Original scientific paper
DOI: /10.5513/JCEA01/24.2.3803
Journal of Central European Agriculture, 2023, 24(2), p.413-421

Effect of liquid organic fertilizer on the chemical composition and nutritive value of natural *Agropyron repens* grassland

Ефект на торенето с течен органичен тор върху химичния състав и хранителната стойност на естествен тревостой Agropyron repens тип

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Received: November 19, 2022; accepted: April 2, 2023

ABSTRACT

The aim of this study was to determine the effect of organic leaf fertilization on the chemical composition, nutritive value, yield of crude protein (CP) and feed units of natural *Agropyron repens* grassland 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: 1500; 2500 and 3500 ml/ha. The obtained results were statistically processed by ANOVA. The applied leaf organic fertilizer Naturamin Plus promoted a positive effect on the productive parameters of natural *Agropyron repens* grassland but had no significant effect on the chemical composition and nutritive value of the harvested biomass. The results demonstrated that yield of CP, feed units for milk (FUM) and feed units for growth (FUG) increased significantly by fertilization with a dose of 2500 ml/ha – respectively 22.2%, 29.7% and 29.4% more compared to the control.

Keywords: fertilization, natural grassland, chemical composition, yield of crude protein, feed units for milk, feed units for growth

РЕЗЮМЕ

Целта на това изследване е да се определи ефекта на органичното листно торене върху химичния състав, хранителната стойност, добива на суров протеин и кръмни единици на естествен тревостой *Agropyron repens* тип в района на Централна Южна България (305 m надморска височина). Опитът е проведен с листен органичен тор Натурамин Плюс, през периода 2018-2019 г. Проектиран е по блоковия метод в 4 повторения и са изпитани 3 дози тор: 1500; 2500 и 3500 ml/ha. Получените резултати са статистически обработени чрез ANOVA. Приложеният листен органичен тор Натурамин Плюс има положителен ефект върху продуктивните параметри на естествения тревостой *Agropyron repens* тип и не оказва съществено влияние върху химичния състав и хранителната стойност на събраната биомаса. Резултатите показват, че добивът на суров протеин, кръмни единици за мляко и кръмни единици за растеж се увеличава значително при торене с доза от 2500 ml/ ha – съответно с 22.2%, 29.7% и 29.4% повече в сравнение с контролата.

Ключови думи: торене, естествен тревостой, химичен състав, добив на суров протеин, кръмни единици за мляко, кръмни единици за растеж

INTRODUCTION

One of the main aspects of modern agriculture is to optimize plant nutrition while minimizing the negative impact of mineral fertilization on the environment. Organic fertilizers and biostimulants are being established as an alternative to reduce the use of mineral fertilization and receive ecologically clean forage production (Călina and Călina, 2015).

Studies show that the application of organic fertilizers and bioproducts at different regimes of grasslands use (hay-use, grazing-use, and hay-grazing-use) has a positive influence on the protein content and nutritive value of the obtained forage, improves the mineral content, leads to an increase in the yield of crude protein and energy (Avarvarei and Chelariu, 2011; Kozhouharov and Lingorski, 2011; Păcurar et al., 2012; Štýbnarová et al., 2012; Tarcău et al., 2012; Kharkevich et al., 2015).

Aiming to reduce to a minimum the negative impact on the environment Chourkova (2013), Pigareva and Zhugdurov (2013), Bozhanska et al. (2017, 2020), Petrova (2017), Wolski et al. (2019), Olszewska (2022) applied liquid biofertilizers to optimize the nutrition of plant species. Established was the effect of the application of different methods, doses and types of biofertilizers and growth regulators on the quality of forage from birdsfoot trefoil (*Lotus corniculatus* L.), red clover (*Trifolium pretense* L.), white clover (*Trifolium repens* L.), sainfoin (*Onobrychis viciifolia* Scop.), perennial cereal grasses and legume grass mixtures, natural grasslands.

Bozhanska et al. (2017, 2020) discovered birdsfoot trefoil (*Lotus corniculatus* L.) and white clover (*Trifolium repens* L.) had a specific reaction to the leaf application of growth regulators and biofertilizers. It was established that the bioproducts had a positive influence on the quality and digestibility of the forage mass. The combination of the tested biofertilizers had led to an increase in the yields of crude protein and feed units for milk, however a more significant impact was reported regarding the yield of feed units for milk in the harvested mass.

