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Original Research

Assessment of New NKSMg Fertilizer Based on Protein Hydrolysate of Keratin in Pot Experiments

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Abstract

We assessed the effectiveness use of the new NKSMg fertilizer on the germination of rapeseed (*Brassica napus* L. var. *napus*). The fertilizer was produced on the basis of protein hydrolysate from processing of poultry feathers. The whole process is based on the digestion of feather waste by acidic hydrolysis, adjusting pH of the reaction medium, and then enriched in select nutrients. The method allows the production of liquid fertilizers that can be used in soil and foliar applications. Pot experiments have shown that the use of NKSMg fertilizer based on protein hydrolysate of keratin had a significant effect on the increase of plant biomass. The contents of nitrogen, phosphorus, potassium, and magnesium in plants increased by 76.2, 5.20, 37.3, and 29.6%, respectively, compared to unfertilized plants. The use of fertilizer also significantly modified the contents of manganese and zinc in tested plants. Technology of production of fertilizers based on protein hydrolysate is currently implemented by Intermag Co., Polish leader in the manufacture of fertilizers, growth stimulators, and other preparations designed for professional agriculture and horticulture.

Keywords: fertilizers, feather waste, hydrolysis, rapeseed, pot experiments

Introduction

The poultry industry is one of the largest and fastest growing agro-based industries in the world [1-5]. Worldwide poultry meat production in 2012 reached more than 100 million tons – one fifth of which was in the United States. European Union annual production of poultry meat was nearly 13 million tons, and average consumption amounted to about 23 kilos per capita per year [6]. Within the last 20 years, the poultry industry has taken over 10% of meat market share, which now accounts for one third of global meat production. This can be attributed to an increasing demand for poultry meat and also egg products [1, 4-6]. A global overview of the poultry sector is shown in Table 1.

However, a major problem that the poultry industry is facing is the large-scale accumulation of waste [1, 4, 7]. Daily slaughter of poultry in a large Polish slaughterhouse is shown in Table 2 [8]. The main keratin waste generated by the poultry industry is feathers [9, 10]. In Poland, about 70,000 tons of this waste is produced annually [11], while on a global scale it is more than 4 million tons [7]. Keratin, a primary component of feathers, is a fibrillar protein resistant to mechanical and chemical treatments due to the presence of a large number of disulphide bonds in its structure. Native keratin is also resistant to the action of typical proteolytic enzymes, such as pepsin, trypsin, and papain. The duration of biodegradation of feathers in the environ-

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Table 1. Global overview of the poultry sector [6].

	Production (000't)							Consumption (kg per capita)						
	1998	2002	2006	2008	2010	2011	2012	1998	2002	2006	2008	2010	2011	2012
France	2.32	2.15	1.80	1.76	1.75	1.86	1.85	25.1	24.6	23.0	24.5	24.7	25.2	25.5
Germany	0.79	1.03	1.19	1.40	1.62	1.68	1.68	15.2	17.2	16.7	18.3	18.7	19.1	18.5
Poland	0.52	0.80	1.08	1.17	1.31	1.34	1.58	14.0	19.8	23.7	24.6	26.3	27.4	27.6
Spain	1.00	1.33	1.15	1.17	1.28	1.28	1.25	27.5	33.6	31.0	30.5	30.2	30.5	30.0
UK	1.53	1.54	1.54	1.46	1.57	1.56	1.61	28.0	28.9	29.8	26.0	28.6	28.5	28.7
EU-27	8.82^	9.36^	10.5^^	11.5	12.2	12.5	12.7	21.5^	23.2^	22.1^^	23.3	23.1	23.1	23.1
Brazil	4.99*	7.67*	9.71*	11.5*	12.8*	13.4*	13.3*	26.3**	34.3*	37.4*	42.1*	48.1*	49.7*	48.5*
China	11.2	13.3	14.3	16.0	17.5	17.7	18.0	nd	7.30**	7.80**	8.80**	9.10**	9.40**	9.80**
Japan	1.21	1.23	1.37	1.37	1.42	1.38	nd	nd	14.4**	15.5**	15.2**	16.4**	16.6**	16.9**
Russia	0.69	0.94	1.62	1.99	2.55	2.90	3.08	12.2	13.0*	17.4*	19.8*	20.7*	21.3*	22.5*
USA	15.1*	17.0*	18.9*	19.9*	19.5*	19.7*	19.7*	45.9	50.8*	52.3*	52.0*	50.8*	50.9*	49.6*
World	62.3	75.3	83.0	92.5	99.2	102	104	nd	nd	nd	nd	nd	nd	nd

