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ORIGINAL PAPER

SYNERGISTIC EFFECTS OF FOLIAR APPLICATION OF AMINO ACIDS AND SILICON ON THE CONTENT OF MICRO- AND MACROELEMENTS IN PHYTOMASS OF GRASSLAND*

Adam Radkowski[1], Iwona Radkowska[2], Jan Bocianowski[3], Adam Florkiewicz[4]

[1] **Department of Agroecology and Crop Production**
University of Agriculture in Kraków, Kraków, Poland
[2] **Department of Cattle Breeding**
National Research Institute of Animal Production, Balice, Poland
[3] **Department of Mathematical and Statistical Methods**
Poznań University of Life Sciences, Poznań, Poland
[4] **Department of Food Analysis and Quality Assessment**
University of Agriculture in Kraków, Kraków, Poland

Abstract

The experiment involved two foliar preparations of biostimulating properties containing silicon and amino acids. The aim of the study was to asses a synergistic effect of foliar application of amino acids and silicon on the content of micro- and macroelements in phytomass of grassland. It was conducted in the years 2017-2019, at an Experimental Station in Prusy near Kraków belonging to the Department of Agroecology and Crop Production, University of Agriculture in Kraków. The field experiment in a randomized block design was repeated four times and the area of the experimental plots was 10 m^2. The plots were covered with loess-based degraded chernozem of class I in the Polish soil classification system. The experimental variants involved separate treatments with either silicon preparation at a dose of 0.8 dm^3 ha^{-1} or amino acid preparation at a dose of 2.0 dm^3 ha^{-1}, and both preparations together at the mentioned doses. Foliar fertilization with the amino acid preparation and combined fertilization with silicon and amino acids significantly ($p \leq 0.05$) increased the content of the investigated macroelements. The difference was the greatest in the first year of the study. For potassium it reached 19.5% in the plants from the first cut treated with a combination of silicon and amino acids. The com-

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Adam Radkowski, Asst. Prof., Department of Agroecology and Crop Production, University of Agriculture in Kraków, Mickiewicza 21, 31-120 Kraków, adam.radkowski@urk.edu.pl ORCID: 0000-0002-3146-6212

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bination enhanced also the content of zinc, copper, manganese and iron. In summary, joint application of silicon and amino acids positively influenced the concentration of minerals in phytomass of grassland. In such cases fertilization is necessary to improve mineral levels in phytomass of grassland.

Keywords: left-handed amino acids, meadow sward, content of microelements, macroelements.

INTRODUCTION

The geographical location of Poland influences climatic factors and makes the success of plant cultivation largely dependent on water conditions. Unfortunately, for several years now, a steadily increasing precipitation deficit and related droughts have been observed. Droughts, unfavorable distribution of precipitation during the growing season and high temperatures have a negative impact on plant metabolism, yield and chemical composition. Changes in the soil water content cause oxidative stress in plants because the accumulation of reactive oxygen species causes damage to plant cells at the level of chemical compounds (Demidchik 2015). During the process of adaptation to drought stress, plants have developed various mechanisms~~,~~ including drought avoidance, drought tolerance and drought escapes.

Many water-conservation practices are based on physiological responses of plants to water stress, including the use of plant growth regulators (PGRs) to improve stress tolerance and reallocate plant water use, application of anti-transpirants to reduce evaporative losses, application of osmoregulators to improve osmotic adjustment, and adjustment of fertilizer doses to improve drought tolerance.

One of the methods to countermeasure oxidative stress in plants is the use of stimulators, hormones or other stress relieving substances administered by spraying (Przybysz et al. 2010). Preparations referred to as plant growth regulators or stimulators are used increasingly often (Rademacher 2015). Amino acids have a positive effect on plant growth and productivity, and they significantly reduce abiotic stresses (Koukounaras et al. 2013, Kandil et al. 2016). They are the building blocks of proteins and therefore contribute to the growth and development of plants (Hounsome et al. 2008). In addition, amino acids form enzymes, antioxidants and other secondary compounds. However, plants require a considerable amount of energy to absorb and assimilate nitrogen, as nitrate must be first reduced to nitrite and then ammonium, which is then used in the biosynthesis of amino acids (Hull, Liu 2005). Amino acids are easily absorbed and transported through plant tissues (Alhadi et al. 2012). They are mainly used as growth promoters in vegetable and forage crops, but in recent years various biostimulators and products have been developed for grass fertilization (Schmidt et al. 2003), some of which contain amino acids as primary compounds (Ervin et al. 2009, Radkowski, Radkowska 2018).

