of granulometric composition of loamy sand, agricultural suitability complex 3 (very good rye complex), rich in potassium and phosphorus as well as in magnesium. Plot area was 24.75 m^2. Spring wheat cv. Nawra was cultivated after cereal mix, sowing 265 $kg \cdot ha^{-1}$, and wheat Tybalt after winter rape at a rate of 180 $kg \cdot ha^{-1}$, both cultivars with a density of 500 seeds per m^2. The research was carried out in 5-year crop rotation with the crop sequence as follows: spring wheat cv. Nawra (2005), faba bean (2006), winter wheat (2007), winter rape (2008) and in 2009 spring wheat cv. Tybalt.

In the experiment, mineral NPK fertilization was applied to spring wheat at a rate of 60 $kg \cdot ha^{-1}$ before sowing (ammonium saltpeter 34%) as well as 30 $kg \cdot ha^{-1}$ at the stage of 1-2 nodes (urea 46%). Phosphorus and potassium were pre-sown at a rate of 31 kg $P \cdot ha^{-1}$ in the form of triple superphosphate (4%) as well as 83 kg $K \cdot ha^{-1}$ as 60% potassium salt (Table 1).

Every year, manure at a rate of 10 $t \cdot ha^{-1}$, was applied depending on the plant in the group of pre-sowing summer-autumn or spring crops. They were fertilized with different rates of meat and bone meal without or with the addition of effective microorganisms (preparation EM-1). Microbiological preparation was applied in the cultivation of all plants at a rate of 5 $dm^3 \cdot ha^{-1}$ with the division into two rates (the first one, 3 $dm^3 \cdot ha^{-1}$, before sowing and the second one, 2 $dm^3 \cdot ha^{-1}$, before the first treatment: harrowing). EM-1 contains microorganisms described in the papers of Szymański and

Peterson [2003] as well as Valarini *et al.* [2003]. Preparation EM-1 was prepared according to the instruction, i.e. microorganisms in it were activated through incubation with a proper rate of sucrose. Together with mineral and organic fertilizers, defined amounts of nutrients were applied to the soil (Table 2).

Table 1. Experimental design including the following fertilization systems
Tabela 1. Schemat doświadczenia z uwzględnieniem systemów nawożenia

Treatment – Obiekt	System of fertilization – System nawożenia
Without fertilization Bez nawożenia	control – without fertilization – obiekt kontrolny – bez nawożenia
NPK	mineral NPK fertilization – nawożenie mineralne NPK (N – 90 $kg \cdot ha^{-1}$, P – 31 $kg \cdot ha^{-1}$, K – 83 $kg \cdot ha^{-1}$)
FYM – Obornik	farm yard manure 10 $t \cdot ha^{-1}$ – obornik 10 $t \cdot ha^{-1}$
1.0 MMK	meat and bone meal 1.0 $t \cdot ha^{-1}$ – mączka mięsno-kostna w ilości 1,0 $t \cdot ha^{-1}$
1.0 MMK + EM-1	meat and bone meal 1.0 $t \cdot ha^{-1}$ and effective microorganism spraying (EM-1) mączka mięsno-kostna w ilości 1,0 $t \cdot ha^{-1}$ i opryskiwanie efektywnymi mikroorganizmami (EM-1)
1.5 MMK	meat and bone meal 1.5 $t \cdot ha^{-1}$ – mączka mięsno-kostna w ilości 1,5 $t \cdot ha^{-1}$
1.5 MMK + EM-1	meat and bone meal 1.5 $t \cdot ha^{-1}$ and effective microorganism spraying (EM-1) mączka mięsno-kostna w ilości 1,5 $t \cdot ha^{-1}$ i opryskiwanie efektywnymi mikroorganizmami (EM-1)
2.0 MMK	meat and bone meal 2.0 $t \cdot ha^{-1}$ – mączka mięsno-kostna w ilości 2,0 $t \cdot ha^{-1}$
2.0 MMK + EM-1	meat and bone meal 2.0 $t \cdot ha^{-1}$ and effective microorganism spraying (EM-1) mączka mięsno-kostna w ilości 2,0 $t \cdot ha^{-1}$ i opryskiwanie efektywnymi mikroorganizmami (EM-1)
2.5 MMK	meat and bone meal 2.5 $t \cdot ha^{-1}$ – mączka mięsno-kostna w ilości 2,5 $t \cdot ha^{-1}$
2.5 MMK + EM-1	meat and bone meal 2.5 $t \cdot ha^{-1}$ and effective microorganism spraying (EM-1) mączka mięsno-kostna w ilości 2,5 $t \cdot ha^{-1}$ i opryskiwanie efektywnymi mikroorganizmami (EM-1)

