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INFLUENCE OF FERTILIZATION WITH LIQUID ORGANIC FERTILIZER ON THE PRODUCTIVITY AND BOTANICAL COMPOSITION OF NATURAL *Agropyron repens* GRASSLAND

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Abstract

The aim of this study was to establish the influence of organic leaf fertilization on the productivity and botanical composition of natural grassland Agropyron repens type in the region of South-Central Bulgaria (305 m altitude). The experiment was carried out with leaf organic fertilizer Naturamin Plus, during the period 2018-2019. The trial was designed by the block method in 4 repetitions and 3 doses of fertilizer were tested: 1,500; 2,500 and 3,500 ml.ha^{-1}. Results obtained for the yield were statistically processed by ANOVA. The use of the Naturamin Plus in natural grassland Agropyron repens type has been found to have a positive effect on productivity regardless of weather conditions over the years. Average for the period of investigation, more green and dry mass were obtained by treatment with a dose of 2,500 ml.ha^{-1} - respectively 31.6% and 30.7% more compared to the control. In grassland with predominant species Agropyron repens (L.) P. Beauv., the largest share is occupied by perennial cereal grasses, while legumes have a small share. Fertilization with liquid organic fertilizer Naturamin Plus increases the participation of perennial cereal and legume grasses and reduces that of weeds.

Key words*: botanical composition, fertilization, natural grassland, productivity.*

INTRODUCTION

Nowadays, natural grasslands are accepted not only as an enormous natural resource, which allows for natural and low cost raising of ruminant animals but they also have valuable ecologic functions: protect the soil from water and wind erosion, protect groundwater from contamination, decrease the effect of greenhouse gasses by absorbing part of CO_2 in the process of photosynthesis, conserve the biodiversity (Călina & Călina, 2015; Kirilov & Mihovski, 2014; Pavlov, 2005; Pavlov & Mihovsky, 2007; Štýbnarová et al., 2012; Trofimov et al., 2014). For that reason, in the attempts to stabilize the natural grassland yields, contemporary science develops new approaches which include the use of treatments identical to natural, in order to improve the provision of plants with nutrients and their resistance to stress influences in the environment. Fertilization is one of the main agro-technical measures, contributing to the increase of productivity and nutritional value of forage obtained from grasslands. (Avarvarei & Chelariu, 2011; Kharkevich et al., 2015; Kozhouharov & Lingorski, 2011; Păcurar et al., 2012; Štýbnarová et al., 2012).

An important factor, influencing the process of optimization of the grasslands fertilization, is the type of fertilizer and the application method. On the most widely distributed natural grasslands in Central Balkan mountains - a natural meadow (*Chrysopogon gryllus* L. Trin. type) and a natural pasture (*Nardus stricta* L. type) Iliev et al. (2020) tested foliar fertilization with the organic humic fertilizer Biostim (BG). It was established that this method of fertilization was more effective, compared to variable mineral fertilization and had a positive effect on the bioproductive characteristics of both grassland types. The application of foliar fertilizers and biostimulators was an effective method to correct the shortage of nutrients and had a positive effect on the resistance and adaptive mechanisms of plants to outside influences, the grassland density, plant height, productiveness and nutritional value of the obtained forage (Ivanova & Zavarukhina, 2015; Xu & Huang, 2010).

In order to decrease the negative impact on the environment Chourkova (2011; 2013), Churkova & Bozhanska (2016), Bozhanska et al. (2017a; 2017b), Petrova, (2017), Pigareva & Zhugdurov (2013), Wolski et al. (2019) used

liquid biofertilizers to optimize the feed rate of grass species. It was established the effect of application of different treatments, doses and types of biofertilizers and growth regulators in the production of birdsfoot trefoil (*Lotus corniculatus* L.), red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), sainfoin (*Onobrychis viciifolia* Scop.), perennial cereal grasses and legume grass mixtures, natural grasslands. The obtained results determined biofertilizers and growth regulators as an alternative for reducing mineral fertilization and obtaining ecologically clean forage production, and according to the authors, its effectiveness is influenced by the biological characteristics of the grass species and the weather conditions.

