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ORIGINAL PAPER

EFFECT OF BIOLOGICAL AND MINERAL FERTILIZERS ON MACRONUTRIENT CONTENT IN SELECTED FORAGE GRASS SPECIES*

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Abstract

A field experiment was carried out at the experimental facility of the University of Natural Sciences and Humanities in Siedlce, Poland. The experiment was established in autumn 2018 and conducted for the next two years. Three biological fertilizers, based on, respectively, humus extract, vermicompost extract and compost extract, constituted the main experimental factor. In the experiment, each fertilizer was used on its own and in combination with mineral NPK fertilizers. The following grass species were used in the experiment: *Dactylis glomerata, Lolium perenne* and *Festulolium braunii*. According to scientific reports, biological fertilizers positively affect forage quality and quantity. Therefore, it was considered appropriate to study their effects further by comparing the content of macronutrients in selected grass species treated with biological fertilizers formed on the basis of beneficial microorganisms. The total content of P, K, Ca and Mg $(g \text{ kg}^{-1})$ in plant dry matter was determined by ICP-AES. Based on the results, the ratios of $K: (Ca+Mg)$ and $Ca:P$ were calculated. The treatment combinations had a various effect on macronutrient accumulation by the grass species. The application of humus extract significantly increased the P content in plant biomass. The application of mineral fertilization had a positive effect on the K:(Ca+Mg) ion ratio and the optimization of Ca:P. The combination of mineral fertilization with vermicompost extract significantly increased the K content in plants and improved the Ca: P ion ratio. The research results indicate that the use of biological fertilizers cannot replace mineral fertilizers. However, the combination of these two types of treatment led to positive results.

Keyword: compost extract, humus extract, vermicompost extract, *Festulolium braunii*, *Dactylis glomerata, Lolium perenne*, phosphorus, potassium, calcium, magnesium.

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INTRODUCTION

The biological value of forage grass is determined on the basis of its nutritional value, in particular macronutrient content (Ciepiela, Godlewska 2017). The chemical composition of permanent grassland plants is very diverse, but good quality forage should contain adequate amounts of macro and microelements with their appropriate percentage combination. Yet some plant macronutrients, i.e. P and K, even if present in sufficient amounts in many soils, are poorly available to plants (Padmanabhan, Shivendra 2009). The literature (Etesami et al. 2017, Adhikari et al. 2021) reports that bacteria solubilise minerals containing K, converting its compounds into forms available to plants. Many species, usually present in all soils, such as *Acidothiobacillus ferrooxidans, Paenibacillus spp., Bacillus mucilaginosus, B. edaphicus* and *B. circulans* and *Pseudomonas* spp., are involved in this process. In addition, Zhang et al. (2020) found that the presence of *Acidobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Nitrospirae* and *Proteobacteria* in soil bacterial communities was positively correlated with the availability of metals, especially Ca and Mg. However, microbial abundance and diversity are not stable, and their potential for solubilising K-bearing minerals depends on soil and climatic conditions (Etesami et al. 2017). That is why, biological fertilizers are used to introduce beneficial microorganisms into the soil, at the same time providing macro- and micronutrients to plants.

The literature argues that biological fertilizers are the most effective when applied in combination with mineral fertilizers. According to Truba et al. (2017), compost and humus extract applied together with mineral fertilizers reduced the content of cellulose and hemicellulose in cell walls of *Dactylis glomerata* and *Lolium perenne*. Furthermore, Truba et al. (2018) found that NPK fertilizers used together with vermicompost, another biological product, contributed to an increase in the content of total protein in forage, and reducing when used with another one, such as humus extract, reduced the level of crude fibre. Additionally, Jankowski et al. (2018) reported a positive effect of the combined application of the compost extract biological fertilizer with NPK fertilizers on soluble carbohydrate content and on the yield.

Thus, according to scientific reports, biological fertilizers positively affect forage quality and quantity. Therefore, it was considered appropriate to study their effects further by comparing the content of macronutrients in selected grass species treated with biological fertilizers formed on the basis of beneficial microorganisms.

