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EFFECT OF MEAT AND BONE MEAL AND EFFECTIVE MICROORGANISMS ON CONTENT AND COMPOSITION OF PROTEIN IN CROPS PART II. FABA BEAN AND WINTER WHEAT*

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Abstract. In the field experiment carried out in the years 2005-2009 at the Eperimental and Plant Production Station in Bałcyny near Ostróda, effect of meat and bone meal was studied applied with or without the addition of effective microorganisms and NPK, manure and with no fertilization on the amount and fraction composition of protein in the faba bean seeds cv. Titus and winter wheat grain cv. Oliwin. The research was conducted in 5-year crop rotation. It was found that protein content in faba bean seeds increased only under conditions of mineral fertilizations (P and K), while in winter wheat also after application of manure and meat and bone meal, especially at a rate of 2.5 $t \cdot ha^{-1}$ with the addition of effective microorganisms. Under the effect of meat and bone meal fertilization, in the protein of wheat grain, there occurred an increase in the proportion of protein fractions, albumins and globulins. Application of meat and bone meals without the addition of effective microorganisms or with its addition in faba bean fertilization is pointless with regard to lack of their actual effect on the content and composition of protein, compared with mineral and organic fertilization.

Key words: organic fertilizer, quality of faba bean seeds, storage protein, structural protein, wheat grain quality

INTRODUCTION

In the long-term experiments with fertilizers, there occurs a phenomenon of after--effect of both mineral and organic fertilizers. Obtaining high yields with proper quality parameters requires providing plants with conditions similar to optimum, especially including supplementation of nutrients in appropriate proportions. Therefore, systematic supplementation of soil supplies with these nutrients is necessary [Czuba 2000]. Most frequently fertilization with nitrogen, phosphorus and potassium is used, ignoring other

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nutrients essential to plants, including microelements. They may be applied, among others, through natural and organic fertilizers: manure, composts or wastes, e.g. meat and bone meals. Release of nutrients from organic fertilizers is much slower than from mineral fertilizers. Therefore, yield-forming activity of meat and bone meal and organic fertilizers, occurs in later years with reference to the accumulation of their activity through increase and improvement of soil fertility [Stępień and Mercik 2002, Jeng *et al.* 2004, Mazur and Mazur 2006]. Systematically applied animal meal increases the amount of available forms of nutrients included in the composition of sorption complex and may constitute the main source of these components in fertilization of crops [Valenzuela *et al.* 2001, Jeng *et al.* 2006].

The aim of the research was determination of the effect of mineral fertilization, manure and meat and bone meal with and without the addition of effective microorganisms on the composition of protein fractions in faba bean and winter wheat cultivated in five-year crop rotation. In the view of the assumed research aims, null hypothesis (H_o) was verified, assuming lack of significant differences between levels of the tested factor. In case of rejecting the null hypothesis (H_o), an alternative hypothesis was taken (H_A) on the occurrence of significant differences between methods of fertilization in formation of protein fractions in seeds of faba bean and winter wheat grains.

MATERIAL AND METHODS

Information concerning the location, setting up method and conducting of field experiment, as well as the scheme of fertilization is described in detail in the first part of the paper [Stępień and Wojtkowiak 2011]. In winter wheat cultivation cv. Oliwin as well as faba bean cv. Titus, meat and bone meal was applied at the following rates: 1.0, 1.5, 2.0 and 2.5 $t \cdot ha^{-1}$ with and without the addition of effective microorganisms (EM-1), manure fertilization (10 $t \cdot ha^{-1}$) or mineral fertilization (NPK) as well as without fertilization. In winter wheat in soil fertilized with minerals, nitrogenous fertilizers were applied before sowing at a rate of 30 $kg \cdot ha^{-1}$ as well as at stages of shooting and heading at rates of 30 $kg \cdot ha^{-1}$ each. Phosphoric fertilizers (super triphosphate 46%) and potassic fertilizers (potassium salt 60%) were applied both under the cultivation of faba bean and winter wheat, before sowing at a rate of 31 kg $P \cdot ha^{-1}$ as well as 83 kg $K \cdot ha^{-1}$. In the cultivation of faba Bean cv. Titus no nitrogen fertilization was applied. Sowing and cultivation treatments as well as the harvest were carried out according to agrotechnical recommendations suitable for a given plant species.

In grain samples, the content of particular protein fractions was determined after their extraction with the method of Wieser *et al.* [1998]. The results were analyzed with the use of a computer program HPLC 3D ChemStation and was presented in $mAU \cdot s^{-1}$ units. Detailed description of the method is presented in I part of the paper [Stępień and Wojtkowiak 2011].

