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ORIGINAL PAPER

EFFECTS OF FERTILIZATION WITH AN AMINO ACID PREPARATION ON THE DRY MATTER YIELD AND CHEMICAL COMPOSITION OF MEADOW PLANTS*

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Abstract

Amino acids are among the most important organic compounds in living organisms. They play a vital biological role as building blocks of proteins, enzymes, nucleic acids, antioxidants, hormones and other components. Plants are capable of self-synthesis of amino acids, but this process requires much energy and time. Therefore, the use of these compounds as biostimulators may save energy and improve dynamics of plant development. The application of amino acids in plant production is a new concept of nutrient supply. The aim of the experiment was to investigate the effects of fertilization with amino acids on the productivity and content of macro- and micronutrients in meadow plants. The study was carried out in 2014-2016, on a farm located in the Province of Małopolska, Kraków district. A field experiment in a randomized block design with four replicates was established on a permanent meadow. The area of each experimental plot was 10 m^2. The soil on the experimental meadow was loess underlain by Haplic phaeozem and classified as class I in the Polish soil classification system. The experimental factor was spraying with an amino acid formulation in the form of Microfert fertilizer at two doses: 1.8 and 4.5 $dm^3\ ha^{-1}$. Foliar fertilization with the higher dose of amino acids (4.5 $dm^3\ ha^{-1}$) significantly ($p \leq 0.05$) improved the plant height, which was manifested by a higher dry matter yield. The greatest difference of 1.29 t ha^{-1} DM as compared with control was observed in the first year of the study. The plants fertilized with amino acids also absorbed more phosphorus, potassium, magnesium and calcium. The higher dose of amino acids improved the plant uptake of zinc, copper, manganese and iron. In conclusion, fertilization with amino acids improved weight of plant aerial parts, plant yield and the use of nutrients.

Keywords: biostimulators, productivity, meadow plants, micronutrients, macronutrients.

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INTRODUCTION

Nowadays, in addition to basic mineral fertilization, modern plant cultivation increasingly often involves different formulations called plant growth regulators or biostimulators (Shekari, Javanmardi 2017). These products improve the development of entire plants or specific organs, accelerate the course of life processes and enhance plant resistance to stress factors such as high temperature, low humidity and salt (Vernieri et al. 2005).

Some of the most important active substances present in biostimulator formulations are amino acids (Calvo et al. 2014). They play a vital biological role as building blocks of proteins, enzymes, nucleic acids, antioxidants, hormones and other important components (Shukla et al. 2014). In plant production, amino acids may directly or indirectly affect the plant growth and yield (Mohamed 2006, Nikiforova et al. 2006). A positive effect of amino acids on mitigation damage to plants caused by abiotic stresses has also been observed (Kowalczyk, Zielony 2008, Sadak et al. 2015).The greatest advantage of using amino acids as biostimulators is their good mobility and easy transportation within plants (Kandil et al. 2016). The role of amino acids in plant growth has been shown through their ability to induce the activity of some enzymes responsible for protein and carbohydrate synthesis. It was also confirmed that amino acids might act as buffers in a plant cell and help to maintain desired pH (Davis 1982). These substances also have a positive effect on photosynthesis, respiration, and water cycle as well as the concentration of ascorbic acid (Meijer 2003, Nikiforova et al. 2006). All these processes may improve the plant height, leaf area, tillering and yield (Kandil et al. 2016).

Foliar application seems to be the most effective method of amino acid treatment. It contributes to alleviating soil deficiencies and overcoming the soil's inability to transfer nutrients to plants. According to Garcia and Hanway (1976), foliar feeding may be eight to ten times more effective than soil feeding.

Research literature indicates that amino acid application is beneficial for different crops yield. However, no information can be found on the effects of amino acids on meadow plants. Therefore, the aim of this study was to determine the effect of Microfert®, an amino acid formulation, on the productivity and content of mineral components in meadow plants.