Also, silicon exerts a stimulating effect on plant growth and development (Fauteux et al. 2006, Ma, Yamaji 2006). Silicon strengthens cell walls, increases the resistance of plants to fungal diseases and to adverse conditions, including low temperature and water shortage (Ma et al. 2004, Ma, Yamaji 2006). Studies on the influence of silicon on the increase in plant yield have involved mainly vegetables and other crops, and their results confirmed beneficial effects of such fertilization (Janas, Borkowski 2009). A drawback to using this element is the instability of its liquid forms that are soluble in water and can be assimilated by plants, therefore, stable liquid forms for extra-root administration have been developed. There is very little scientific research on the possibility of using growth stimulators in fertilization of grassland. Therefore, the aim of the study was to determine the effect of foliar application of amino acids and silicon, particularly in combination, on the content of micro- and macroelements in meadow sward.

MATERIAL AND METHODS

Study site and soil analysis

A field experiment in a split-block design with four replications was conducted on a temporary meadow in 2017-2019. The plot area was 10 m^2. The meadow lay at the Experimental Station in Prusy (50°07′ N, 20°05′ E), belonging to the Department of Agroecology and Crop Production, University of Agriculture in Kraków. The plot soil was degraded chernozem developed from loess, classified as a very good wheat complex with the textural composition of clay loam. The soil was slightly acidic with pH (pH_{KCl}=6.3), average content of phosphorus (56 mg P kg^{-1} soil), low content of potassium (104 mg K kg^{-1} soil), and very high content of magnesium (143 mg kg^{-1} soil).

Prior to the experiment, the dominant species were perennial ryegrass (*Lolium perenne* L.), meadow fescue (*Festuca pretenses* Huds.) and Kentucky bluegrass (*Poa pratensis* L.) – Table 1. The share of *Fabaceae* plants was 6% and they were represented by red clover (*Trifolium pratense* L.). The experimental meadow also harbored six other species of dicotyledonous plants that accounted for 8% of its flora.

Weather conditions

Annual rainfall in the study period (2017-2019) amounted to 634.8, 809.6 and 728.6 mm, respectively (Table 2). Mean rainfall during the plant growing season (April - September) was 338.6 mm in 2017, 572.2 mm in 2018, and 533.6 mm in 2019. Mean annual temperature in the years 2017–2019 reached 9.6, 9.3 and 10.6°C, and between April and September 16.2, 15.6 and 16.6°C, respectively (Table 2).

Table 1

Floristic composition of the experimental meadow prior to the experiment
(% share in surface coverage)

Species	**Portion (%)**
Grasses	**86**
Perennial ryegrass (*Lolium perenne* L.)	26
Meadow fescue (*Festuca pratensis* Huds.)	18
Kentucky bluegrass (*Poa pratensis* L.)	15
Orchard grass (*Dactylis glomerata* L.)	11
Red fescue (*Festuca rubra* L.)	7
Timothy grass (*Phleum pratense* L.)	5
Rough bluegrass (*Poa trivialis* L.)	4
Fabaceae	**6**
Red clover (*Trifolium pratense* L.)	6
The other dicotyledonous	**8**
English plantain (*Plantago lanceolata* L.)	2
Broadleaf plantain (*Plantago major* L.)	1
Common yarrow (*Achillea millefolium* L.)	1
Common dandelion (*Taraxacum officinale* F. H. Wigg.)	2
Hedge bedstraw (*Galium mollugo* L.)	1
Shepherd's purse (*Capsella bursa pastoris*)	1

Table 2

Rainfall and mean air temperature at the Experimental Station in Prusy,
University of Agriculture in Kraków in the years 2017-2019

Month	Rainfall (mm)				Mean temperature (°C)			
	2017	2018	2019	sum of 2017-2019	2017	2018	2019	average of 2017-2019
January	21.2	9.6	44.2	25.0	-2.1	-4.9	-2.1	-3.0
February	80.6	22.4	12.8	38.6	3.9	0.2	3.1	2.4
March	34.6	43.8	21.4	33.3	4.7	6.4	6.2	5.8
April	58.6	111	76.2	82.1	9.5	7.6	10.3	9.1
May	41.4	83.8	205	110.1	14.5	14	12.4	13.6
June	59.8	45.2	22.4	42.5	18.8	18.8	22.2	19.9
July	92.8	84.4	53.2	76.8	19.6	19.2	19.2	19.3
August	62.0	83.8	88.2	78.0	18.5	20.3	20.5	19.8
September	24.0	164	88.6	92.2	16.3	13.5	14.7	14.8
October	104.4	83.0	36.0	74.5	7.7	9.9	11.3	9.6
November	36.2	48.4	43.0	42.5	3.8	4.4	6.1	4.8
December	19.2	30.2	37.6	29.0	0.3	1.9	3.2	1.8