According to data from Churkova (2013) fertilizing grassland birdsfoot trefoil (*Lotus corniculatus* L.) with boron humate significantly increased the content of crude protein, while phosphorus humate decreased crude fiber. Treatment of birdsfoot trefoil with boron humate had the most significant effect on the feed units for milk and feed units for growth.

Through the leaf application of natural microfertilizers can be overcome the deficit of a number of microelements in meadow and pasture grasslands. The improved mineral content of the biomass is a prerequisite for satisfying the needs of animals and receiving high quality animal production (Ivanova and Zavarukhina, 2015; Godlewska and Ciepiela, 2016; Ciepiela and Godlewska, 2017).

Confirmed were the positive effects of applying biostimulators in the growing of Orchard grass (*Dactylis glomerata* L.), Braun's festulolium (*Festulolium braunii*) and Italian ryegrass (*Lolium multiflorum* Lam.) on a background of varying nitrogen regime on fiber fraction content and dry matter digestibility. The application of both natural and synthetic biostimulators allowed for the improvement of the nutritive value, increase of the absorption of dry matter and digestibility of grass forage (Ciepiela et al., 2016; Godlewska and Ciepiela, 2021).

The leaf application of biostimulators had a positive influence on the chemical composition of the biomass from Perennial ryegrass (*Lolium perenne* L.) – increased the content of crude protein, phosphorous, and chlorophyll in the leaves, while simultaneously decreased the build-up of crude fibre, K and Ca in plants. This is valuable in the practice, as it allows for the production of high quality green forage for animals, while at the same time minimizing the use of mineral fertilizers and the harmful impact on the environment (Olszewska, 2022).

The aim of the present study was to determine the effect of the liquid organic fertilizer Naturamin Plus (Daymsa, ES) on the chemical composition, nutritive value, yield of crude protein and feed units of 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 (Shanin, 1977). The soil in the area is Gleic Chromic Luvisols, with low humus content (3.42% – 3.93%), low acidity, low available nitrogen (31.3 – 38.1 mg/kg soil) and phosphorus content (3.1 – 4.3 mg/100 g soil) but with high available potassium content (42 – 44 mg/100 g soil).

The effect of the combined fertilizer Naturamin Plus was tested. Naturamin Plus has the following composition: total 400 g/l amino acids, free amino acids – 200 g/l, Nitrogen (N) – 75 g/l, Iron (Fe) – 12 g/l, Manganese (Mn) – 7.5 g/l; Boron (B) – 1.3 g/l, Copper (Cu) – 1.2 g/l, Molybdenum (Mo) – 0.5 g/l, Zinc (Zn) – 2.5 g/l. The combined fertilizer Naturamin Plus is certified according to European Council Regulation (EC) No 834/2007 for use in organic production.

Fertilization with the liquid organic fertilizer Naturamin Plus in each of the trial years was made during the time of grass growing (height 10-15 cm). Naturamin Plus is applied as an aqueous solution in doses of 1500, 2500, and 3500 ml/ha diluted in water up to 300 l/ha. A handheld sprayer pump with a fine dispersion nozzle was used. The experimental plots were harvested via mowing in the spike growth phase of the dominant grass species *Agropyron repens* (L.) P. Beauv. The cutting height was 5-6 cm. During the years of the experiment, the plots were mowed twice. The samples were collected from each plot, during each harvest. The collected samples were dried, ground and subjected to chemical analysis.

The following parameters were determined: chemical composition of the dry matter (DM), nutritive value of biomass for ruminants, feed units for milk (FUM) and feed units for growth (FUG). The chemical composition of the DM was determined per Weende (AOAC, 2007) – crude protein (CP), crude fat or ether extract (EE), crude fibre (CF), ash and nitrogen-free extract (NFE). The nutritive value of biomass for ruminants was calculated on the basis of the chemical composition, using empirical equations (Todorov et al., 2004, 2007):

GE = 0.0242 CP + 0.0366 EE + 0.0209 CF + 0.017 NFE;

ME = 0.0152 DP + 0.0342 DEE + 0.0128 DCF + 0.0159 DNFE;

where: GE – gross energy, ME – metabolizable energy, DP – digestible protein, DEE – digestible ether extract, DCF– digestible crude fibre, DNFE – digestible nitrogen free extract. Digestibility coefficients of biomass were obtained from the reference data of Todorov et al. (2007).

The FUM and FUG were calculated as follows:

FUM = ME (0.075 + 0.039q);
FUG = ME (0.0382 + 0.104q);
q = ME/GE.