MATERIAL AND MATHODS

A field experiment was carried out at the experimental facility of the University of Natural Sciences and Humanities in Siedlce, Poland. With plots of 3 m² arranged in a split-plot design, the experiment was established in autumn 2018 and conducted for the next two years (2019-2020). On the basis of the FAO classification, the soil was determined as a Technosol (Schad et al. 2014), with its pH of 6.6 and the following macronutrient content (g kg⁻¹): C_{org} – 12.30, N_{total} – 1.250, P – 0.790, K – 1.060, Ca – 1.820, $Mg - 1.260$.

Three biological fertilizers, based on, respectively, humus extract, vermicompost extract and compost extract, constituted the main experimental factor. They were approved for organic farming by the Institute of Soil Science and Plant Cultivation in Puławy, and their composition is presented in Table 1. In the experiment, each was used on its own and in combination with mineral NPK fertilizers. Once a year in the spring, the application of biological fertilizers was carried out according to the manufacturers' recommendations at the following doses: compost extract -0.6 l ha⁻¹, vermicompost extract – 15 l ha⁻¹, humus extract – 50 l ha⁻¹. Phosphorus mineral fertilizer

Table 1

Soil conditioner composition based on manufacturers' data

was applied once in the spring $(80 \text{ kg } \text{ha}^1)$, while nitrogen $(150 \text{ kg } \text{ha}^1)$ and potassium (120 kg ha^{-1}) doses were split into three equal parts: the first before the growing season and second and third after the first and second harvests. Sown in autumn 2018, the following grass species were used in the experiment: *Dactylis glomerata* var. Bora, *Lolium perenne* var. Info and *Festulolium braunii* var. Sulino.

Table 2 presents the amount of precipitation and the average air temperature during the two growing periods. Based on meteorological data, Table 2

Year	Month								
	Apr.	May	June	July	Aug.	Sept.	Oct.	means	
Temperature $(^{\circ}C)$									
2019	13.1	17.0	18.3	20.4	20.6	15.9	9.6	16.4	
2020	8.6	11.7	19.3	19.0	20.2	15.5	12.0	15.2	
Means	10.9	14.4	18.8	19.7	20.4	15.7	10.8	15.8	
Multiannual means	8.5	14.0	17.4	19.8	18.9	13.2	7.9	14.2	
Precipitation (mm)									
2019	5.9	59.8	35.9	29.7	49.3	17.4	9.5	29.6	
2020	6.0	63.5	118.5	67.7	18.0	38.8	17.6	47.2	
Means	6.0	61.7	77.2	48.7	33.7	28.1	13.6	38.4	
Multiannual means	33.0	52.0	52.0	65.0	56.0	48.0	28.0	47.7	

Average monthly air temperature (°C) and monthly total precipitation (mm)

optimal hydrothermal conditions were recorded only sporadically. In the years of study, months with optimal weather were followed by periods of excess or shortage of water. In turn, as indicated by meteorological data, in the first and second year of research a shortage of rain was recorded during most of the growing period.

The total content of P, K, Ca and Mg $(g \ kg_1)$ in plant dry matter was determined by ICP-AES. Based on the results, the ratios of K:(Ca+Mg) and Ca:P were calculated. The results of the research were processed statistically using three factor analysis of variance. Verification of the significance of experimental factor impact on tested characteristics was done with the Fisher-Snedecor test, and the Tukey's test was used to evaluate differences between means. The calculations were done with the Statistica 13 Program.

RESULTS AND DISCUSSION

According to Table 3, plants treated with humus extract contained the most P $(3.667 \text{ g kg}^{-1})$, and, noteworthy, the biological fertilizer based on it contained its greatest amount too (Table 1). However, the above value did

Effects of the treatment on P content (g kg^{-1}) over two growing periods

Means in rows marked with the same small letters do not differ significantly.