The aim of the present study was to determine the influence of the liquid organic fertilizer Naturamin Plus (Daymsa, ES) on the productiveness, botanical composition, and agronomic efficiency of fertilization in natural grassland *Agropyron repens* (L.) P. Beauv. type in the ecological conditions of South-Central Bulgaria.

MATERIALS AND METHODS

The study was conducted in two vegetation seasons in the period 2018–2019 on natural grassland *Agropyron repens* (L.) P. Beauv. type, located in the area of village Yagoda, Stara Zagora region, 305 m altitude. The trial was designed by the block method in 4 repetitions and the size of the experimental plot was 10 m^2. The soils in the area are Gleic Chromic Luvisols, with low humus content (3.42-3.93%), low acidity, low available nitrogen (31.3-38.1 $mg.kg^{-1}$ soil) and phosphorus content (3.1-4.3 $mg.100\ g^{-1}$ soil) but with high available potassium content (42-44 $mg.100\ g^{-1}$ soil).

Tested was the influence of the combined fertilizer Naturamin Plus, which has the following composition: total 400 $g.l^{-1}$ amino acids, free amino acids - 200 $g.l^{-1}$, Nitrogen (N) - 75 $g.l^{-1}$, Iron (Fe) - 12 $g.l^{-1}$, Manganese (Mn) - 7.5 $g.l^{-1}$; Boron (B) - 1.3 $g.l^{-1}$, Copper (Cu) - 1.2 $g.l^{-1}$, Molybdenum (Mo) - 0.5 $g.l^{-1}$, Zinc (Zn) - 2.5 $g.l^{-1}$. The preparation is certified according to European Council Regulation (EC) No 834/2007 for use in organic production. The following doses were used: 1,500, 2,500 and 3,500 $ml.ha^{-1}$.

Fertilization with the liquid organic fertilizer Naturamin Plus was made a single time during grassland vegetation in each of the trial years at the time of growing (height 10-15 cm) of the grassland. A small sprayer pump with fine dispersion nozzle with 300 $l.ha^{-1}$ spraying solution was used for spraying. The experimental plots were harvested via mowing in the spike growth phase of the dominant grass species *Agropyron repens* (L.) P. Beauv. During the years of the experiment the plots were mowed twice.

The following indicators were evaluated: green mass and dry matter yield; botanical composition of the grassland (%) was determined via weight analysis of fresh grass samples taken immediately before mowing; the share of the main botanic groups (perennial cereal grasses, legume grasses and weeds); agronomic efficiency of fertilization (AE), partial fertilization productivity (PFP).

Agronomic efficiency of fertilization was calculated the following way: ratio of the difference between yield with fertilization (YF) and yield without fertilization (YF_0) divided by the fertilization rate (F).

Formula for agronomic efficiency of fertilization: $(AE) = (YF - YF_0)/F$;

Partial fertilization productivity (PFP) was calculated the following way: ratio of the yield (Y) to the applied fertilizer (F).

Formula for partial fertilization productivity: $(PFP) = Y/F$.

Data processing was performed by a two-way dispersion analysis (Lidanski, 1988), using MS Excel software - 2010.

The results for the yield were statistically processed with ANOVA LSD test for statistical significance of the differences, standard deviation and coefficient of variation (Lidanski, 1988), using MS Excel software - 2010.

RESULTS AND DISCUSSIONS

Weather conditions during the period of the study are shown on Table 1. Water is one of the main factors determining the growth and development of grassland. Regarding rainfall, the years of the study are characterized as moderately favorable. The multiannual (1936-2017) average rainfall during the vegetation period (April-September) was 299 mm. In

2018, the quantity of rainfall during active grassland vegetation was with 10.5 mm above normal, and in 2019 with 14.7 mm above normal. This had a favorable influence on the complex biochemical processes related with the growth and development of the plants and helped for the formation of two undergrowth.

The average of annual air temperature calculated for the multiannual period (1936-2017) was 12.5ºC. Due to the warm winter and relatively small altitude, spring arrives comparatively early in the region. Already in the first ten days of March (4-7 March) the temperatures permanently stay above 5ºC, and as a result, the active vegetation of the grassland begins. The air temperature during the active vegetation (April-September) on average for the multiannual period (1936-2017) was 19.4ºC. The temperature during vegetation in the trial period (2018-2019) was with 1.0-1.5ºC higher than the multiannual average in both years of the study.