The obtained yields of protein and feed units from biomass of natural *Agropyron repens* grassland were calculated on the basis of the obtained yields of DM established by Zhelyazkova and Gerdzhikova (2022), and content of CP, FUM and FUG in DM.

The results for the chemical composition and yields 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 DISCUSSION

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.

Table 1. Climate conditions of South-Central Bulgaria

Years	Months												I-XII	IV-IX
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII		
	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, °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

The average of annual air temperature calculated for the multiannual period (1936-2017) was 12.5 °C. Already in the first ten days of March (4-7 March) the temperatures permanently stay above 5 °C, 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 19.4 °C. The temperature during vegetation in the trial period (2018-2019) was with 1.0-1.5 °C higher than the multiannual average in both years of the study.

The protein nutritive value of forages depends mainly on the content of CP. From the studies (Table 2) is seen that the content of CP in the grassland biomass average for the period 2018-2019 was 116.03 g/kg DM.

The content of CP in the DM of the biomass average for the period 2018-2019 was highest in the control – 124.71 g/kg DM. From the data obtained it is evident that fertilization of the natural grassland with Naturamin Plus led to a decrease of the CP content in the biomass. The lowest CP content average for the period of the study was obtained by leaf fertilization with a dose of 2500 ml/ha. Compared with the control the decrease was on average with 10.8% (13.49 g/kg DM). After treatment with Naturamin Plus in doses 2500 and 3500 ml/ha the differences with the control were proven ($P<0.05$), while for the dose of 1500 ml/ha the difference with the control was not proven.

The DM of the grass biomass obtained from natural *Agropyron repens* grassland, contains ether extract (EE) on average 22.74 g/kg DM. The content of the EE is low, therefore the variation between the different variants is non-significant.

Crude fibres (CF) as a second main component are on average 229.56 g/kg DM. It is known that there is a negative correlation between the content of CP and CF. From the obtained data is seen that fertilizing the natural grassland with Naturamin Plus led to an increase of the content of CF in the obtained biomass. The highest content of CF on average for the period of the study was found in leaf fertilization in a dose of 2500 ml/ha and the increase compared with the untreated variant was on average with 12.4% (26.62 g/kg DM) and is well proven statistically ($P<0.001$). This is probably due to the increased participation of perennial cereal grasses in the observed grassland of the *Agropyron repens* L. type under the effect of fertilization with the liquid organic fertilizer Naturamin Plus in the dose of 2500 ml/ha (Zhelyazkova and Gerdzhikova, 2022). After treatment with Naturamin Plus in doses of 1500 and 3500 ml/ha the differences in the CF content compared with the control are also proven ($P<0.05$).

The total Ash content in the grass biomass on average for the period of the study was 89.35 g/kg DM. The variation here was non-significant and there were

no proven differences between the different doses of treatment.

Nitrogen free extract (NFE) were on average 542.32 g/kg DM. Fertilization with the liquid organic fertilizer Naturamin Plus had little effect on the quantity of NFE. The differences between the fertilized variants have not been proven statistically.

The total gross energy (GE – the calorific value from complete combustion of the food) of the biomass obtained from natural *Agropyron repens* grassland, was 17.63 MJ/kg DM (Table 3). In the years of the experiment, as well as after treatment with increasing doses of the liquid organic fertilizer Naturamin Plus this indicator had little variation (17.61 – 17.65 MJ/kg DM).

Animals do not fully use the potential energy of forages. A significant part is lost with undigested waste, metabolism, etc. The quantity of ME (physiologically useful for animals energy) for ruminant animals in the grass biomass is 8.81 MJ/kg DM. It also stayed near constant and didn't change during the experiment duration or under the influence of the applied leaf fertilizer in increasing doses. On average for the years of the experiment, the ME made up to 49.97% of the total (gross) energy content of the grass biomass.

Grass biomass from natural *Agropyron repens* grassland contained on average 0.83 FUM and 0.79 FUG in a kg DM. The content of FUM and FUG remained almost identical and did not change significantly in the different years of the study and under the influence of the applied leaf fertilizer in increasing doses.

No significant differences in the energy values were found, despite the lower content of CP as a result of the applied fertilization. The fertilization with increasing doses of the liquid organic fertilizer Naturamin Plus had no influence on the nutritive value of the grass biomass for ruminant animals.