^ To 2004: EU-15, ^^ To 2006: EU-25
* Only chicken and turkey meat, ** Only chicken meat
nd – No data

Table 2. Daily slaughter of poultry in a large Polish slaughterhouse [8].

Type of poultry	Daily slaughter (pcs)	Waste generated (t)		
		Blood	Mixture of slaughter wastes*	Feathers
Chickens	50	6-7	15	13-15
Geese	35	6	15	4
Ducks	35	6	15	4

*Bones, skin, blood, intestines, raw feather, adipose, and muscle tissue

ment is from two to three years, which is a serious ecological problem [9, 10, 12]. In addition, a limited number of disposal companies, affecting the low competitiveness of the market and the lack of alternative solutions for reducing the amount of animal by-products for poultry producers, contributes to the persistence of the price of their purchase at the level of €100 per ton. For a medium-sized poultry slaughterhouse, producing 20-30 tons of waste per day, this costs about €1 million annually [13].

The occurrence of the epidemic of bovine spongiform encephalopathy (BSE) has led to the introduction of a series of legal regulations in order to limit the use of processed animal protein in the feeding of livestock. The first ban, introduced in 1988 in the UK, referred to the use of meat and bone meal deriving from ruminants in cattle nutrition. In 1994 a ban on cattle, sheep, and goat feeding was introduced regarding meat and bone meal from mammals. In 2001 the use of processed animal protein in feeding of livestock intended for the production of raw materials and food products of animal origin was totally halted (Regulation (EC) No. 999/2001). An exception was fish meal, which was permitted for the feeding of animals other than ruminants. In the following years more precise legal acts regarding bans on the use of products of animal origin in nutrition of livestock were introduced (Commission Regulation (EC) No. 808/2003, Commission Regulation (EC) No. 1234/2003, Regulation (EC) No. 1069/2009, Commission Regulation (EU) No. 595/2010, Commission Regulation (EU) No. 142 /2011) [14, 15].

In view of environmental protection and applicable laws as well as the high cost of disposal, and also the prevention of the waste of valuable organic matter, it is necessary to develop an effective feather waste management that is safe for the environment and economically viable.

This paper presents a method of feather waste processing for the fertilizer NKSMg and assessment of its effect for the plants (rapeseed) in pot experiments.

Materials and Methods

Production of Fertilizer

The basis for the production of fertilizer was feather waste, which was obtained from a Polish poultry slaughterhouse. Before hydrolysis the material was washed in deionized water, dried, and comminuted.

The method of producing NKSMg fertilizer is based on digestion of feather waste in 30% sulfuric acid. The process is carried out for four hours at 80ºC. As a result of hydrolysis the mixture of short peptides and amino acids is obtained. The next step of the process is pH regulation to 6 with magnesium oxide, and then adding urea and potassium sulphate. This method enables the production of liquid fertilizers that can be used for both soil and foliar application.

The method is protected with a block patent whose right to dispose of the know-how is Wroclaw University of Technology (Poland) [16-18].

Testing Fertilizer on Plants

Pot experiments were conducted in Swojec Experimental Station at the University of Environmental and Life Sciences in Wroclaw. Tests were performed at the Wroclaw University of Technology.

The objective of the studies was to determine the effect of NKSMg (8:1:2:0.5) fertilizer based on protein hydrolysate, referred to in this article as Fertilizer FH, on the germination of plants. The tests were carried out on rapeseed (variety Monolith). Fertilization was applied 14 days before sowing plants.