Table 1. Climate conditions of South-Central Bulgaria

Years	Months													
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	I-XII	IV-IX
Total rainfall, mm														
2018	19.0	115.0	89.9	2.9	99.3	85.2	75.2	27.0	19.9	40.8	30.8	21.6	626.6	309.5
2019	39.9	15.3	5.3	57.9	63.5	108.7	52.0	11.0	20.6	14.2	11.7	4.0	404.1	313.4
1936-2017	41.7	35.8	37.2	45.1	62.8	61.7	51.2	43.5	34.7	42.7	48.9	47.8	553.1	299.0
Average temperature, ^{0}C														
2018	2.4	3.6	6.7	15.7	19.1	21.8	23.7	25.1	19.7	14.4	7.8	2.0	13.5	20.9
2019	2.3	4.5	8.9	11.6	17.4	23.4	23.9	25.2	20.6	14.2	11.7	4.0	14.0	20.4
1936-2017	1.0	2.8	6.6	12.0	17.1	21.2	23.7	23.3	19.0	13.0	7.4	2.7	12.5	19.4

Productivity is the most important and synthesizing indicator for the application of a particular technology. During the study, the yield of green mass, from natural grassland *Agropyron repens* (L.) P. Beauv. type, varied based on the treatment with the liquid organic fertilizer Naturamin Plus. As shown on Table 2, for the two years period the annual average yield varied from 11,643.8 to 15,706.3 kg.ha^{-1}.

Table 2. Green mass yield by the years and average for the period 2018–2019 (kg.ha^{-1})

Variant	Dose	2018		2019		Average	
	ml.ha^{-1}	kg.ha^{-1}	%	kg.ha^{-1}	%	kg.ha^{-1}	%
Control (untreated)		10,191.7	100.0	13,812.5	100.0	12,002.1	100.0
Naturamin Plus	1,500	11,275.0a	110.6	16,487.5*ab	119.4	13,881.3a	115.7
Naturamin Plus	2,500	13,608.3**b	133.5	17,975.0**b	130.1	15,791.7**b	131.6
Naturamin Plus	3,500	11,500.0*a	112.8	14,550.0a	105.3	13,025.0a	108.5
Average		11,643.8	114.2	15,706.3	113.7	13,675.0	113.9
LSD.P< 0.05		1,301.7	11.2	2,464.7	17.8	1,883.2	15.7
LSD.P< 0.01		1,870.3	16.1	3,541.2	25.6	2,705.7	22.5
LSD.P< 0.001		2,751.3	23.6	5,209.4	37.7	3,980.4	33.2
SD						2,809.03	
CV						20.54	
SE						496.57	
Min						9,850	
Max						21,500	

*Different letters indicate statistically significant differences among variants at P < 0.05

*, **, *** – Statistically significant differences of the variants and control at P< 0.05; 0.01 and 0.001, respectively

Annually as well as average for the studied period, the lowest yield was obtained in the untreated control. The highest, statistically well-proven green mass yield (P<0.01) average for the period of the study was obtained in the variant treated with dose 2,500 ml.ha^{-1}. In the different years of the study, the green mass yield from this variant surpassed the control with 30.1% (4,162.5 kg.ha^{-1}) to 33.5% (3,416.6 kg.ha^{-1}).

Treatment with the dose 1,500 ml.ha^{-1} also had a positive influence: the obtained green mass yield was on average with 15.7% (1,879.2 kg.ha^{-1}) higher than in the untreated control. However, this was statistically proven (P<0.05) only in 2019.

In treatment of the grassland with leaf fertilizer Naturamin Plus in dose 3,500 ml.ha^{-1} both by years, as well as average for the studied period were obtained positive differences in the green mass yield, compared to the basic variant, but they were not proven statistically.

Dry matter yield (Table 3) from natural grassland *Agropyron repens* (L.) P. Beauv. type based on leaf fertilization with the liquid organic fertilizer Naturamin Plus varied from 2,491.1 to 3,149.7 kg.ha^{-1} in the different years of the study, and it was higher in 2019 when the combination of the climate factors - rainfall and temperatures – was more favorable for the development of the grassland. The lack of rainfall in April 2018, combined with the air temperatures, which were 3.7°C above usual for the month, had a negative impact on the biomass productivity of first undergrowth and consequently on the annual green mass and dry matter yield for 2018.