Table 2. Chemical composition of biomass of natural *Agropyron repens* grassland, treated with liquid organic fertilizer (average for the period 2018-2019, g/kg DM, n=32)

Variant, Dose, ml/ha	Crude protein X ± S_x	Ether extract X ± Sx	Crude fiber X ± S_x	Ash X ± S_x	NFE X ± S_x
Control	124.71 ± 12.51	23.75 ± 2.71	215.43 ± 17.01	92.03 ± 10.43	544.09 ± 13.04
NP 1500	116.75 ± 14.31^{a}	22.38 ± 2.19^{a}	231.11 ± 17.45^{*a}	90.01 ± 7.41^{a}	539.75 ± 8.02^{a}
NP 2500	111.22 ± 12.31^{*a}	22.99 ± 1.09^{a}	242.07 ± 18.66^{***b}	87.76 ± 10.05^{a}	535.95 ± 4.94^{a}
NP 3500	111.44 ± 12.41^{*a}	21.83 ± 1.44^{a}	229.62 ± 16.41^{*a}	87.59 ± 8.34^{a}	549.51 ± 6.25^{a}
Average	116.03	22.74	229.56	89.35	542.32
LSD *P*<0.05	9.56	3.62	11.49	9.82	14.78
LSD *P*<0.01	13.74	5.20	16.51	14.11	21.24
LSD *P*<0.001	20.22	7.65	24.29	20.75	31.25
SD	23.78	3.59	32.63	16.46	16.29
CV	20.50	15.78	14.21	18.42	3.00
SE	5.95	0.90	8.16	4.12	4.07
Min	79.34	18.22	182.85	63.24	506.38
Max	159.14	31.76	277.12	119.86	563.44

NFE – Nitrogen free extract; NP – Naturamin Plus; LSD – The least significant differences; SD – Standard deviation; CV – Coefficient of variation; SE – Standard error;
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

Table 3. Nutritive value of biomass of natural *Agropyron repens* grassland, treated with liquid organic fertilizer (average for the period 2018-2019)

Variant, Dose, ml/ha	GE, MJ/kg DM	ME, MJ/kg DM	FUM, per kg DM	FUG, per kg DM
Control (untreated)	17.64	8.84	0.84	0.80
Naturamin Plus 1500	17.65	8.81	0.83	0.79
Naturamin Plus 2500	17.61	8.78	0.83	0.79
Naturamin Plus 3500	17.64	8.83	0.83	0.80
Average	17.63	8.81	0.83	0.79

DM – Dry matter; GE – Gross energy; ME – Metabolizable energy; FUM – Feed units for milk; FUG – Feed units for growth

An important element of the productivity of forage cultures are the yields of CP and feed units for milk, realized with the obtained biomass per unit area. From the biomass of *Agropyron repens* grassland were obtained on average 326.3 kg/ha CP (Table 4). During the years of the experiment the yields of CP varied from 325.5 to 327.2 kg/ha. Lower were yields of CP in 2018 when the lack of rainfall in April, along with the air temperatures with 3.7 °C above the norm for the month led to the higher protein content of the biomass but had a negative impact on the yearly DM yield (Zhelyazkova and Gerdzhikova, 2022).

Table 4. Yield of CP in the biomass of natural *Agropyron repens* grassland, treated with liquid organic fertilizer (by the years and average for the period 2018-2019, kg/ha, n=32)

Variant, Dose, ml/ha	2018		2019		Average	
	kg/ha	%	kg/ha	%	kg/ha	%
Control (untreated)	308.5	100.0	302.9	100.0	305.7	100.0
Naturamin Plus 1500	327.6	106.2	335.9	110.9	331.7[a]	108.5
Naturamin Plus 2500	364.9	118.3	382.0	126.1	373.4[**b]	122.2
Naturamin Plus 3500	301.0	97.6	288.0	95.1	294.5[a]	96.4
Average	325.5		327.2		326.3	
LSD *P*<0.05					41.1	13.4
LSD *P*<0.01					59.0	19.3
LSD *P*<0.001					86.9	28.4
SD					39.5	
CV					12.0	
SE					7.0	
Min					284.2	
Max					429.8	

LSD – The least significant differences; SD – Standard deviation; CV – Coefficient of variation; SE – Standard error
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

Yields of CP were highest at treatment with 2500 ml/ha, where the excess compared with the control was on average with 22.2% (67.8 kg/ha). The excess in CP yield after treatment with this dose was due to the higher absolute value of the DM yield (established were statistically well proven differences (P<0.01) by Zhelyazkova and Gerdzhikova (2022)).