Means in columns marked with the same capital letters do not differ significantly.

not differ significantly from that recorded on the control plot $(3.494 \text{ g kg}^{-1})$. The average P content was higher in the first year (2019) than in the second one (2020), when it decreased by about 9%.

Among the grass species, *Festulolium braunii* was richest in P (3.542 g kg-1), but the content was 7% lower in *Dactylis glomerata* and 2.0% lower in *Lolium perenne* (Table 3). According to Vervoort et al. (1998), grass species differ in their potential of P uptake from the soil. *Dactylis glomerata* is considered to be one of the species that most intensively absorbs this chemical element. In the present experiment, P content in *Festulolium braunii* was similar across treatments. In the case of *Dactylis glomerata*, compost extract contributed to a slight decrease in the amount of P, while humus extract increased it slightly. Dynamic changes in P content across treatments were noted in the biomass of *Lolium perenne*. It was found that the content of this macronutrient increased significantly after applying humus extract. According to Alburquerque et al. (2007), its overall increase in *Lolium perenne* was observed after the use of compost, but treatments involving nitrogen fertilizer reduced it. In addition, the authors argued that the use of compost could improve the availability of P for plants.

Comparing the K content in the dry matter of grass species across treatment combinations, it was found that the largest amount of the element was on the plots with vermicompost extract applied together with mineral fertilizers (Table 4). This value was significantly higher, by 10.3%, than that on the plot treated with mineral fertilizers only. Of all biological fertilizers, the one with vermicompost extract contains the least K in its composition. However, its mixture of microorganisms probably contributed to the activation of this chemical element in the soil and to its higher uptake by plants. Etesami et al. (2017) point out that many microorganisms convert insoluble

Table 3

Species/years	Fertiliser effect $(g \ kg^{-1})$								
	$\mathbf{0}$	NPK	CE	VE	HE	$CE+NPK$		VE+NPK HE+NPK	Means
Means for species									
Festulolium $\frac{1}{2}$	31.87^{4a}	35.50^{4a}	31.55^{Aa}	32.88^{Aa}	32.15^{4a}	34.62^{Aa}	34.98^{Aa}	31.62^{Aa}	33.15^{4}
Dactylis glomerata	33.62^{Aa}	30.60^{4a}	31.87^{Aa}	34.13^{Aa}	32.17^{Aa}	33.17^{Aa}	33.58^{Aa}	31.73^{Aa}	32.61^{A}
Lolium perenne	29.633^{Ab}	29.35^{Ab}	34.38^{Aab}	33.88 ^{Aab}	31.80^{Aab}	33.50^{Aab}	36.73^{Aa}	35.57^{Aab}	33.11^{A}
Means for growing seasons									
2019	29.37^{Ab}	33.04^{Aab}	32.49 ^{Aab}	34.99^{Aab}	31.784b	32.92^{Aab}	37.39^{4a}	31.60^{Ab}	32.95^{A}
2020	34.04^{Aa}	30.59^{4a}	32.71^{Aa}	32.28^{Aa}	32.30^{4a}	34.60^{4a}	32.81^{Ba}	34.34^{Aa}	32.96 ⁴
Mean	31.71^{ab}	31.82^{b}	32.60^{ab}	33.63^{ab}	32.040^{ab}	33.76^{ab}	35.10^a	32.97^{ab}	

Effects of the treatment on K content $(g \ kg¹)$ over two growing periods.

Means in rows marked with the same small letters do not differ significantly.

Means in columns marked with the same capital letters do not differ significantly.

K into forms available to plants. However, in terms of the average K content, no significant variation in this element was demonstrated across growing seasons (Table 4).

The content of K in the dry matter of plants across treatment combinations, with biological fertilizers used either alone or together with mineral fertilizers, did not differ significantly and ranged from 32.04 g kg⁻¹ to 35.10 g kg⁻¹. Similarly, no significant differences were found between the K content across different grass species.