Table 2. Doses of nutrients in fertilizers, $kg \cdot ha^{-1} \cdot year^{-1}$
Tabela 2. Dawki składników pokarmowych w nawozach, $kg \cdot ha^{-1} \cdot rok^{-1}$

Specification Wyszczególnienie	NPK* $kg \cdot ha^{-1}$	FYM Obornik 10 $t \cdot ha^{-1}$	MMK 1.0 $t \cdot ha^{-1}$	MMK 1.5 $t \cdot ha^{-1}$	MMK 2.0 $t \cdot ha^{-1}$	MMK 2.5 $t \cdot ha^{-1}$
N	90.0	51.0	66.5	99.8	133.0	166.3
P	31.0	12.1	39.8	59.7	79.6	99.5
K	83.1	49.0	83.1	83.1	83.1	83.1
Mg	–	8.0	2.0	3.0	4.0	5.0
Ca	–	34.0	19.0	28.5	38.0	47.5
Na	–	3.2	5.6	8.4	11.2	14.0
Cu	–	0.050	0.010	0.015	0.020	0.025
Fe	–	3.850	0.510	0.765	1.020	1.275
Mn	–	0.450	0.003	0.005	0.006	0.008
Zn	–	0.250	0.099	0.149	0.198	0.248

* for explanations see Table 1 – objaśnienia w tabeli 1

Sowing, cultivation treatments and harvest of spring wheat were carried out according to agricultural requirements suitable for this plant species. Weeds were controlled only mechanically. There was no disease or pest control.

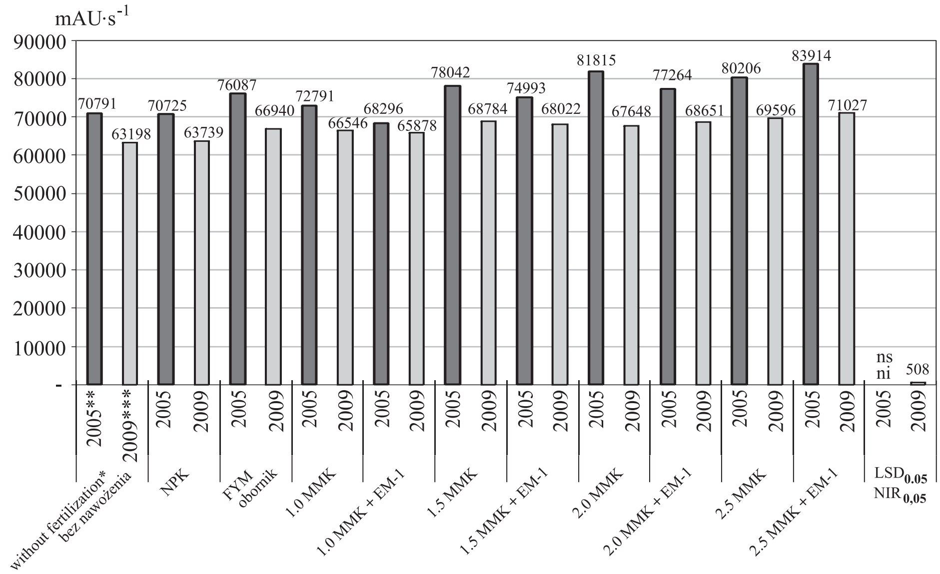
In particular years of research, annual rainfall total was usually in the range from 500 to 700 mm, while the lowest rainfall was observed in the years of spring wheat cultivation (501 and 577 mm). 2005 was not favourable for optimum moisture content in soil and release of nutrients included in fertilizers. In the year of setting up the experiment, rainfall total was lower by 17%, whereas in April and June on average by half, compared with the rainfall from the long-term period. Low rainfall in August (53% of the long-term period) was favorable for ripening of spring wheat and made the harvest easier. In 2009 water deficiency was observed only in April (11% of the amount from the long-term period), however it probably had no significant effect on the growth of spring wheat as well as on the moisture content of soil enabling break down of fertilizers with regard to water reserves accumulated in March when there was 234% rainfall, compared with the means from the long-term period (data according to the research station in Bałcyny).