The substrate for growing plants was soil of light loamy sand with low organic matter content. It was characterized by acidic pH and low levels of soluble forms of potassium, high content of phosphorus, and very high magnesium. The contents of macro- and micronutrients in the soil were not modified before the beginning of the study. In order to improve the properties of the soil, pH was adjusted by incubating with the addition of calcium carbonate at a dose determined on the basis of a single hydrolytic acidity. The experiment was conducted in pots with a capacity of 0.5 kg of soil, and the bottoms of the pots were lined with a layer of gravel aerated by a vertical drain. Time of plant vegetation in the experiment was established so that the period of nutrient uptake from fertilizers was about three weeks.

The fertilizing effect of the product on the basis of protein hydrolysate was compared to the control object (unfertilized) and the two most commonly used nitrogen fertilizers: ammonium nitrate and urea. Cultivated plants were fertilized with a nitrogen dose of 0.4 g N per kg of soil. Fertilizers were applied (in solution form after dilution with water to a volume of 20 cm^3) on the entire surface of the soil in the pot, and then watered with 50 cm^3 of distilled water. Tests were carried out in quadruplicates.

To obtain the highest biomass and nutrient uptake, plants were sown at high density, with 40 pieces of rapeseed per pot. However, due to the very uneven emergence of plants, thinning was conducted, which left in the pots 18 of strongest plants. During the incubation of soil with a deacidifying substance and tested fertilizers, and also during plant growth, soil moisture was maintained at 60% of field water capacity. A collection of rapeseed occurred on the day 20 after the emergence of plants.

Analytical Methods

Feathers were subjected to analysis of elemental composition. Nitrogen was determined by the Kjeldahl method, mercury by atomic absorption spectrometry (AMA-254, Altec), and other macro-and micronutrients and toxic elements by inductively coupled plasma optical emission spectrometry (Vista MPX, Varian) after digestion in aqua regia.

In order to determine the amino acid composition in the hydrolysate of keratin obtained by digestion feathers, chromatographic analysis was carried out. The analysis was performed on an amino acid analyzer AAA 400 (Ingos).

In the pot experiments, fresh and dry mass of plants was determined and also the analysis of macro-and micronutrients in the biomass was performed. The nitrogen content in the plants was analyzed by the colorimetric method after mineralization in sulphuric/salicylic acid. The content of the other macro- and microelements was determined after dry mineralization of the plant material in a muffle furnace at 450ºC, and after the obtained ash was digested in nitric acid. Phosphorus was determined by the colorimetric method, other components were determined by atomic absorption spectrometry using SpectrAA-220 FS (Varian).

Statistical Analysis

The statistical analysis of study results was conducted with Tukey's test for the reasonable significant difference (RIR) at the significance level 0.05. The calculations were made using the STATISTICA program version 10.

Results

Multielemental analysis has shown that the feathers used for the production of fertilizer are a rich source of nitrogen (14.8%) and sulfur (2.35%), as well as micronutrients such as iron (203 mg/kg) and zinc (92.8 mg/kg). On the other hand, the content of toxic metals such as arsenic, cadmium, chromium, mercury, nickel, and lead is at an extremely low level. The elemental composition of the used feathers is presented in Table 3.

As a result of performing the hydrolysis process of feathers, a highly mineralized mixture of short peptides and amino acids was obtained. Hydrolysate, which was the base of the fertilizer, contained significant amounts of amino acids such as proline, serine, glycine, leucine, glutamic acid, aspartic acid, valine, and arginine (respectively 35.5, 33.5, 31.6, 30.4, 27.0, 24.4, 23.8, and 20.6 g/kg). The detailed content of amino acids in the protein hydrolysate from poultry feathers is shown in Table 4.