Distribution of meteorological conditions in the years of cultivation of faba bean and winter wheat was diversified. The range of annual rainfall total in the year of faba bean cultivation was 722.0 mm (data according to the research station in Bałcyny) and was by about 20% higher than the mean value from the analyzed long-term period. In the period from April to September, the rainfall total oscillated around the level of 475.3 mm. Faba bean is distinguished from the leguminous plants by high water requirements. It is a plant from the oceanic climate and for the proper development, rainfall of about

400 mm is necessary during growth [Prusiński *et al.* 2008]. In 2007 rainfall total (490.2 mm), and also its distribution was beneficial for wheat cultivation. Only at the beginning of its growing season (in September) there occurred almost twice higher rainfall.

For statistical calculations of result data, program STATISTICA 9.0 by Statsoft was used. Apart from basic statistical parameters, analysis of normal data distribution with Shapiro-Wilk test with $P \leq 0.05$, and next homogenous groups were determined statically with Tukey test.

RESULTS AND DISCUSSION

In faba bean cultivation, the protein content, and also its composition, were affected more effectively by mineral fertilization (with phosphorus and potassium) compared with fertilization with manure and meat and bone meal (Table 1). This method of fertilization contributed to the increase in the total protein content, above all as a result of the increase in the number of globulin fractions. Application of meal had no beneficial effect on the protein content in faba bean seeds. However, it may be assumed that the nutritive value of this protein was better with regard to the lower accumulation of defective prolamin fractions which are characterized by low content of exogenous amino acid, lysine. Some authors [Stępień and Mercik 2002] emphasize the relatively poor yield-forming activity of total nitrogen which is applied into the soil together with meal. It probably results from the fact that nitrogen occurs in them in the form which is least available for plants. On the other hand, Górecka *et al.* [2009] think that the yield--forming activity of animal waste is similar to the effects obtained after application of mineral fertilizers at the same balanced rate.

Table 1. True protein content (peak surface expressed in $mAU \cdot s^{-1}$) in faba bean seeds
Tabela 1. Zawartość frakcji białek prostych (powierzchnia pików wyrażona w $mAU \cdot s^{-1}$) w nasionach bobiku

Specification Wyszczególnienie	Albumins Albuminy	Globulins Globuliny	Prolamins Prolaminy	Glutenins Gluteniny	Total protein Suma białek
Without fertilization Bez nawożenia	76 665^{cd}	37 796^{e}	11 324^{g}	8 099^{cde}	133 884^{fg}
NPK	75 431^{bcd}	41 721^{f}	12 260^{h}	8 427de	137 838^{g}
FYM – Obornik	76 072^{cd}	20 082^{ab}	7 868^{bc}	6 825^{abc}	110 846^{c}
1.0 MMK	77 538^{cd}	31 058^{d}	9 919^{f}	7 955^{bcde}	126 470^{ef}
1.0 MMK + EM-1	75 861^{bcd}	26 239^{c}	9 111^{e}	9 131^{e}	120 341de
1.5 MMK	68 810^{abc}	23 605^{bc}	8 606de	6 989^{abcd}	108 010^{bc}
1.5 MMK + EM-1	79 114^{d}	22 714^{bc}	9 742^{f}	7 596^{abcde}	119 166de
2.0 MMK	62 175^{a}	20 759^{ab}	8 583de	7 461^{abcd}	98 977^{a}
2.0 MMK + EM-1	66 380^{ab}	18 268^{a}	8 150^{cd}	7 520^{abcd}	100 317^{ab}
2.5 MMK	78 966^{d}	20 069^{ba}	7 319^{b}	6 423^{ab}	112 776^{cd}
2.5 MMK + EM-1	68 759^{abc}	17 844^{a}	5 432^{a}	6 293^{a}	98 328^{a}

NPK – mineral fertilization – nawożenie mineralne
MMK – meat and bone meal – mączka mięsno-kostna
EM-1 – effective microorganisms – efektywne mikroorganizmy
a, b… – homogenous groups – grupy jednorodne

The applied fertilization affected the higher diversification of the total protein content and its particular fractions in winter wheat grain cv. Oliwin (Table 2).