MATERIAL AND METHODS

Study site and soil analysis

The study was conducted on a farm located in the Province of Małopolska, Kraków district. A field experiment in a randomized block design with four replicates was established on a permanent meadow. The area of each experimental plot was 10 m^2. The soil on the experimental meadow was loess underlain by Haplic phaeozem and classified as class I. It was moderately rich in absorbable forms of phosphorus (12.6 mg 100 g^{-1} soil), potassium (16.2 mg 100 g^{-1} soil) and magnesium (8.1 mg 100 g^{-1} soil).

Prior to the experiment, dominant grass species included English ryegrass (*Lolium perenne*), meadow fescue (*Festuca pratensis*), orchard grass (*Dactylis glomerata*), Kentucky bluegrass (*Poa pratensis*), red fescue (*Festuca rubra*), and timothy grass (*Phleum pratense*) – Table 1. The share of *Fabaceae* plants was 14% and they were represented by red clover (*Trifolium pratense*) and white clover (*Trifolium repens* L.). The experimental meadow also comprised nine species of dicotyledonous plants, which accounted for 12% of its flora.

Table 1

Botanical composition of the experimental meadow sward prior to the experiment

Grasses	**74%**
English ryegrass (*Lolium perenne* L.)	21%
Meadow fescue (*Festuca pratensis* Huds.)	18%
Orchard grass (*Dactylis glomerata* L.)	15%
Kentucky bluegrass (*Poa pratensis* L.)	8%
Red fescue (*Festuca rubra* L.)	5%
Timothy grass (*Phleum pratense* L.)	5%
Rough bluegrass (*Poa trivialis* L.)	2%
Fabaceae	**14%**
Red clover (*Trifolium pratense* L.)	9%
White clover (*Trifolium repens* L.)	5%
Dicotyledons	**12%**
Common yarrow (*Achillea millefolium* L.)	2%
Common dandelion (*Taraxacum officinale* F. H. Wigg.)	2%
Shepherd's purse (*Capsella bursa pastoris*)	2%
English plantain (*Plantago lanceolata* L.)	1%
Broadleaf plantain (*Plantago major* L.)	1%
Red dead-nettle (*Lamium purpureum* L.)	1%
White campion (*Melandrium album* (Mill.))	+
Black knapweed (*Centaurea jacea* L.)	+
Chickweed (*Stellaria media* (L.) Vill.)	+
Germander speedwell (*Veronica chamaedrys* L.)	+
Hedge bedstraw (*Galium mollugo* L.)	+
Common knotgrass (*Polygonum aviculare* L.)	+

Botanical composition was evaluated by using Klapp's method.

Weather conditions

Annual rainfall in the study period (2014-2016) amounted to 684.7, 555.7 and 626.7 mm, respectively (Table 2). Mean rainfall during the plant growing period (April–September) was 508.0 mm in 2014, 317.7 mm in 2015, and 321.6 mm in 2016. Mean annual temperature in the years 2014-2016 was 6.1, 6.4 and 6.9°C, respectively, and 10.9, 12.1 and 12.1°C between April and September.

Table 2

Rainfall and average air temperature at the Plant Breeding Station in Polanowice in 2014-2016.

Month	2014	2015	2016
	monthly rainfall (mm)		
1	21.0	42.5	23.0
2	23.2	38.0	94.0
3	33.5	55.0	42.5
4	39.5	11.0	67.0
5	76.0	89.0	38.3
6	102.0	34.0	35.8
7	163.0	93.0	80.6
8	117.0	43.0	82.0
9	10.5	47.7	18.0
10	51.0	56.0	99.1
11	26.0	40.5	33.5
12	22.0	6.0	13.0
Total	684.7	555.7	626.7
Total April-September	508.0	317.7	321.6
	average monthly air temperature (°C)		
1	-2.8	-2.0	-1.3
2	-1.55	-1.9	3.0
3	2.57	0.8	2.0
4	5.1	4.7	5.3
5	10.3	10.3	10.9
6	13.65	14.2	15.5
7	9.6	16.9	17.0
8	16.7	16.1	14.0
9	10.1	10.6	10.1
10	7.27	4.5	5.5
11	2.88	0.8	1.3
12	-0.2	2.4	-0.6
Average	6.1	6.4	6.9
Average April-September	10.9	12.1	12.1