The application of all fertilizers tested in the experiment resulted in a significant increase in the accumulation of plant biomass. The use of fertilizer FH increased the fresh mass of plants by 31.4% and dry mass by 21.3% compared to the control object (not fertilized). A slightly better effect was obtained only in the case of ammonium nitrate fertilization with an increase of 31.4% and 27.7% for fresh and dry mass, respectively, whereas the use of urea resulted in an increase of fresh mass of rapeseed by 24.8% and dry mass by 14.9%. Compared to the application of fertilizer FH, plant yield was lower by 5.0% and 5.3%. The resulting yields in the pot experiments are presented in Table 5.

Table 3. Elemental composition of the feathers.

Macronutrients	Content (% d.m.)	Micronutrients	Content (mg/kg d.m.)	Toxic elements	Content (mg/kg d.m.)
N	14.8	B	3.15	As	4.15
P	0.061	Cu	12.0	Cd	< 0.187
K	0.056	Fe	203	Cr	1.77
Ca	0.168	Mn	14.0	Hg	0.021
Mg	0.034	Mo	< 1.94	Ni	2.45
S	2.35	Zn	92.8	Pb	1.81

Table 4. Content of amino acids in the protein hydrolysate from poultry feathers.

Amino acid	Content (g/kg)	Amino acid	Content (g/kg)
Aspartic acid	24.4	Leucine	30.4
Threonine	13.2	Tyrosine	11.4
Serine	33.5	Phenylalanine	16.3
Glutamic acid	27.0	Histidine	3.65
Proline	35.5	Lysine	3.58
Glycine	31.6	Arginine	20.6
Alanine	18.0	Cysteine	11.5
Valine	23.8	Methionine	0.23
Isoleucine	14.7	Tryptophan	< LOD*

*Limit of detection (LOD): < 50 pmol (0.5 μmol/dm³)

After the application of fertilizers, in all three variants a significant increase in the content of nitrogen in the tissues of plants cultivated in the experiment was observed (Fig. 1). N content in plants compared to unfertilized plants increased by 76.2%, 57.5%, and 44.5%, respectively, for the fertilizer FH, ammonium nitrate, and urea. The use of fertilizer based on protein hydrolysate also significantly modified the content of potassium and magnesium (Figs. 2 and 3). The content of K in plants increased from 57.2 g/kg (the control object) to 78.5 g/kg (increase of 37.3%), and Mg from 3.93 g/kg to 5.09 g/kg (29.6%). Compared to the application of ammonium nitrate and urea, the content of potassium increased by 21.5% and 20.9%, respectively, while magnesium content increased by 12.6% and 11.1%. The use of fertilizer FH, ammonium nitrate, and urea also resulted in an increase in the content of phosphorus in plants, respectively by 5.20%, 7.87%, and 13.8% compared to unfertilized plants, but these differences were not statistically significant (Fig. 4).

In Table 6 the average content of microelements in harvested plants depending on the applied fertilizer is presented. Fertilizer FH significantly modified the content of micronutrients such as manganese and zinc in the aerial parts of rapeseed. The use of fertilizer on the basis of feather waste increased the content of Mn from 80.5 mg/kg (the control object) to 391 mg/kg (increase of 386%), and Zn from 83.8 mg/kg to 232 mg/kg (177%). For other experimental objects statistically significant differences also were found in the content of these micronutrients in plants. The average manganese content in rapeseed fertilized with