In the research, the content and composition of protein fractions was determined. The grain sample was ground in the laboratory mill IKA A10 into the particle size finer than 250 μm. 100 mg of flour were applied to Eppendorf test-tubes each time, and four protein fractions were extracted according to Wieser *et al.* [1998]. Albumins were extracted with the use of distilled water, globulins with a mixture of NaCl and $HKNaPO_4$, prolamins with 60% ethanol, and glutenins in the mixture consisting of 50% propanol-1 + 2 m of urea + tris-HCl and 1 DTE under nitrogen. Determination was carried out in Hewlett Packard apparatus. Detection was carried out with the wave length of 210 μm. The results were analyzed with the use of a computer program HPLC according to 3D ChemStation manufactured by HP company, and they are presented in $mAU \cdot s^{-1}$ units.

Obtained results were statistically elaborated with the use of STATISTICA 9.0 program by Statsoft company. Single-factor analysis of variance was used, which was in accordance with the mathematical model of the experiment design of randomized blocks. Analysis of variance was carried out with the use of Tukey test on the significance level where $P \leq 0.05$.

RESULTS AND DISCUSSION

It was found that fertilization with NPK, manure and meat and bone meal applied to plants in the experiment, affected the increase in the content and change in composition of particular protein fractions. Protein content in spring wheat grain cv. Nawra and Tybalt cultivated in crop rotation oscillated within a wide range, depending on the cultivar as well as on the level of fertilization intensity (Fig. 1). However, a significant effect of methods of fertilization on the amount of protein was found only in 2009. Under the effect of meat and bone meal, protein content in grain of both wheat cultivars increased compared with the soil fertilized with NPK or manure. Its highest accumulation occurred in wheat grain cv. Nawra cultivated in 2005 after application of the meat and bone meal alone at a rate of 2.0 and 2.5 $t \cdot ha^{-1}$ as well as at a rate of 2.5 $t \cdot ha^{-1}$ with addition of effective microorganisms and was higher by 15.6, 13.3 and 18.5% compared with the control sample. Nogalska and Czapla [2009], also proved that together with the increase of the meal rate, the mean nitrogen content also increased in meadow fescue, which as a result could have had an effect on the higher accumulation of protein in plants. In the conducted experiment, in wheat cv. Tybalt cultivated in the following year of crop rotation (2009), meal fertilization mostly only to a slight degree affected the protein content, more of it (by 12.4%) was after application of meal at a rate of 2.5 t·ha^{-1} together with effective microorganisms (EM-1). According to some authors [Kwiatkowski *et al.* 2006, Kołodziejczyk *et al.* 2009], increase in the cultivation intensity, especially in the fertilization level, improves technological quality of the grain through the increase in protein concentration. In the research of Gąsiorowska and Makarewicz [2008], significantly highest total protein content was characteristic of the spring wheat grain fertilized with a rate of 160 and 60 N kg·ha^{-1}. On the other hand, Nowak *et al.* [2004] think that not all wheat cultivars react to the application of mineral fertilizers with the change of the grain quality traits.



ns – ni – non-significant difference – różnica nieistotna
* for explanations see Table 1 – objaśnienia w tabeli 1
** in 2005 cultivar Nawra – w 2005 r. odmiana Nawra
*** in 2009 cultivar Tybalt – w 2009 r. odmiana Tybalt

Fig. 1. Effect of fertilization on true protein content (peak surface expressed in mAU·s^{-1}) in spring wheat grain

Rys. 1. Wpływ nawożenia na sumę białek prostych (powierzchnia pików wyrażona w mAU·s^{-1}) w ziarnie pszenicy jarej

Evaluated cultivars, similarly to the research of Konopka *et al.* [2007a], differed with the content and composition of protein (Table 3). In the protein of wheat grain cv. Nawra (2005), there were usually more analyzed protein fractions, especially constitutional proteins (albumins and globulins). The applied mineral fertilization with NPK as well as organic fertilization with manure resulted in the increase in storage protein content (gliadins and glutenins), while to a larger degree in glutenins after application of NPK (by 19.0%) and manure (by 14.7%) in the wheat grain cv. Nawra. More beneficial was the effect of meal at a rate of 1.5 $t \cdot ha^{-1}$ in the cultivation of wheat cv. Tybalt (2009), as it caused the increase in accumulation (by 6.2%) of constitutional protein fractions (albumins and globulins). In the first year of spring wheat cultivation, meat and bone meal at a rate above 1.0 $t \cdot ha^{-1}$ with the addition and without the addition of EM-1 preparation resulted in an increase in the gliadin content. Most favourable effect had a rate of 2.5 $t \cdot ha^{-1}$ with the addition of EM-1, significantly increasing the content of this protein fraction by 26.2%. Fertilization applied in the experiment in the form of a meat and bone meal usually also favourably affected the increase in the content of glutenin protein fraction. Occurring changes in the protein composition in mentioned spring wheat cultivars fertilized with meal can also be explained among others through the effect of microelements included in them. In the research of Domska *et al.* [2009] under the effect of the applied microelements, protein content also increased, mainly of storage protein fractions in wheat grain and triticale grain.