Material and experimental design

The experiment involved four variants: control (no preparation), spraying with a silicon preparation Optysil® at a dose of 0.8 dm^3 ha^{-1}, spraying with an amino acid preparation in the form of Agro-Sorb® Folium fertilizer at a dose of 2 dm^3 ha^{-1}, and a combination of both preparations at their respective doses of 0.8 and 2 dm^3 ha^{-1} per each regrowth. The doses of the preparations were established based on results described in our previous papers (Radkowski et al. 2020).

Optysil® contains 93.6 g Si per 1 L of the solution, so the actual Si dose was 74.9 g Si ha^{-1}. As per the decision of the Ministry of Agriculture and Rural Development No. S-514/15, this fertilizer is classified as mineral growth stimulator and it is manufactured by Intermag Ltd. company from Olkusz.

The amino acid preparation Agro-Sorb® Folium is a growth stimulator with 18 biologically active, free amino acids (L-alpha) obtained by enzymatic hydrolysis. The preparation features a high concentration of biologically active free amino acids that account for at least 9.3% – a minimum of 100 g in 1000 ml. They include aspartic acid 0.450%, serine 0.321%, glutamic acid 1.814%, glycine 2.743%, histidine 0208%, arginine 0.131%, threonine 0.332%, alanine 0.524%, proline 0.347%, cysteine 0.435%, tyrosine 0.174%, valine 0.551%, methionine 0.349%, lysine 0.661%, isoleucine 0.308%, leucine 0.180%, phenylalanine 0.218%, and tryptophan 0.05% (data confirmed by chemical analysis). Agro-Sorb® Folium, the fertilizer used in the experiment, is manufactured by Biopharmacotech Ltd. Limited Partnership, based in Częstochowa.

The solutions used for spraying were prepared by solving appropriate amount of the preparation in such an amount of water so as to obtain 300 dm^3 ha^{-1} of the working solution. The plots were sprayed once the spring vegetation commenced. 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 cut. Phosphorus was applied once in the spring at 34.9 kg P ha^{-1} in the form of enriched superphosphate 17.4% P, and potassium for the first and second cut at 49.8 kg K_2O ha^{-1} as 49.8% K potassium salt.

The collected plant material was analyzed for its chemical composition and dry weight content (after oven-drying at 105°C).

The content of minerals, i.e. calcium, magnesium, potassium, and sodium, was determined by atomic absorption spectrometry with FAAS atomization (Varian AA240FS Varian Inc., Palo Alto, USA) according to PN-EN 15505:2009 standard, while iron, manganese and zinc according to PN-EN 14084:2004 standard. The content of copper, molybdenum, lead and boron was determined with a validated method of atomic absorption spectrometry with electrothermal atomization, using ET-AAS graphite cuvette (Varian AA240Z Varian Inc., Palo Alto, USA), as per PN-EN 14084: 2004 standard.

Total phosphorus content was established based on UV-VIS spectrophotometry and staining with ammonium monovanadate (V) and ammonium heptomolybdate following the sample mineralization as described in PN-ISO 13730:1999 standard.

Statistical analysis

Normality of the distribution of the content of each element was assessed with the Shapiro-Wilk test. Three-way analysis of variance (ANOVA) was used to verify zero hypotheses on no effects of fertilization, year, cut and interactions fertilization×year, fertilization×cut, year×cut and fertilization×year××cut on the content of each element. Significance of differences between the compared average content of every element was determined by the Fisher's least significant differences (LSDs). Assessment of the relationships between the content of individual elements was based on correlation coefficients. The results of the correlation analysis were plotted as a correlation matrix. Individual hypotheses were tested at a significance level α=0.05. All the analyses were conducted using the GenStat 18 statistical software package.

RESULTS AND DISCUSSION

The factor most strongly differentiating the content of the elements (except for Na) was the year of the study (Table 3). The cut significantly affected the levels of most elements, except for Na and Mo (Table 3).