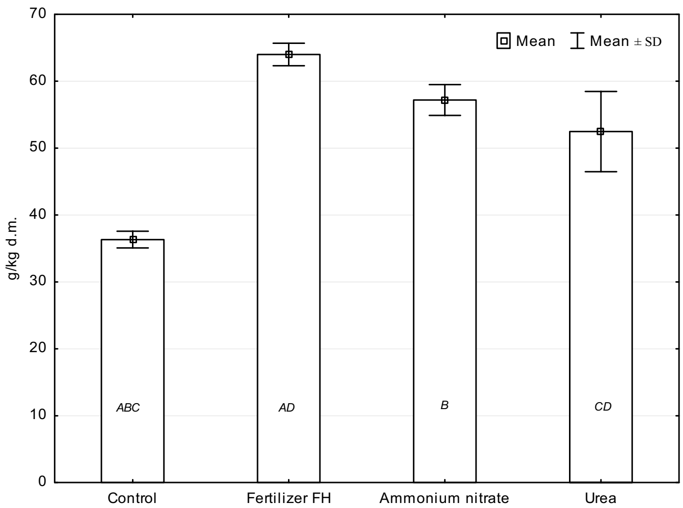

Fig. 1. Content of nitrogen in the harvested plants depending on the type of fertilizer used.
A, B, C, D – Values designated with the same letters indicate high significant differences according to Tukey's test (p≤0.01)

Table 5. Resulting yields in the pot experiments.

Fertilizer	Repetition	Yield of fresh mass (g)	Yield of dry mass (g)	Part of dry mass (%)
Control (unfertilized)	1	12.4	0.98	7.9
	2	11.4	0.96	8.4
	3	12.4	0.93	7.5
	4	12.0	0.89	7.4
Fertilizer FH	1	15.7	1.11	7.1
	2	15.2	1.11	7.3
	3	16.7	1.19	7.1
	4	15.9	1.15	7.2
Ammonium nitrate	1	16.3	1.29	7.9
	2	16.7	1.19	7.1
	3	15.0	1.11	7.4
	4	15.6	1.19	7.6
Urea	1	14.7	1.03	7.0
	2	15.1	1.11	7.4
	3	14.8	0.99	6.7
	4	15.9	1.18	7.4
Mean				
Control (unfertilized)		12.1 ± 0.47^{ABC}	0.94 ± 0.04^{AaB}	7.8 ± 0.5^{a}
Fertilizer FH		15.9 ± 0.62^{A}	1.14 ± 0.04^{A}	7.2 ± 0.1
Ammonium nitrate		15.9 ± 0.75^{B}	1.20 ± 0.07^{B}	7.5 ± 0.3
Urea		15.1 ± 0.54^{C}	1.08 ± 0.08^{a}	7.1 ± 0.3^{a}

$^{A, B, C}$ – Values designated with the same letters in a single column indicate high significant differences according to Tukey's test ($p \leq 0.01$)
^{a} – Values designated with the same letters in a single column indicate significant differences according to Tukey's test ($p \leq 0.05$)

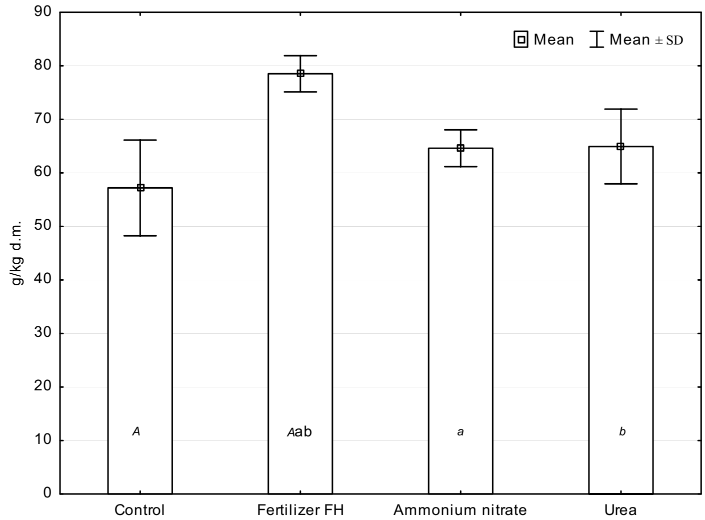

Fig. 2. Content of potassium in the harvested plants depending on the type of fertilizer used.
A – Values designated with the same letters in a single column indicate high significant differences according to Tukey's test ($p \leq 0.01$)
a, b – Values designated with the same letters in a single column indicate significant differences according to Tukey's test ($p \leq 0.05$)

Table 6. Microelement content in the harvested plants.