Material and experimental design

The study included three variants: control (no treatment) and experimental spraying with the investigated preparation Microfert at two doses: 1.8 and 4.5 dm^3 ha^{-1}. Each dose was divided into three equal batches. The first batch, 0.6 dm^3 ha^{-1} for the variant of 1.8 dm^3 ha^{-1} and 1.5 dm^3 ha^{-1} for the variant of 4.5 dm^3 ha^{-1} was applied after the spring plant growth had begun. The next batches, applied during regrowth, were of identical concentrations.

The solutions used for spraying were prepared by dissolving an appropriate amount of the amino acid preparation in such a volume of water so as to obtain 300 dm^3 ha^{-1} of the working solution.

Microfert, manufactured by Intermag Ltd., is a biostimulating fertilizer that contains amino acids, peptides and chelated micronutrients. The amino acid content of this formulation is at the level of 440 g kg^{-1} of which 160 g kg^{-1} are free amino acids such as alanine 53 g, arginine 2 g, phenylalanine 10 g, glycine 102 g, histidine 3 g, isoleucine 4 g, aspartic acid 20 g, glutamic acid 60 g, leucine 18 g, hydroxylysine 6 g, hydroxyproline 28 g, lysine 22 g, methionine 3 g, ornithine 26 g, proline 50 g, serine 2 g, threonine 3 g, tyrosine 7 g, and valine 21 g kg^{-1}.

Other components of the preparation are: total nitrogen (N) 93.2, soluble organic nitrogen (N) 88.2, organic carbon (C) 277.2, boron (B) 0.63, cobalt (Co) 0.013, iron (Fe) 2.52, manganese (Mn) 1.26, molybdenum (Mo) 0.063 and zinc (Zn) 0.13 g kg^{-1}. Due to their properties, amino acids are combined in the order determined by the DNA structure. This way each plant is capable of producing proteins that are currently required and direct them as desired into sites where metabolic processes should be improved.

During the study, basic mineral fertilization was applied in the form of 80 kg N ha^{-1} for the first cut and 60 kg N ha^{-1} as 34% N ammonium nitrate for the second and third cut. Phosphorus was applied once in the spring at 35.2 kg P ha^{-1} in the form of enriched superphosphate 17.6% P, and potassium was applied for the first and third cut at 49.8 kg K ha^{-1} as 49.8% K potassium salt.

The average plant height before harvesting was assessed with a measuring tape at 10 sites per plot. Regrowth energy and plant density were determined on a nine-point scale, assuming 9 for the most favourable and 1 for the least favourable rate (Domański et. al. 1998). Meadow plants were harvested at the heading stage (first cut) and after 6-7 weeks (second and third cuts). Directly after harvesting, the yield was weighed and 0.5 kg of green mass was randomly sampled for chemical analysis. According to the AOAC method, average weighed samples, after drying and grinding, were analyzed for the content of organic and mineral matter.

Statistical analysis

The significance of differences between the compared average yields of dry weight and content of individual elements was determined by the Duncan's test using Statistica 10 PL software (StatSoft, Inc. 2011). The analysis of variance and the Duncan's test were performed at a significance level $\alpha = 0.05$.

RESULTS

The effects of the doses of the amino acid stimulator on the plant height, regrowth energy and density are presented in Table 3. The stimulator treatment was found to positively affect all investigated traits. The experimental plants were higher than the control ones and showed greater regrowth energy. In addition, the plots fertilized with 1.8 and 4.5 dm^3 ha^{-1} of the amino acid preparation showed higher plant density.