Table 3

Mean squares from three-way analysis of variance for the investigated chemical elements

Source of variation	Fertilization (F)	Year (Y)	Cut (C)	F × Y	F × C	Y × C	F × Y × C	Residual
d.f.	3	2	1	6	3	2	6	48
P	0.0535**	0.310***	0.540***	0.0009	0.0008	0.003	0.0007	0.008
K	12.76***	22.59***	413.05***	0.532	0.5	1.16	0.199	0.893
Ca	0.549	1.397**	65.91***	0.013	0.077	0.175	0.004	0.26
Mg	0.502	1.565**	4.228***	0.01	0.045	0.034	0.009	0.209
Na	0.003	0.0111	0.0002	0.0002	0.0002	0.011	0.0001	0.004
Cu	2.052**	5.964***	4.019**	0.072	0.075	0.499	0.057	0.389
Zn	86.75**	526.52***	410.69***	1.35	15.86	46.5	2.59	20.06
Mn	4791	40701***	138741***	1830	872	3896	1233	1838
Fe	1045	3542**	6591**	14	178.5	93.7	5.9	552.7
Mo	0.0164**	0.0592***	0.0032	0.0001	0.0052	0.00001	0.00002	0.0034
B	0.709	16.04**	45.98***	0.746	1.88	1.995	0.802	2.76
Pb	0.064	0.367***	1.620***	0.015	0.117*	0.036	0.017	0.037
* $p<0.05$; ** $p<0.01$; *** $p<0.001$; d.f. – number of degrees of freedom								

The study demonstrated significant influence of silicon and amino acid preparations on the chemical composition of phytomass of grassland. Also, ANOVA results indicated significant effects of fertilization on the concentration of P, K, Cu, Zn, and Mo (Table 3). Fertilization with the amino acid preparation and a combination of amino acids with silicon the most strongly affected potassium content in grassland phytomass (Table 4). This element was the most abundant in plants treated both with amino acids and silicon. They contained by 14% more potassium than those harvested from non-fertilized plots (mean for three years of the study). For the variant fertilized with amino acid preparation alone, the difference when compared with control was 7%.

Table 4

Mean content of macroelements (g kg^{-1} DM) in the phytomass of grassland depending on fertilization

Year	Cut	Fertilization	P	K	Ca	Mg	Na
2017	I	control	2.388	17.910	3.250	5.049	0.218
		silicon	2.422	18.600	3.415	5.083	0.229
		aminoacid	2.490	19.300	3.522	5.186	0.236
		silicon+aminoacid	2.490	21.400	3.564	5.489	0.248
	II	control	2.136	13.200	5.254	4.469	0.268
		silicon	2.230	13.930	5.356	4.674	0.281
		aminoacid	2.295	13.980	5.595	4.670	0.277
		silicon+aminoacid	2.328	15.170	5.905	4.724	0.295
2018	I	control	2.146	16.050	3.004	4.513	0.291
		silicon	2.174	16.790	3.160	4.562	0.313
		aminoacid	2.235	17.070	3.138	4.654	0.310
		silicon+aminoacid	2.245	17.780	3.239	4.895	0.325
	II	control	1.990	11.850	4.715	4.011	0.261
		silicon	2.018	12.440	4.807	4.194	0.274
		aminoacid	2.085	12.750	4.924	4.231	0.270
		silicon+aminoacid	2.088	13.270	5.092	4.326	0.283
2019	I	control	2.290	17.170	3.182	4.841	0.273
		silicon	2.322	18.060	3.380	4.874	0.278
		aminoacid	2.378	18.380	3.433	4.972	0.311
		silicon+aminoacid	2.388	19.160	3.520	5.276	0.321
	II	control	2.126	12.660	5.037	4.285	0.281
		silicon	2.149	13.330	5.135	4.481	0.297
		aminoacid	2.209	13.350	5.364	4.677	0.300
		silicon+aminoacid	2.235	14.250	5.585	4.835	0.309
$LSD_{0.05}$			0.151	1.551	0.837	0.750	0.101

The content of copper and zinc in plants sprayed with silicon and amino acids increased in relation to the control by 15% and 14%, respectively (mean for three years of the study).