Fertilizer	Cu	Fe	Mn	Zn
	(mg/kg d.m.)			
Control (unfertilized)	8.00±1.50	128±11.8	80.5±23.2 [A]	83.8±18.8 [Aa]
Fertilizer FH	8.40±0.86	155±38.6	391±72.6 [ABC]	232±15.9 [ABC]
Ammonium nitrate	6.30±1.05	167±25.4	150±28.8 [B]	109±4.57 [B]
Urea	6.20±1.33	150±30.9	117±17.2 [C]	126±19.1 [aC]

Explanations, see Table 5

Table 7. Pot experiments: statistical evaluation of the impact of NKSMg fertilizer based on protein hydrolysate from poultry feathers for biomass and chemical composition of rapeseed (in comparison to other fertilizers).

Trait		Fertilizer		
		Control (not fertilized)	Ammonium nitrate	Urea
Yield of fresh mass		+	○	○
Yield of dry mass		+	○	○
Part of dry mass		○	○	○
Macroelement content	N	+	○	+
	P	○	○	○
	K	+	+	+
	Mg	+	○	○
Microelement content	Cu	○	○	○
	Fe	○	○	○
	Mn	+	+	+
	Zn	+	+	+

○ Not significant difference, + Significant difference (increase)

fertilizer FH was about 161% higher than for plants fertilized with ammonium nitrate, and 234% for plants fertilized with urea, while the average zinc content was higher, respectively, by 113% and 84.1%. The contents of copper and iron were also higher in plants fertilized with fertilizer FH compared to unfertilized plants, respectively by 5.0% and 21.1%, but these differences were not statistically significant.

Discussion

The use of feather waste enables the recovery of nutrients and is one of the recommended strategies for dealing with waste [19]. Poultry feathers are a rich source of nitrogen, acting as the main growth factor, and sulfur is often mentioned as a deficient component in agricultural soils. Feather waste is also a source of desired micronutrients in plant cultivation, particularly iron and zinc. The content of toxic metals is at an extremely low level – far ahead of the limit values set by the requirements for organic-mineral fertilizers [20] (Table 3).

Poultry feathers are made of over 90% keratin proteins [10, 12]. This is a cheap material to obtain fertilizers based on amino acids, which are well known in the agricultural industry, used primarily as chelates of metal ions.

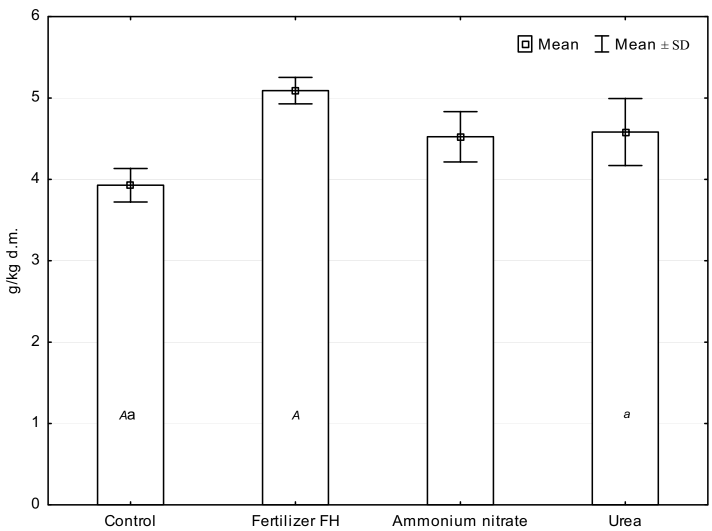

Fig. 3. Content of magnesium in the harvested plants depending on the type of fertilizer used.
A – Values designated with the same letters in a single column indicate high significant differences according to Tukey's test (p≤0.01)
a – Values designated with the same letters in a single column indicate significant differences according to Tukey's test (p≤0.05)