Positive was also the difference after treatment with a dose of 1500 ml/ha – the excess in the yield of CP compared with the untreated plants was on average with 8.5% but the difference was not statistically proven. The lowest yields of CP, by years of the study and average for the period, were obtained after treatment with Naturamin Plus in a dose of 3500 ml/ha – with 3.6% lower than the control but the differences with the control were not statistically proven.

In the years of the experiment from the biomass of *Agropyron repens* grassland were obtained on average 2348.7 n/ha FUM (Table 5) and 2241.2 n/ha FUG (Table 6). Analogically to the yield of CP, lower yields of FUM were realized in 2018, when the climatic conditions had a negative effect on the yields of the first cut and therefore on the yearly yield for 2018 (Zhelyazkova and Gerdzhikova, 2022).

The highest, statistically well proven yield of FUM and FUG per hectare (P<0.001) in the different years and on average for the period of the study was obtained after treatment with Naturamin Plus in a dose of 2500 ml/ha. On average for the period of the experiment the yield of feed units for milk from this variant exceeded the control with 29.7% for FUM (613.2 n/ha) with 29.4% for FUG (580.5 n/ha).

Table 5. Yield of FUM in the biomass of natural *Agropyron repens* grassland, treated with liquid organic fertilizer (by the years and average for the period 2018-2019, n/ha, n=32)

Variant, Dose, ml/ha	FUM					
	2018		2019		Average	
	kg/ha	%	kg/ha	%	kg/ha	%
Control (untreated)	1880.0	100.0	2252.1	100.0	2066.0	100.0
Naturamin Plus 1500	2090.0	111.2	2707.2	120.2	2398.6^{**a}	116.1
Naturamin Plus 2500	2352.8	125.1	3005.6	133.5	2679.2^{***b}	129.7
Naturamin Plus 3500	1953.4	103.9	2548.8	113.2	2251.1^{a}	109.0
Average	2069.0		2628.4		2348.7	
LSD P<0.05					202.8	9.8
LSD P<0.01					291.4	14.1
LSD P<0.001					428.6	20.7
SD					403.9	
CV					17.2	
SE					71.4	
Min					1838.0	
Max					3381.9	

LSD – The least significant differences; SD – Standard deviation; CV – Coefficient of variation; SE – Standard error
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

Table 6. Yield of FUG in the biomass of natural *Agropyron repens* grassland, treated with liquid organic fertilizer (by the years and average for the period 2018-2019, n/ha, n=32)

Variant, Dose, ml/ha	FUM					
	2018		2019		Average	
	kg/ha	%	kg/ha	%	kg/ha	%
Control (untreated)	1796.6	100.0	2150.6	100.0	1973.6	100.0
Naturamin Plus 1500	1993.5	111.0	2581.5	120.0	2287.5^{**a}	115.9
Naturamin Plus 2500	2244.7	124.9	2863.5	133.1	2554.1^{***b}	129.4
Naturamin Plus 3500	1865.6	103.8	2433.9	113.2	2149.7^{a}	108.9
Average	1975.1		2507.4		2241.2	
LSD *P*<0.05					193.4	9.8
LSD *P*<0.01					277.8	14.1
LSD *P*<0.001					408.7	20.7
SD					383.8	
CV					17.1	
SE					67.9	
Min					1756.5	
Max					3221.9	

LSD – The least significant differences; SD – Standard deviation; CV – Coefficient of variation; SE – Standard error
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

In second place was fertilization with a dose of 1500 ml/ha – with 16.1% higher yield of FUM and with 15.9% higher yield of FUG compared with the control (*P*<0.01). Closest to the control values of yield for FUM and FUG, by years and average for the period of the study, were obtained after fertilization of the grassland with Naturamin Plus in a dose of 3500 ml/ha.

CONCLUSIONS

The applied leaf organic fertilizer Naturamin Plus promoted a positive effect on the productive parameters of natural *Agropyron repens* grassland regardless of weather conditions over the years. The results demonstrated that yield of CP, FUM and FUG increased significantly by treatment with a dose of 2500 ml/ha – respectively 22.2%, 29.7% and 29.4% more compared to the control.

The use of the liquid organic fertilizer Naturamin Plus in natural grassland had no significant effect on the chemical composition and nutritive value on the biomass for ruminants.

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