Table 3. True protein content (peak surface expressed in $mAU \cdot s^{-1}$) in spring wheat grain
Tabela 3. Zawartość frakcji białek prostych (powierzchnia pików wyrażona w $mAU \cdot s^{-1}$) w ziarnie pszenicy jarej

Specification* Wyszczególnienie	Protein fractions – Frakcje białek					
	Albumins and globulins Albuminy i globuliny		Gliadins Gliadyny		Glutenins Gluteniny	
	2005**	2009***	2005	2009	2005	2009
Without fertilization Bez nawożenia	19075^{a}	12826^{be}	27928^{b}	26453^{g}	23789^{b}	23919^{f}
NPK	17512^{b}	11526^{a}	28410^{bc}	27934^{b}	28303^{f}	24279^{df}
FYM – Obornik	19024^{a}	12778^{e}	29783^{ce}	28457^{c}	27280^{a}	25705^{abc}
1.0 MMK	20048^{a}	12177^{ac}	29001^{bc}	29167^{ad}	23742^{b}	25202^{abde}
1.0 MMK + EM-1	16443^{b}	12554^{d}	26100^{f}	28852^{d}	25753c	24473^{def}
1.5 MMK	19998^{a}	13629^{g}	31846^{ad}	29693^{e}	26198^{cd}	25462^{abce}
1.5 MMK + EM-1	16277^{b}	12046^{a}	32045^{ad}	30139^{h}	26671^{ad}	25837^{abc}
2.0 MMK	19636^{a}	12519^{d}	33042^{a}	29282^{a}	29137^{f}	25848^{abc}
2.0 MMK + EM-1	20020^{a}	12995^{b}	30798^{de}	29369^{ae}	26447^{cde}	26287^{bc}
2.5 MMK	19852^{a}	12335^{c}	33129^{a}	30860^{f}	27226^{a}	26401^{c}
2.5 MMK + EM-1	20089^{a}	12085^{a}	3525^{g}	30618^{f}	28574^{e}	28324^{g}

a, b… – homogenous groups – grupy jednorodne
* for explanations see Table 1 – objaśnienia w tabeli 1
** in 2005 cultivar Nawra – w 2005 r. odmiana Nawra
*** in 2009 cultivar Tybalt – w 2009 r. odmiana Tybalt

Meat and bone meal is a rich source of nutrients for plants, above all of nitrogen, phosphorus, magnesium and calcium as well as microelements and an organic substance. Jeng *et al.* [2004] proved that nitrogen applied in the form of meat and bone meal in 80% fulfills fertilization requirements of cereals for this element. Domska and Wojtkowiak [2004] observed that in the grain of plants fertilized with nitrogen (80 $kg \cdot ha^{-1}$) the content of storage proteins and proteids increased. According to Warechowska [2004], protein content in the cereal grain depends to a large degree on the weather conditions in particular years of research. Also other authors [Konopka *et al.* 2007b] indicate the effect of meteorological conditions, and above all deficiency of water, on the content and composition of protein in wheat grain. Based on the conducted research with three wheat cultivars they proved that in case of water deficiency, accumulation of albumins and globulins, γ gliadins as well as glutenins, both low- and high-molecular weight, decreases, while most sensitive was wheat cv. Nawra. In own research, the content and composition of protein in cereal grain could have been affected by among others cultivar properties because, as it was proved in the conducted research in 2005, despite lower rainfall in April and June than in 2009, in the wheat grain cv. Nawra there were more albumins and globulins and in some cases also more gliadins or glutenins compared with wheat cv. Tybalt.

One of the most important factors affecting technological quality of wheat is the content and composition of gluten proteins in grains [Makarska and Kowalczyk 2000]. Above all they affect the improvement of baking properties of flour. In the tested grain samples, proportion of storage protein fractions of gliadins oscillated within the range from 38.2 to 44.3%, and glutenins from 33.6 to 39.8% (Table 4). Higher percentage part of these fractions occurred usually in the grain protein of wheat cv. Tybalt cultivated in 2009. High percentage part of gliadins was characteristic of the grain protein of both cultivars after application of a rate of 1.5 $t \cdot ha^{-1}$ meal with effective microorganisms, and glutenins of grain cv. Tybalt fertilized with a rate of 2.5 $t \cdot ha^{-1}$ with the addition of EM-1. When evaluating the technological quality of cereal products, attention should also be paid to the proportion of complete constitutional proteins (structural proteins), albumins and globulins. In this case, it was higher in the protein of wheat cv. Nawra. Most beneficial changes, i.e. high proportion of both constitutional and storage proteins, occurred in the grain of wheat fertilized with meal at a rate of 1.5 $t \cdot ha^{-1}$. Addition of effective microorganisms EM affected in some cases the increase in the proportion of gliadins and glutenins at the expense of albumins and globulins.