Ranging from 1.490 to 1.531 g $kg⁻¹$, the average Mg content in plants did not differ significantly. The application of biological fertilizers resulted in comparable Mg content in forage, which indicates that a complex interaction between the product, plant, soil and environment may have occurred (Coelho et al. 2019). The average Mg content in plants (Table 5) was higher in the first year (2019) than in the second one (2020), when it decreased by about 4.7%. In addition, no significant differences in Mg content were noted across treatments.

The highest content of Ca in dry matter (Table 6) was in plants treated with mineral fertilizers only, 24.7% more than in control. Biological fertilizers used alone or in combination with mineral fertilizers did not contribute to a significant increase in the Ca content. Of all fertilizers, only the one with humus extract contains Ca (Table 1) in the amount of 3 mg $kg¹$, but this did not affect the amounts of this chemical element in grass. Like in the case of P and Mg, the average Ca content in the grass species (Table 6) was higher in the first year (2019) than in the second one (2020), when it decreased by about 14.5%. The lower concentration of macronutrients in the second year of research can also be explained by a significant increase in plant biomass as a result of treatment, which may have resulted in the

Species/years	Fertiliser effect $(g \; kg^{-1})$								
	$\overline{0}$	NPK	CE	VE	HЕ	$CE+NPK$		VE+NPK HE+NPK	Means
Means for species									
Festulolium brauni	1.467^{Aa}	1.617^{Aa}	1.433^{Aa}	1.383^{Aa}	1.450^{Aa}	1.550^{Aa}	1.650^{Aa}	1.550^{Aa}	1.513^{4}
Dactylis glomerata	1.650^{Aa}	1.600^{Aa}	1.500^{Aa}	1.483^{Aa}	1.483^{Aa}	1.467^{Aa}	1.483^{Aa}	1.583^{Aa}	1.531^{A}
Lolium perenne	1.400^{Aa}	1.417^{Aa}	1.417^{Aa}	1.567^{Aa}	1.517^{Aa}	1.500^{Aa}	1.550^{Aa}	1.550^{Aa}	1.490 ⁴
Means for growing seasons									
2019	1.589^{4a}	1.600^{4a}	1.511^{Aa}	1.533^{Aa}	1.478^{Aa}	1.467^{Aa}	1.567^{Aa}	1.622^{Aa}	1.546^{4}
2020	1.422^{Aa}	1.489^{4a}	1.389^{Aa}	1.422^{Aa}	1.489^{Aa}	1.544^{Aa}	1.556^{Aa}	1.500^{Aa}	1.476^B
Mean	1.506^{a}	1.544^a	1.450^a	1.478^a	1.483^{a}	1.506^a	1.561^a	1.561^a	

Effects of the treatment on Mg content $(g \ kg_1)$ over two growing periods

Means in rows marked with the same small letters do not differ significantly. Means in columns marked with the same capital letters do not differ significantly.

Table 6

Effects of the treatment on Ca content (g kg-1) over two growing periods.

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Means in columns marked with the same capital letters do not differ significantly.

dilution effect. This decrease may also have been due to the progressive uptake of macronutrients by plants, thus lowering their amounts in the soil (Alburquerque et al. 2007).

According to studies by Tilvikiene et al. (2018), the Ca content in *Dactylis glomerata* treated with organic fertilizer was lower than when it was treated with mineral fertilizer. In the present experiment, the species of *Lolium perenne* had its highest content in dry matter (6.05 g kg⁻¹), on average,

Table 5

23.3% higher than in *Dactylis glomerata* and 7.0% higher than in *Festulolium braunii*.