Table 2. True protein content (peak surface expressed in mAU·s^{-1}) in winter wheat grain
Tabela 2. Zawartość frakcji białek prostych (powierzchnia pików wyrażona w mAU·s^{-1}) w ziarnie pszenicy ozimej

Specification Wyszczególnienie	Albumins and globulins Albuminy i globuliny	Gliadins Gliadyny	Glutenins Gluteniny	Total protein Suma białek
Without fertilization Bez nawożenia	11565^{a}	26305^{ab}	16721^{a}	54591^{a}
NPK	12386^{a}	27333^{bcd}	21601^{g}	61320^{cd}
FYM – Obornik	11850^{a}	27643^{bcde}	19597^{cde}	59090^{bc}
1.0 MMK	15963^{c}	27948^{bcdef}	21698^{g}	65609^{f}
1.0 MMK + EM-1	16074^{cd}	24833^{a}	18109^{b}	59016^{bc}
1.5 MMK	16483^{cde}	29311^{ef}	18090^{b}	63883^{ef}
1.5 MMK + EM-1	16933^{ef}	29486^{f}	18998^{bcd}	65416^{a}
2.0 MMK	16681^{cdef}	28947^{def}	18523^{bc}	64150^{ef}
2.0 MMK + EM-1	16890^{def}	27984^{bcdef}	19805de	64679^{f}
2.5 MMK	14115b	26664^{b}	21142^{fg}	61920de
2.5 MMK + EM-1	17478^{f}	28541^{cdef}	20171^{ef}	66189^{f}

for explanations see under Table 1 – objaśnienia pod tabelą 1

Protein content oscillated from 54591 to 66189 mAU·s^{-1} and was highest after application of the meat and bone meal at a rate of 1.5 t·ha^{-1} with the addition of EM-1. Its slightly lower accumulation occurred just after application of the rate of 1.0 t·ha^{-1} of the meat and bone meal alone. In the protein of plants fertilized with meal, there occurred an increase in the accumulation of structural protein fractions, albumins and globulins, and also gliadins, whereas to the largest degree after their application with EM-1, at a rate of 1.5 as well as 2.5 t·ha^{-1}. According to Hessey [2003] and Kurek and Ozimek [2008], activity of microbiological preparations favorably affects the plant growth through availability of nutrients present in the soil. Kucharski and Jastrzębska [2005], however, point to their negative effect on the growth and development of lettuce. Their application decreased the commercial quality of the product due to the occurrence of necrotic spots on leaves. Animal meal applied in plant fertilization is a rich source of nitrogen, phosphorus, and calcium, and also of the following microelements: copper, zinc, iron and manganese [Tenuta and Lazarovits 2003, Jeng *et al.* 2006, Ylivainio *et al.* 2008]. According to Jeng *et al.* [2004], nitrogen included in the meal, is immediately available for crop plants, though according to Delin and Engstrom [2010] nitrogen included in the meal occurs mainly in the form of protein compounds, and directly after its application it is not available to plants. On the other hand, Hanáčkova *et al.* [2008] proved that accumulation of inorganic nitrogen in the soil after application of animal biowastes does not differ from its level occurring after application of standard manure rates. In own research, manure fertilization at a rate of 10 t·ha^{-1} affected the increase in the total protein content in the soil, mainly of protein fractions of glutenins, by 17.2% compared with the soil which was not fertilized. According to Domska *et al.* [2003], manure fertilization at a rate of 30 t·ha^{-1} under forecrop most favorably affected the content of true protein in barley grain, fraction composition and amino acid composition as well as the degree of protein utilization. Application of a higher rate of microelements however caused deterioration of the nutritive value of protein in barley grain as a result of the increase of the prolamin content in it at the expense of glutelins. Barczak and Kozera [2003] indicate changes in the fraction composition of protein in spring barley grain under the effect of fertilization with microelements. In this case, there occurred an increase in the content of constitutional proteins (albumins and globulins). Particularly beneficial was the effect of zinc, manganese and copper. In contrast to the research of mentioned above authors, Wołoszyk *et al.* [2003] proved a beneficial effect of NPK fertilization on the accumulation of glutenins in winter wheat grain.

Nutritive value of plant protein is determined not only by the content of particular protein fractions characterized by diversified, more or less valuable amino acid composition, but above all by their mutual relation expressed as a percentage part in relation to the total protein content. In tested samples of faba bean seeds, relatively high proportion of albumin fractions was present, in the range from 54.7 to 70.0% (Table 3). Under the effect of the meal (compared with mineral fertilization with P and K), and after application of their higher rate (2.5 $t \cdot ha^{-1}$) also compared with manure fertilization, proportion of this fraction increased, however in some cases at the expense of globulins.