Table 3

Average plant height, regrowth energy and density depending on a Microfert dose

Cut	Microfert dose (dm^3 ha^{-1})		
	control	1.8	4.5
Average plant height (cm)			
I	72*b*	76*ab*	79*a*
II	32*b*	33*b*	34*a*
III	36*b*	38*ab*	40*a*
Regrowth energy (9-point scale)			
Spring	7.9*b*	8.3*b*	8.6*a*
First cut	7.4*b*	7.7*b*	8.2*a*
Second cut	7.0*b*	7.4*b*	7.8*a*
Third cut	7.7*b*	7.9*b*	8.2*a*
Plant density for individual cuts (9-point scale)			
I	7.3*b*	7.6*b*	7.9*a*
II	7.2*b*	7.5*b*	7.8*a*
III	7.5*b*	7.8*b*	8.1*a*

a, *b* – values in rows marked with different letters differ significantly ($P \leq 0.05$) according to the Duncan's multiple range test.

The three-year experiment involving treatment of meadow plants with Microfert generally significantly increased dry matter yields (Table 4). The meadow was harvested thrice during each vegetation season. Yielding was highly variable depending on the experimental variant, and dry matter yield ranged from 1.66 to 6.42 t ha^{-1}.

The largest dry matter yield was obtained from the plots fertilized with the triple dose of the amino acid formulation (variant 3) – Table 4. In the first year of the study, the yield was by 12.3% higher than in control,

Table 4

The effect of Microfert application on dry matter yield (t ha^{-1}) of meadow plants

Dose of Microfert (dm^3 ha^{-1})	Cut			Total
	first	second	third	
2014				
Control	5.74*b*	2.54*b*	2.27*b*	10.56*b*
1.8	6.05*ab*	2.55*ab*	2.57*ab*	11.16*b*
4.5	6.42*a*	2.71*a*	2.73*a*	11.86*a*
V(%) variation coefficient	5.56	3.59	9.14	5.79
2015				
Control	4.94*b*	1.66*b*	3.73*b*	10.33*b*
1.8	4.97*b*	1.68*b*	3.98*ab*	10.63*b*
4.5	5.43*a*	1.82*a*	4.30*a*	11.55*a*
V(%) variation coefficient	5.37	5.07	7.14	5.87
2016				
Control	5.04*b*	1.85*b*	3.72*b*	10.61*b*
1.8	5.24*b*	1.90*ab*	3.94*ab*	11.08*ab*
4.5	5.52*a*	1.94*a*	4.29*a*	11.75*a*
V(%) variation coefficient	4.58	2.38	7.22	5.14

a, b – values in columns separately for years, marked with different letters differ significantly ($P \leq 0.05$) according to the Duncan's multiple range test, SEM – standard error of the mean.

in the second year by 11.8%, and in the third by 10.7%. Abundant rainfall occurring in the first year of the study contributed to more intensive productivity of the meadow, which resulted in a significant increase in the yield on the plots treated with Microfert. Reversely, lower rainfall in the second and third year of the study, particularly in April 2015 and May 2016, resulted in the first cut yield reduction which could not be balanced off by the use of amino acids.

The study demonstrated the greatest yield improvement on the plots fertilized with 4.5 dm^3 ha^{-1} of the amino acid preparation. Total dry matter yield from the first, second and third cut was by 1.29, 1.22, and 1.14 t ha^{-1} DM greater than in control in the first, second and third year, respectively.

The plants growing on the plots fertilized with amino acids had higher content of total protein than the control ones. The difference was 5% for the dose of 1.8 dm^3 ha^{-1} and 10.5% for the dose of 4.5 dm^3 ha^{-1} (Table 5).

A reverse relationship was noticed for raw fiber, which was less abundant in the amino acid fertilized plants than in the control ones (Table 5).