A synthetic criterion for assessing forage quality is the ratio of $K:(Ca+Mg)$, which should range from 1.800 to 2.200 (Grzegorczyk et al. 2013). Among all treatment combinations, the most favourable K: $(Ca+Mg)$ ratio (4.109) was recorded in plants treated with mineral fertilizers only (Table 7). The least

Table 7

The K:(Ca+Mg) ratio over two growing periods

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Means in columns marked with the same capital letters do not differ significantly.

favourable ones were noted after the application of compost extract on its own (5.161) and its combination with mineral fertilizers (5.128). However, the K:(Ca+Mg) ratio was not satisfactory for any treatment combination. This was due to the low content of Mg in the forage and the high content of K, which increased the ratio value. In the first year (2019), the ratio of K:(Ca+Mg) for all treatment combinations was similar (Table 7). In the following year (2020), months with water scarcity accounted for a smaller part of the growing season. This may have had an effect on the availability for plants of Ca and Mg in the soil, which increased the $K:(Ca+Mg)$ ratio, especially for plots with compost extract.

Of all species, the most favourable ratio was in *Lolium perenne* (4.700), while in the forage of *Dactylis glomerata* it was higher (5.300). However, the ratio of K:(Ca+Mg) was too wide in all grass species, which was due to the low content of Ca and Mg and the high content of K. Studying the effect of a biological fertilizer on hybrid alfalfa, Sosnowski et al. (2014) recorded the average ratio value of 0.910 - 1.060.

According to the National Research Council (1996), the Ca:P ratio in forage should range from 1.000 to 2.000. Table 8 shows that the ratio in all plots was close within the above standards, ranging from 1.129 to 1.923. Yet

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The Ca:P ratio over two growing periods

Table 8

Means in rows marked with the same small letters do not differ significantly.

Means in columns marked with the same capital letters do not differ significantly.

the optimal Ca:P ratio in terms of forage quality was in forage from plots with vermicompost extract used in combination with mineral fertilizers and from those treated with NPK fertilizers only (1.795-1.797). A low value of the Ca:P ratio was recorded in control plants and in those treated with compost extract in combination with NPK fertilizers.

In the first year of research, the ratio of Ca to P turned out to be narrow in control plants and in those treated with humus extract, compost extract on its own and compost extract in combination with NPK fertilizers. Unlike the others, the hums extract fertilizer contains both P and Ca in its composition, which in the plant-soil system might have caused the narrowing of the ratio. The Ca:P ratio in forage from the other plots was satisfactory, ranging from 1.691 to 1.838.

In the second year, the Ca:P ratio varied significantly across treatments and ranged from 1.324 to 1.923. The narrowest ratio was in grass treated with compost extract applied together with mineral fertilizers (1.324). Forage from the remaining plots was within the standards recommended by the National Research Council (1996). The high ratio variation in the second year could have been caused by hydrothermal conditions. Of all species, the most favourable Ca: P ratio was in *Lolium perenne*, with 1.8 as the average of all treatments, while in others it was narrower.

According to Grzegorczyk et al. (2017), Ca and P levels in the diet of animals should be balanced to increase their intake. Ciepiela and Godlewska (2017) also investigated effects of a biostimulant on the ratio of Ca and P ions in grass forage. The authors found that it was 2.64 on average for *Dactylis glomerata*, and 2.78 for *Festulolium braunii*. In turn, Grzegorczyk et al. (2016) found that the Ca:P ratio in herbs and legumes,

ranging from 3.9 to 7.8, significantly exceeded recommended norms. Finally, Ayan et al. (2010) emphasize that the Ca:P ratio above 2.0 in the diet of animals adversely affects their health.

CONCLUSIONS

1. The application of humus extract significantly increased the P content in plant biomass. The application of mineral fertilization had a positive effect on the K:(Ca+Mg) ion ratio and the optimization of Ca:P. The combination of mineral fertilization with vermicompost extract significantly increased the K content in plants and improved the Ca: P ion ratio.

2. Of all grass species *Lolium perenne* contained the most favourable content of macronutrients and the highest ratios.

3. The research results indicate that the use of biological fertilizers cannot replace mineral fertilizers. However, the combination of these two types of treatment led to positive results. In view of the above, it is advisable to continue research on the effects of biological fertilizers on forage grasses.

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