Highest dry matter yield was obtained from fertilization with Naturamin Plus in dose 2,500 ml.ha^{-1}. The yield was on average with 30.7% (760 kg.ha^{-1}) higher than the untreated control. The obtained differences are well proven statistically (P<0.001) for the years of the study.

Fertilization of the natural grassland with Naturamin Plus in dose 1,500 ml.ha^{-1} contributed to obtain higher dry matter yield but the statistical significance of the difference compared to the untreated variant was low (P<0.05).

Similarly to the results obtained for green mass, in treatment with the highest dose (3,500 ml.ha^{-1}) of the organic fertilizer Naturamin Plus, the effect on the dry matter yield was not statistically proven.

Table 3. Dry matter yield by the years and average for the period 2018-2019 (kg.ha^{-1})

Variant	Dose	2018		2019		Average	
	ml.ha^{-1}	kg.ha^{-1}	%	kg.ha^{-1}	%	kg.ha^{-1}	%
Control (untreated)		2,257.8	100.0	2,685.2	100.0	2,471.5	100.0
Naturamin Plus	1,500	2,520.0*a	111.6	3,245.5*ab	120.9	2,882.7*a	116.6
Naturamin Plus	2,500	2,840.8***b	125.8	3,622.2***b	134.9	3,231.5***b	130.7
Naturamin Plus	3,500	2,345.8a	103.9	3,046.1a	113.4	2,695.9a	109.1
Average		2,491.1	110.3	3,149.7	117.3	2,820.4	114.1
LSD.P< 0.05		252.2	11.2	419.4	15.6	335.8	13.6
LSD.P< 0.01		362.2	16.0	602.6	22.4	482.4	19.5
LSD.P< 0.001		532.8	23.6	886.5	33.0	709.7	28.7
SD						486.14	
CV						17.24	
SE						89.94	
Min						2,207.42	
Max						4,075.6	

*Different letters indicate statistically significant differences among variants at P < 0.05

*, **, *** – Statistically significant differences of the variants and control at P< 0.05; 0.01 and 0.001, respectively

Leaf fertilization with Naturamin Plus has a significant influence on the botanical composition of grassland.

Perennial cereal grasses participate on average with 49.54% in the first year (Table 4), while their participation in grassland in the second year of the study increased with 15.86% and reached up to 65.44% from the grassland.

Average for the period of the study the participation of perennial cereal grasses in the grassland was 57.49%.

In second place in the composition of the grassland was the participation of weeds, where was observed the opposite tendency - decrease of their participation from the first year to the second year of the study.

Table 4. Botanical composition of the natural grassland *Agropyron repens* type, treated with leaf fertilizer Naturamin Plus, by the years and average for the period 2018-2019 (%)

Variant	Dose ml.ha^{-1}	Groups	2018	2019	Average
Control (untreated)		Perennial cereal grasses	38.06	58.58	48.32
		Legumes	4.75	0.28	2.51
		Weeds	57.19	41.14	49.17
Naturamin Plus	1,500	Perennial cereal grasses	58.49	72.69	65.59
		Legumes	3.88	1.18	2.53
		Weeds	37.63	26.13	31.88
Naturamin Plus	2,500	Perennial cereal grasses	53.76	69.04	61.40
		Legumes	9.44	3.59	6.52
		Weeds	36.79	27.37	32.08
Naturamin Plus	3,500	Perennial cereal grasses	47.87	61.46	54.66
		Legumes	9.93	3.53	6.73
		Weeds	42.20	35.02	38.61
Average		Perennial cereal grasses	49.54	65.44	57.49
		Legumes	7.00	2.14	4.57
		Weeds	43.45	32.42	34.99

They occupied on average 43.45% in the first year and decreased to 32.42% in the second year.

Perennial cereal grasses had the highest participation share after treatment with Naturamin Plus in doses 1,500 and 2,500 ml.ha^{-1}. While weeds decreased the share of their participation after treatment with dose 1,500 ml.ha^{-1} Naturamin Plus. These results demonstrate the positive influence of Naturamin Plus for leaf fertilization.