Table 4. Percentage part of protein fractions in total protein content of spring wheat grain
Tabela 4. Procentowy udział poszczególnych frakcji białka w stosunku do ogólnej zawartości białka w ziarnie pszenicy jarej

Specification* Wyszczególnienie	Albumins and globulins Albuminy i globuliny		Gliadins Gliadyny		Glutenins Gluteniny	
	2005**	2009***	2005	2009	2005	2009
Without fertilization Bez nawożenia	26.9	20.3	39.4	41.9	33.6	37.8
NPK	24.8	18.1	40.2	43.8	35.1	38.1
FYM – Obornik	25.0	19.1	39.1	42.5	35.9	38.4
1.0 MMK	27.5	18.3	39.8	43.8	32.6	37.9
1.0 MMK + EM-1	24.1	19.1	38.2	43.8	37.7	37.2
1.5 MMK	25.6	19.8	40.8	43.2	33.6	37.0
1.5 MMK + EM-1	21.7	17.7	42.7	44.3	35.6	38.0
2.0 MMK	24.0	18.5	40.4	43.9	35.6	38.2
2.0 MMK + EM-1	25.9	18.9	39.9	42.8	34.2	38.3
2.5 MMK	24.8	17.7	41.3	44.3	33.9	37.9
2.5 MMK + EM-1	23.9	17.0	42.0	43.1	34.1	39.8

* for explanations see Table 1 – objaśnienia w tabeli 1
** in 2005 cultivar Nawra – w 2005 r. odmiana Nawra
*** in 2009 cultivar Tybalt – w 2009 r. odmiana Tybalt

CONCLUSIONS

1. Meat and bone meal applied without or with the effective microorganisms significantly affected the increase in the protein content in spring wheat grain.
2. The greatest effect on the protein content in wheat cv. Tybalt showed fertilization of the soil with meat and bone meal at a rate of 2.5 t·ha^{-1} used with or without EM-1.
3. Beneficial effect on the protein composition of both wheat cultivars (high proportion of both constitutional and storage proteins) showed fertilization with meal at a rate of 1.5 t·ha^{-1}.
4. Under the effect of meat and bone meal applied at a rate from 1.0 to 2.5 t·ha^{-1} usually the content of gliadins and glutenins increased, while the amount of albumins and globulins decreased in the grain of both spring wheat cultivars.

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WPŁYW MĄCZEK MIĘSNO-KOSTNYCH I EFEKTYWNYCH MIKROORGANIZMÓW NA ZAWARTOŚĆ I SKŁAD BIAŁKA W ROŚLINACH CZ. I. PSZENICA JARA

Streszczenie. Celem pracy było określenie wpływu mączek mięsno-kostnych stosowanych samodzielnie bądź z efektywnymi mikroorganizmami – w porównaniu z nawożeniem obornikiem, nawozami mineralnymi lub brakiem nawożenia – na zawartość i skład białka prostego ziarna pszenicy jarej odmian Nawra i Tybalt. Badania prowadzono w pięcioletnim zmianowaniu roślin w latach 2005-2009 w Zakładzie Produkcyjno-Doświadczalnym w Bałcynach koło Ostródy. Stwierdzono, że zastosowane nawożenie zarówno NPK, obornikiem, jak i mączką mięsno-kostną w dawkach od 1,0 do 2,5 $t \cdot ha^{-1}$ wpłynęło na istotny wzrost zawartości białka w ziarnie pszenicy jarej odmiany Tybalt uprawianej w 2009 r. Pod wpływem zastosowanej mączki mięsno-kostnej zwiększała się przeważnie zawartość gliadyn i glutenin w ziarnie pszenicy jarej obu odmian. Najbardziej korzystnie na skład białka (duży procentowy udział białek konstytucyjnych i zapasowych w stosunku do ogólnej zawartości białka) oddziaływało nawożenie mączką w dawce 1,5 t·ha^{-1}.

Słowa kluczowe: białka budulcowe, białka zapasowe, EM-1, jakość ziarna pszenicy, Nawra, Tybalt

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