The study presents weighted averages for the macro- and micronutrient content over the entire experimental period (2014-2016). The content of the investigated elements varied depending on the amino acid dose and was as follows: 2.90-3.16 g P kg^{-1} DM, 29.88-33.29 g K kg^{-1} DM, 8.31-9.25 g Ca kg^{-1} DM, 3.38-3.59 g Mg kg^{-1} DM, and 0.11-0.12 g Na kg^{-1} DM (Table 6). The content of micronutrients changed within the following range: 78.87-90.26 mg Zn kg^{-1} DM, 4.30-4.75 mg Cu kg^{-1} DM, 45.22-51.46 mg Mn kg^{-1} DM, and

Table 5

The content of total protein and crude fiber depending on a Microfert fertilization dose

Cut	Biostimulator dose (dm^3 ha^{-1})		
	Control	1.8	4.5
Total protein (g kg^{-1} DM)			
I	124.2*b*	131.4*ab*	133.5*a*
II	131.0*b*	138.3*ab*	147.6*a*
III	142.1*b*	147.6*b*	157.7*a*
Mean	132.4	139.1	146.3
Crude fiber (g kg^{-1} DM)			
I	28.8*a*	28.4*a*	27.6*a*
II	25.7*a*	25.2*a*	24.8*a*
III	27.4*a*	27.0*a*	26.6*a*
Mean	27.3	26.9	26.3

a, *b* – values in rows marked with different letters differ significantly ($P \leq 0.05$) according to the Duncan's multiple range test.

Table 6

Average weighted content of macro- and micronutrients in meadow plants depending on a Microfert dose

Element	Biostimulator dose (dm^3 ha^{-1})		
	control	1.8	4.5
Macronutrients (g kg^{-1} DM)			
P	2.90*b*	3.04*ab*	3.16*a*
K	29.88*b*	31.74*ab*	33.29*a*
Ca	8.31*b*	8.50*b*	9.25*a*
Mg	3.38*b*	3.54*ab*	3.59*a*
Na	0.11*a*	0.11*a*	0.12*a*
Micronutrients (mg kg^{-1} DM)			
Zn	78.87*b*	87.47*ab*	90.26*a*
Cu	4.30*b*	4.63*ab*	4.75*a*
Mn	45.22*b*	47.31*b*	51.46*a*
Fe	98.51*b*	101.34*b*	106.05*a*

a, *b* – values in rows marked with different letters differ significantly ($P \leq 0.05$) according to the Duncan's multiple range test.

98.51-106.05 mg Fe kg^{-1} DM. The results presented in Table 6 indicate considerable differences in the content of macro- and micronutrients in the investigated meadow plants. Our analysis confirmed that, greater amounts of all nutrients except sodium were found in the plants fertilized with the higher dose of amino acids (4.5 dm^3 ha^{-1}) as compared with the control plants.

DISCUSSION

Preparations containing amino acids as biostimulators are mainly used in cultivation of fruit and vegetables. Research demonstrated positive influence of these biostimulators on plant yield and growth and confirmed their ability to alleviate the negative effects of environmental stress (El-Zohiri, Asfour 2009, Sadak et al. 2015, Kandil et al. 2016). The results of our study confirmed the usefulness of this method for meadow plant fertilization.

The main reason why amino acid fertilization is so effective is that these components are sources of nitrogen easily available to plants (Thon et al. 1981). Moreover, it is also absorbed more quickly than inorganic nitrogen. The effectiveness of this fertilization may be also explained by the fact that some amino acids, e.g. phenylalanine or ornithine, may stimulate biosynthesis of gibberellins (Shekari, Javanmardi 2017). It is worth pointing out that amino acids play a crucial role in plant metabolism and assimilation of proteins, which are among the most essential factors promoting proper cell formation. The effect of undisturbed physiological processes is an increase in fresh and dry matter.

Greater density of the meadow plants reported in our study for the plots fertilized with amino acids was consistent with the report by Kandil et al. (2016). These authors applied amino acids in the form of a foliar spray on wheat (*Triticum aestivum* L.) and found that this procedure yielded the highest number of spikes/m^2.