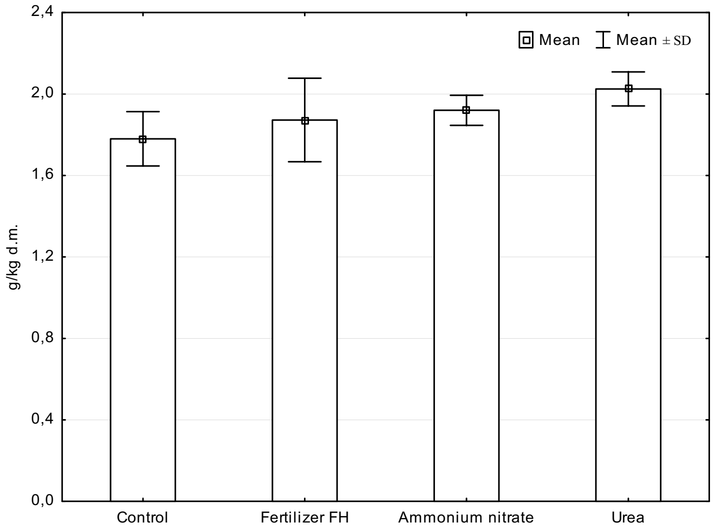

Fig. 4. Content of phosphorus in the harvested plants depending on the type of fertilizer used.

Microelements chelated with amino acids form very small, electrically neutral molecules, which accelerate their absorption and transport within the plant [21-23]. They also act as biostimulators of plant growth. Amino acids are the basic building blocks of protein and fulfill multiple functions in the plant: structural, metabolic, and transport function. Plants are capable of the production of amino acids themselves, but their synthesis is the process that requires large amounts of energy. Therefore, the application of ready-made amino acids allows the plants to save this energy and dynamise the pace of their development or reconstruction, especially in critical times of plant development, such as after transplantation, during flowering and climatic stress (frost, drought), or plant diseases [24-26]. For the production of fertilizer protein hydrolysate of keratin from feather waste was used. Hydrolysate was obtained by digestion of feathers in 30% sulfuric acid. The total content of amino acids in this hydrolysate was 31.9% (Table 4).

Pot experiments have shown the great effect of the produced NKSMg fertilizer in cultivation of plants. Table 7 presents a summary of statistical evaluation of the impact of fertilizer on the basis of protein hydrolysate for biomass and chemical composition of rapeseed, compared to the control object as well as the used ammonium nitrate and urea. The use of this fertilizer resulted in a significant increase in the accumulation of plant biomass compared to the unfertilized object. Studies have also shown that this fertilizer can be used to supplement the plant with nitrogen, potassium, and magnesium effectively. Plant analysis showed a significant increase in the contents of nitrogen, potassium, and magnesium in the tissues of tested plants compared to the control object, potassium compared to ammonium nitrate, and nitrogen and potassium compared to urea. Furthermore, fertilization significantly increased the content of manganese and zinc compared to all fertilizers used. Increased efficiency of fertilizer FH was associated with the presence of amino acids obtained by hydrolysis of keratin. Amino acids are good chelating agents and they acted as carriers of micronutrients contained in the used soil.

Conclusions

The presented method of producing liquid fertilizers on the basis of feather waste is the solution for the problem of this waste. It offers many benefits: eliminates the toxic and sanitary problems of feather waste, provides recovery of valuable nutrients from feathers (amino acids, macro- and microelements), enables production of fertilizers (the composition of which is adapted to the needs of the crops), and also allows reuse of industrial sulfuric acid waste.

Currently, registration trials for the fertilizers and also stimulators based on protein hydrolysate of keratin from feathers are carried out in order to introduce them to the market by Intermag Co. (Poland). Tests include physicochemical and microbiological analysis, and also field experiments of agricultural crops (wheat, corn, rapeseed, soy), vegetable crops (tomatoes, lettuce, beet sugar), and fruit crops (apples, cherries). The planned registration is in 2015. Market launch of a new range of fertilizers as well as stimulators on the basis of short peptides and amino acids will allow for the possibility of extending the standard fertilization in order to intensify the yields, and also the application in a situation of shortage of any of the nutrients.

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