Relationships between the content of the investigated elements were assessed based on correlation coefficients. Significant positive correlations were found for P and K (0.720), P and Mg (0.499), P and Fe (0.340), P and Mo (0.291), P and Pb (0.534), K and Mg (0.512), K and Fe (0.550), K and Pb (0.656), Ca and Cu (0.436), Ca and Zn (0.466), Ca and Mn (0.570), K and B (0.443), Mg and Cu (0.288), Mg and Fe (0.273), Mg and Pb (0.317), Cu and Zn (0.544), Cu and Mn (0.371), Cu and Mo (0.333), Zn and Mn (0.536), Zn and Fe (0.236), Zn and Mo (0.342), Zn and B (0.344), Mn and Mo (0.499), Mn and B (0. 340), and Fe and Pb (0.630) – Table 5. Significant negative correlations were perceived for P and Ca (-0.409), K and Ca (-0.621), K and Mn (-0.446), K and B (-0.314), Ca and Mg (-0.335), Ca and Fe (-0.252), and Ca and Pb (-0.446) – Table 5.

Beneficial effects of silicon on plants and yield efficiency are partly because of its interactions with such macroelements, such as N, P and K (Singh et al. 2005). The favorable effects of silicon fertilization might be owed to better and more effective osmoregulation, improved water balance, reduction of transpiration-related losses, maintaining proper supply of necessary nutrients, reduced intake of toxic ions and improved performance of antioxidant mechanisms. Ma (2004) and Ma, Yamaji (2006) also reported that silicon improved plant resistance to fungal diseases and adverse environmental conditions, such as low temperature and water shortage. The use of silicon in fertilization of chickpeas, a well-known papilionaceous plant, improved the binding of atmospheric nitrogen by 51% in the plants exposed to water deficit, and by 47% under optimal conditions. Mali, Aery (2008) suggested that positive influence of silicon on nitrogen fixation might be due to a higher content of leghemoglobin – an oxygen fixing hemeprotein present in root nodules of papilionaceous plants.

Experimental studies also confirmed beneficial effects of amino acids on the growth of phytomass of grassland (Ahmed et al. 2011, Kandil et al. 2016, Radkowski, Radkowska 2018, Radkowski et al. 2020). Their high fertilization efficiency stems from the fact that they serve as a source of easily available nitrogen for plant cells, absorbed faster than inorganic nitrogen. Amino acids also play a crucial role in plant metabolism and absorption of proteins, which are one of the most important factors facilitating proper cell formation and eventually increase in fresh and dry matter. Enhanced yield following amino acid treatment was reported in numerous studies (El-Zohiri, Asfour 2009, Ahmed et al. 2011). Amino acids accelerate protein synthesis and increase the content of ascorbic acid, which positively affect plant growth and yield (Alaru et al. 2003, Meijer 2003).

Our study demonstrated that combined fertilization of grassland phytomass with amino acids and silicon improved the content of minerals.

As for animal nutrition standards, high quality feed should contain at least 3.0 g P kg^{-1} DM, 17-20 g K kg^{-1} DM, 7.0 g Ca kg^{-1} DM, 2.0 g Mg kg^{-1} DM, 1.5-2.5 g Na kg^{-1} dry matter (Falkowski et al. 2000).

Table 5

Correlation coefficients between content of macroelements and microelements in the phytomass of grassland

Elements	P	K	Ca	Mg	Na	Cu	Zn	Mn	Fe	Mo	B
K	0.720***										
Ca	-0.409***	-0.621***									
Mg	0.499***	0.512***	-0.335**								
Na	-0.099	-0.107	0	0.045							
Cu	0.12	-0.036	0.436***	0.288*	0.084						
Zn	0.059	-0.115	0.466***	-0.106	-0.011	0.544***					
Mn	-0.061	-0.446***	0.570***	-0.134	-0.009	0.371**	0.536***				
Fe	0.340**	0.550***	-0.252*	0.273*	-0.146	0.138	0.236*	-0.058			
Mo	0.291*	0.029	0.142	0.165	-0.017	0.333**	0.342**	0.499***	0.012		
B	-0.065	-0.314**	0.443***	-0.077	-0.2	0.227	0.344**	0.340**	-0.081	0.021	
Pb	0.534***	0.656***	-0.446***	0.317**	-0.097	0.067	0.142	-0.116	0.630***	0.087	-0.116

* $p<0.05$; ** $p<0.01$; *** $p<0.001$

In our study, the potassium content reached desired values in all variants from the first cut. The levels of phosphorus, calcium and sodium were below optimal values and only magnesium concentration exceeded the expectations. As for microelements, their amounts meeting the demands set out for animal feed in applicable standards are: 50 mg kg^{-1} DM for Zn and Fe, 10 mg kg^{-1} DM for Cu, and 60 mg kg^{-1} DM for Mn (Falkowski et al. 2000).

