

## **EFFECT OF APPLICATION OF DIFFERENT AMOUNTS OF NITROGEN AND STAGES OF PLANT DEVELOPMENT AT CUTTING ON YIELD AND FORAGE VALUE OF GRASSLANDS**

Muamer Bezdrob<sup>1</sup>, Armin Jamakovic<sup>1</sup>, Saud Hamidović<sup>1</sup>, Teofil Gavrić<sup>1</sup>, Aleksandar Simić<sup>2</sup>, Nermin Rakita<sup>1</sup>

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### **Summary**

Grasslands in Bosnia and Herzegovina account for over 50% of the total agricultural land. However, although grasslands occupy large areas they are not optimally used. Inadequate use without the application of nitrogen fertilizer as well as cutting in the late stages of plant development have a negative impact on producing good quality forage and achieving high and stable yields. As the quality of biomass and grassland productivity largely depend on soil fertility, botanical composition, and stage of plant development at cutting, the aim of the research was to determine the influence of the application of different amounts of nitrogen fertilizer and the stage of plant development at cutting on dry mass yield, crude proteins and grassland microbial activity.

The results of the research show that the stage of plant development at cutting and the application of different amounts of nitrogen have a significant impact on the dry mass and crude protein yields. The dry matter yield ranged from 7.54 t ha<sup>-1</sup> in the N<sub>0</sub> variant (grass earing stage) to 13.19 t ha<sup>-1</sup> in the N<sub>90</sub> variant (grass flowering stage). Cutting at the grass earing stage can produce a considerably higher amount of good quality forage mass from the grassland, as well as the application of 70 to 90 kg ha<sup>-1</sup> of nitrogen.

**Key words:** *grasslands, nitrogen, plant development stage, dry mass yield, crude protein yield.*

### **INTRODUCTION**

Given that grasslands in Bosnia and Herzegovina make up over half of the total agricultural land, they represent an important resource for the production of roughage. Unfortunately, our grasslands are low-productive and the resulting bulk forage is of poor quality. In order to improve and enhance the condition of the grasslands, it would be necessary to make much more effort in grassland management method, i.e. to ensure

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<sup>1</sup>Faculty of Agriculture and Food Sciences of University of Sarajevo, Bosnia and Herzegovina

<sup>2</sup>Faculty of Agriculture, University of Belgrade, Zemun, Serbia

\*Correspondence: [m.bezdrob@ppf.unsa.ba](mailto:m.bezdrob@ppf.unsa.ba)

that higher yields and better quality of bulk forage are achieved in the production on these areas.

This implies the application of appropriate grassland management measures: fertilization (mineral and organic), cutting at the optimal stages of plant development, and adequate use of forage from the grasslands, etc. A number of authors in their research results point out the significance of the application of different amounts of nitrogen which affect the productivity and quality of the grasslands (Mijatović *et al.*, 1975; Stjepanović *et al.*, 1998; Mandić *et al.* 2001; Sima and Pacurar, 2002; Bijelić *et al.*, 2011; Tomić *et al.*, 2011). Soil microorganisms also play a key role in creating and maintaining grasslands soil fertility. The amount and activity of soil microorganisms is a significant factor for its fertility. Numerous microorganisms are present in the soil, especially from the group of bacteria and fungi. Beneficial microorganisms decompose organic matter in the soil thus making it accessible to plants and contributing to increased soil fertility, which results in higher yields and better quality of forage (Parr, 1994; Jose *et al.*, 2001).

## MATERIAL AND METHODS

The experimental trial was set up in early April 2018 on a grassland that was previously sown in 2011, located at the Butmir agricultural estate near Sarajevo, using a random block system, in four replications. The size of the plot was 5 m<sup>2</sup>. Over 80% of grasses were represented on the examined grassland. Timothy (*Phleum pratense* L.) had the highest representation on the grassland, followed by orchard grass (*Dactylis glomerata* L.). In the experiment, Nitrogen fertilizer (27% Calcium ammonium nitrate – CAN) was applied in early spring. The experiment examined the effect of applying different amounts of nitrogen 0 (N<sub>0</sub> control), 50 (N<sub>50</sub>), 70 (N<sub>70</sub>), and 90 (N<sub>90</sub>) in two phases of cutting (cutting at the grass earing phase and cutting at the grass flowering phase) and microbial activity of the soil. The total dry mass yield was determined on the basis of the green mass yield obtained at cutting, and the dry matter content. The dry matter content was determined by drying the samples in a dryer to a moisture content ranging from 13 to 15%. The content of total nitrogen in plant mass was determined by the Kjeldahl method. Crude protein content was determined based on the level of total nitrogen in dry matter multiplied by factor 6.25. Crude protein yield (CP) was determined on the basis of dry mass yield and the content of crude proteins in it. Soil microbiological activity: under grassland it included examination of systematic groups of microorganisms; the total number of microorganisms, fungi and actinomycetes on appropriate nutrient media. As part of the microbiological analysis, isolation and identification of fungi by genus was performed. The results of the research were processed by statistical methods of analysis of variance and the differences between the media were tested by the Tukey test. The weather conditions in the research year were quite favorable. They are characterized by high air temperatures and an even distribution of precipitation during the vegetation period. The total amount of precipitation in 2018

was 1043.3 mm, which is 111.6 mm more than the multi-year average. The mean annual air temperature was 1.9°C higher compared to the multi-year average (9.5°C).