Table 3. Percentage part of protein fractions in total protein content in faba bean seeds and winter wheat grain
Tabela 3. Procentowy udział frakcji białka w stosunku do ogólnej zawartości białka w nasionach bobiku i w ziarnie pszenicy ozimej

Specification Wyszczególnienie	Faba bean – Bobik				Winter wheat – Pszenica ozima		
	Albumins Albuminy	Globulins Globuliny	Prolamins Prolaminy	Glutenins Gluteniny	Albumins and globulins Albuminy i globuliny	Gliadins Gliadyny	Glutenins Gluteniny
Without fertilization Bez nawożenia	57.3	28.2	8.5	6.1	21.2	48.2	30.6
NPK	54.7	30.3	8.9	6.1	20.2	44.6	35.2
FYM – Obornik	68.3	18.1	7.1	6.2	20.0	46.8	33.2
1.0 MMK	61.1	24.6	7.8	6.3	24.3	42.6	33.1
1.0 MMK + EM-1	63.4	21.8	7.6	7.6	27.2	42.1	30.7
1.5 MMK	63.7	21.9	8.0	6.5	25.8	45.9	28.3
1.5 MMK + EM-1	66.4	19.1	8.2	6.4	25.9	45.1	29.0
2.0 MMK	62.8	21.0	8.7	7.5	26.0	45.1	28.9
2.0 MMK + EM-1	66.2	18.2	8.1	7.5	26.1	43.3	30.6
2.5 MMK	70.0	17.8	6.5	5.7	22.8	43.1	34.1
2.5 MMK + EM-1	69.9	18.2	5.5	6.4	26.4	43.1	30.5

for explanation see under Table 1 – objaśnienia pod tabelą 1

Fertilization with PK, manure and meal had no effect however on bigger changes in the percentage part of other fractions of storage proteins, i.e. prolamins and glutenins in faba bean seeds. Content and quality of protein in papilionaceous plants has a particularly great significance, as they cover almost 15% of arable lands in the world, and they provide for the human food requirements of protein in approximately 33% [Księżak and Borowiecki 2001].

In tested samples of winter wheat grain under the effect of meal fertilization, percentage part of the most valuable fraction of structural proteins, albumins and globulins, increased. Gliadins prevailed in the grain with a lower proportion of glutenins. Fractions of storage protein play a significant role in forming the baking quality [Knapowski and Ralcewicz 2004]. Mineral fertilization (NPK) and manure fertilization at a rate of 10 t·ha^{-1} caused the increase in protein in glutenin proportion by 4.61 and 2.54%, compared with the control test. Stępień and Adamiak [2005] confirmed the 36% increase in the protein yield after manure fertilization compared with plants without fertilization. Barczak and Kozera [2003], Domska *et al.* [2003], Knapowski and Ralcewicz [2004], in their research indicate the effect of nitrogen fertilization on the content of storage protein fractions, gliadins and glutenins. Wooding *et al.* [2000] as well as Johansson *et al.* [2001] found that increase in the nitrogen rates results in the increase of grain yield as well as protein accumulation, but at the same time decreases its quality. Many authors point to the course of weather conditions as a factor which mostly varies the technological quality of winter wheat grain [Stankowski *et al.* 2004, Zwolak 2005, Podolska *et al.* 2007, Podolska 2008].

CONCLUSIONS

1. Protein content in faba bean seeds cv. Titus increased only under conditions of mineral fertilization (PK), while in the grain of winter wheat cv. Oliwin also after fertilization with manure and meat and bone meal, and to the highest degree after application of meal at a rate of 2.5 t·ha^{-1} together with the addition of effective microorganisms.
2. Under the effect of meat and bone meal fertilization, proportion of structural protein fractions of albumins and globulins increased in the protein of winter wheat grain.
3. Application of meat and bone meal, both without and with the addition of effective microorganisms did not cause any changes in the protein composition of faba bean seeds.

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

Streszczenie. W doświadczeniu polowym założonym w latach 2005-2009 w Zakładzie Produkcyjno-Doświadczalnym w Bałcynach koło Ostródy badano wpływ mączki mięsno-kostnej stosowanej samodzielnie lub z udziałem efektywnych mikroorganizmów oraz NPK, obornika i braku nawożenia na ilość i skład frakcyjny białka nasion bobiku odmiany Titus i ziarna pszenicy ozimej odmiany Oliwin. Badania prowadzono w pięcioletnim zmianowaniu. Stwierdzono, że zawartość białka w nasionach bobiku zwiększyła się jedynie w warunkach nawożenia mineralnego (P i K), natomiast w pszenicy ozimej również po zastosowaniu obornika i mączki mięsno-kostnej, szczególnie w dawce 2.5 $t \cdot ha^{-1}$ z dodatkiem efektywnych mikroorganizmów. Pod wpływem nawożenia mączką mięsno-kostną w białku ziarna pszenicy zwiększał się udział frakcji białek albumin i globulin. Stosowanie mączek mięsno-kostnych bez dodatku efektywnych mikroorganizmów lub z ich dodatkiem w nawożeniu bobiku jest niecelowe ze względu na brak efektywnego oddziaływania na zawartość i skład białka w porównaniu z nawożeniem mineralnym i organicznym.

Słowa kluczowe: białka budulcowe, białka zapasowe, jakość nasion bobiku, jakość ziarna pszenicy, nawóz organiczny

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