When assessing the investigated sward against these criteria, we found favorable influence of foliar application of silicon and amino acids on the microelement content (Table 6). Foliar fertilization with these compounds enhanced the content of such microelements as copper, zinc, manganese, iron, and molybdenum. Studies by others (Hellal et al. 2012, Segalin et al. 2013)

Table 6

Mean content of microelements (mg kg^{-1} DM) in the phytomass of grassland depending on fertilization

Year	Cut	Fertilization	Cu	Zn	Mn	Fe	Mo	B	Pb
2017	I	control	5.397	35.250	185.900	119.900	0.481	11.180	1.262
		silicon	5.623	38.070	204.600	143.100	0.505	10.620	1.423
		aminoacid	5.747	37.630	185.200	140.900	0.457	10.380	1.378
		silicon+aminoacid	6.227	40.340	220.400	146.600	0.505	10.300	1.526
	II	control	6.253	40.910	254.000	106.300	0.525	10.960	1.106
		silicon	5.930	39.230	382.300	113.300	0.485	11.680	1.190
		aminoacid	6.812	42.030	293.200	115.700	0.441	11.880	1.106
		silicon+aminoacid	7.040	47.520	323.600	122.300	0.557	12.030	1.170
2018	I	control	4.767	31.300	154.100	99.800	0.400	9.300	1.050
		silicon	5.000	31.670	164.600	119.000	0.420	8.830	1.183
		aminoacid	5.133	32.100	164.700	117.200	0.380	8.630	1.423
		silicon+aminoacid	5.400	35.770	175.300	121.900	0.420	8.570	1.547
	II	control	4.951	34.030	211.300	95.100	0.437	10.500	0.920
		silicon	5.200	32.630	218.000	99.700	0.403	11.100	0.990
		aminoacid	5.256	34.970	230.600	100.600	0.367	11.270	0.920
		silicon+aminoacid	5.627	37.530	252.400	101.200	0.463	8.900	0.817
2019	I	control	4.702	25.000	119.200	98.400	0.395	9.170	1.036
		silicon	5.123	27.900	128.500	117.400	0.414	8.710	1.167
		aminoacid	5.272	27.210	138.700	115.600	0.375	8.520	1.204
		silicon+aminoacid	5.637	28.570	150.800	120.300	0.414	8.450	1.217
	II	control	5.367	33.570	208.400	87.200	0.431	10.360	0.907
		silicon	5.463	32.190	213.700	95.400	0.398	10.950	0.977
		aminoacid	5.667	34.490	223.900	93.800	0.362	11.110	0.907
		silicon+aminoacid	6.133	39.000	234.200	100.000	0.457	11.110	0.806
$LSD_{0.05}$			1.024	7.352	70.390	38.600	0.096	2.727	0.317

confirm increased concentrations of nutrients following fertilization with silicon and amino acids.

Meadow sward examined in our experiment had a low microelement content, probably due to the low share of mineral-rich herbs and legumes (Gaweł, Nędzi 2014). Moreover, the sward flora included species of new cultivars used for overseeding the experimental plots. The new cultivars are more efficient, and their presence could dilute the mineral component concentration (Albayrak, Türk 2013).

In such cases, fertilization is necessary to improve mineral levels in grassland phytomass. Stimulants in the form of amino acid and silicon preparations used in the experiment were helpful and necessary to obtain the highest quality feed.

CONCLUSIONS

1. Combined fertilization with silicon and amino acid preparations considerably affected the potassium content in the phytomass of grassland. Foliar application of these compounds significantly (by 14%) increased mean levels of potassium vs. the non-fertilized variant.

2. Fertilization with a combination of silicon and amino acids improved the content of all macroelements in the investigated meadow sward. As compared with the control variant, their levels rose by 5%, 10%, 9% and 12% for phosphorus, calcium, magnesium, and sodium, respectively.

3. Our study also confirmed the highest concentration of microelements in the plants fertilized with silicon and amino acids. The mean content of microelements in this variant was by 11% higher than in the control.

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Conflict of interest

The authors declare no conflict of interest.

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ORCID:

AR: 0000-0002-3146-6212

IR: 0000-0002-8780-1585

JB: 0000-0002-0102-0084

AF: 0000-0001-7922-3167