The higher yield obtained in amino acid fertilized variants in our experiment corresponded to the results observed in other crops such as soybean or potatoes (Saeed et al. 2005, El-Zohiri, Asfour 2009). The cited studies demonstrated that application of an amino acid preparation improved shoot growth, fresh weight and yield. Foliar fertilization with amino acids resulted in a greater number of leaves, branches, plant height and fresh and dry matter of fenugreek (*Trigonella foenum-graecum* L.) (Tarraf et al. 2015). Similar findings were reported by Gamal El-Din and Abd El-Wahed (2005) in chamomile. They noticed that some amino acids significantly enhanced the plant height, number of branches and flower heads and fresh and dry plant weight.

According to Kandil et al. (2016), amino acids play an important role in plant metabolism and assimilation of proteins necessary for the formation of new cells, and therefore they may increase fresh and dry matter yield. Moreover, amino acid application positively affects the content of chlorophyll and its constituents in plants (Shehata et al. 2011). It also has a beneficial influence on plant chemical composition. Studies conducted in such plants as lettuce, cabbage, onion, bok choy and other leafy crops showed that this fertilization resulted in partial reduction of nitrates (NO_3) in leaves and an increase in total nitrogen content (Gunes et al. 1994, 1996, Chen, Gao 2002, Wang et al. 2007).

The higher content of total protein in meadow plants fertilized using the amino acid preparation found in our study corresponded to the results obtained by Zewail (2014). The author found that the content of crude protein in common bean (*Phaseolus vulgaris* L.) leaves rose parallel to the increasing doses of amino acids. Similar results were obtained by Pooryousef and Alizadeh (2014). In their study, the Aminol-forte fertilizer raised the crude protein content from 17% in control to 22%.

Our study indicated that the use of an amino acid preparation improved the content of phosphorus and potassium in meadow plants. Similar relationships were observed by Abo Sedera et al. (2010). Those authors fertilized strawberries with an amino acid fertilizer at doses of 0.5 and 1.0 g l^{-1}, and consequently reported a significant rise in nitrogen, phosphorus and potassium content in the plant leaves.

Contrary to the above, Shehata et al. (2011), who compared different concentrations of an amino acid preparation, found no differences in the potassium content between the control and experimental variants.

Yunsheng et al. (2015 *a*,*b*) found that the highest percentage of P and K in snap bean was achieved following the spraying of plants with 50 ppm of Asparagine and Glutamine. Sadak et al. (2015) evaluated the effect of amino acid foliar spraying on faba bean growing under seawater salt stress. The results showed that the amino acid spraying significantly improved K_+: Na^+ ratio in leaf tissues. Moreover, an increase in the N, P, K, Mg and Ca content in the leaves was independent of salinity level. Those authors claimed that amino acids promoted K^+ increase and Na^+ reduction and thus improved plant tolerance to salinity.

Amino acids are capable of chelating micronutrients (Dromantienė et al. 2013). These components may improve absorption of various elements in the roots and their translocation and accumulation in the leaves (Hammad 2008). These relationships are consistent with the results of our study that confirmed a higher content of the investigated micronutrients in amino acid fertilized plants than in control ones. Garcia et al. (2011) reported beneficial effects of amino acid fertilization on the mineral composition of tomato leaves, as these biostimulators enhanced the leaf content of Ca^{2+}, K^+, Mg^{2+}, Fe, Cu, and Mn.

CONCLUSIONS

The results of the study showed that foliar fertilization with Microfert containing amino acids in meadow plants at the higher dose (4.5 dm^3 ha^{-1}) significantly ($p \leq 0.05$) improved the plant height, which was manifested by a higher dry matter yield. It was shown that plants fertilized with Microfert absorbed more phosphorus, potassium, magnesium and calcium. Moreover, the use of this preparation at the higher dose affected the plant's uptake of zinc, copper, manganese and iron. To conclude, the addition of amino acids with Microfert improved the development of aerial plant organ weight, plant yield and the use of nutrients.

The research suggests that our knowledge about the quality of meadow plants fertilized with amino acids needs expanding. Taking into account the effect of amino acid fertilizers in plants, it would be interesting to evaluate other parameters in future research, e.g. the LAI – Leaf Area Index or leaf chlorophyll content (SPAD, Minolta).

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