Legumes had the smallest share in the studied grassland - average for the period of the study 4.57%, while their participation varied from 2.14% to 7.00%. The share of legumes in the obtained forage mass was bigger in the first year of the study. Each year of the study, and as average for the period, was observed that with the increase of the share of perennial cereal grasses, was increased the participation of beneficial legume plants in the grassland.

According to the obtained results, legume grasses had the smallest share in the biomass received from the untreated control and in treatment with the low dose of the biofertilizer Naturamin Plus (1,500 ml.ha^{-1}).

Their share increased with the increase of the applied dose of the product Naturamin Plus. Leaf application of the preparation Naturamin Plus had the strongest positive influence on the share of legumes in the first year of the study.

Agronomic efficiency of fertilization characterizes the abilities of plants to increase their yield as a result of the applied fertilization.

Highest values for AE were received after application of 2,500 ml.ha^{-1} dose fertilizer - with 21% more for green mass and 11% more for dry matter - compared to the lower fertilization dose (Table 5).

Table 5. Agronomic efficiency and partial fertilization productivity of fertilization with Naturamin plus of the natural grassland *Agropyron repens* type (kg.ml^{-1})

Variant	Dose ml.ha^{-1}	Agronomic efficiency			Partial fertilization productivity		
		2018	2019	Average	2018	2019	Average
			Green mass				
Naturamin plus	1,500	722.22	1,783.33	1,252.78	7,516.67	10,991.67	9,254.17
Naturamin plus	2,500	1,366.67	1,665.00	1,515.83	5,443.33	7,190.00	6,316.67
Naturamin plus	3,500	373.81	210.71	292.26	3,285.71	4,157.14	3,721.43
Average		820.90	1,219.68	1,020.29	5,415.24	7,446.27	6,430.75
			Dry matter				
Naturamin plus	1,500	174.78	373.52	274.15	1,680.0	2,163.7	1,921.8
Naturamin plus	2,500	233.21	374.78	303.99	1,136.3	1,448.9	1,292.6
Naturamin plus	3,500	25.13	103.11	64.12	670.2	870.3	770.3
Average		144.37	283.80	214.09	1,162.2	1,494.3	1,328.2

Agronomic efficiency frequently lowers with the increase of the applied fertilization rate (Li et al., 2020). The results obtained in this study correspond with this and show that a high rate of fertilization (3,500 ml.ha^{-1}) decreases AE with 77% compared to the lowest one (1,500 ml.ha^{-1}). Agronomic efficiency of fertilization showed the same tendency for the green mass yield and for the dry matter yield.

Partial fertilization productivity is the ratio of the yield to the applied dose fertilizer. In specific conditions of growing, the efficiency of the used fertilizer in most cases decreases with the increase of the applied dose (Dibb, 2000), which was also confirmed in the present study. The highest value of partial fertilization productivity average for the period of the study was established for the lowest applied fertilization rate (1,500 ml.ha^{-1}), surpassing with 32% the values of partial fertilization productivity for fertilization rate 2,500 ml.ha^{-1}, and with 60% for fertilization rate 3,500 ml.ha^{-1}.

CONCLUSIONS

Application of liquid organic fertilizer Naturamin Plus to natural grassland of *Agropyron repens* (L.) P. Beauv. type has a positive influence on the productivity, regardless of the climate conditions during the study period. The strongest effect on the productive characteristics resulted from the treatment with dose 2,500 ml.ha^{-1} - green mass yield increased with 31.6%, and dry matter increased with 30.7%.

Treatment with Naturamin Plus in dose 2,500 ml.ha^{-1} leads to increase in the agronomic efficiency of fertilization. Using higher doses of Naturamin Plus, the effect on the productiveness of grassland and of agronomic efficiency of fertilization decreases.

In natural grasslands *Agropyron repens* (L.) P. Beauv. type, the largest share is occupied by perennial cereal grasses. Fertilization with the liquid organic fertilizer Naturamin Plus increases the participation of cereal and legume grasses and reduces that of weeds.

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MISCELLANEOUS