## RESULTS AND DISCUSSION

### Dry mass yield depending on the applied nitrogen dose and cutting regime

In the research year, the highest yield of dry mass (Table 1) at grass earing phase was achieved by the N<sub>90</sub> variant (11.07 t ha<sup>-1</sup>), followed by the N<sub>70</sub> variant (10.06 t ha<sup>-1</sup>). The dry mass yield of variant N<sub>90</sub> is significantly higher compared to the dry mass yield of variant N<sub>50</sub> and the control variant N<sub>0</sub>. A significantly higher dry mass yield was also achieved by variant N<sub>70</sub> compared to the control. The overall highest dry mass yield at grass flowering stage was achieved by variant N<sub>90</sub> (13.19 t ha<sup>-1</sup>), followed by the variant treated with 70 kg ha<sup>-1</sup> (N<sub>70</sub>) of nitrogen fertilizer (12.28 t ha<sup>-1</sup>). The dry mass yields in all variants in which nitrogen fertilizer was applied were statistically significantly higher compared to the control variant (8.95 t ha<sup>-1</sup>).

Tab. 1. Impact of nitrogen application and plant development stage on dry mass yield (t ha<sup>-1</sup>)

Variant	Cutting regime	
	Earing	Flowering
Control (N <sub>0</sub> )	7.54 c	8.95b
Fertilization N <sub>50</sub> )	8.95bc	11.86a
Fertilization (N <sub>70</sub> )	10.06ab	12.28a
Fertilization (N <sub>90</sub> )	11.07a	13.19a
Average	9.40	11.57

<sup>abc</sup>Values marked with different letters differ significantly (P<0,05) <sup>ns</sup> no significant difference

The dry mass yield at different cutting regimes is characterized by the fact that the highest average annual yield was achieved when cutting at grass flowering phase (11.57 t ha<sup>-1</sup>), whereas the yield in grass earing phase amounted only 9.40 t ha<sup>-1</sup>, which is by 2.16 t ha<sup>-1</sup> less compared to the average yield obtained at the later cutting phase (grass flowering phase). The lowest annual dry mass yield was achieved by the control variant N<sub>0</sub> at both cutting phases, in the amount that ranged from 7.54 t ha<sup>-1</sup> in the grass earing phase, to 8.95 t ha<sup>-1</sup> in the grass flowering phase.

The cutting regime had a significant impact on the dry mass yield. The cutting regime in the grass flowering phase achieved a statistically significantly higher dry mass yield

compared to the grass earing phase (except for the control variant N<sub>0</sub>), which is quite consistent with the results of the research by Alibegović-Grbić *et al.* (2010).

### Crude protein yield depending on the applied nitorgen dose and cutting regime

The overall average annual yield of crude proteins (Table 2) in the grass earing phase was (1109.40 kg ha<sup>-1</sup>). The highest total annual protein yield was achieved by variant N<sub>90</sub> (1504.32 kg ha<sup>-1</sup>). The lowest crude protein yield was achieved by the control variant N<sub>0</sub> (789.18 kg ha<sup>-1</sup>) without the application of nitrogen. All variants treated with nitrogen achieved a significantly higher protein yield compared to the control variant N<sub>0</sub>. Variant N<sub>90</sub> achieved a statistically significantly higher yield of crude proteins compared to variants N<sub>50</sub> and N<sub>70</sub>. The average annual yield of crude protein in the grass flowering phase was (1031.14 kg ha<sup>-1</sup>). The highest total annual protein yield was achieved by variant N<sub>90</sub> (1152.95 kg ha<sup>-1</sup>), followed by variant N<sub>50</sub> (1125.18 kg ha<sup>-1</sup>), while the lowest one was achieved by variant N<sub>0</sub> (767.11 kg ha<sup>-1</sup>). The yield of crude proteins where nitrogen was applied is statistically significantly higher compared to the control where no nitrogen was applied. Additionally, the N<sub>90</sub> variant achieved a significantly higher yield compared to the N<sub>50</sub> and N<sub>70</sub> variants.

Tab. 2. Impact of nitrogen and plant development stage on crude protein yield (kg ha<sup>-1</sup>)

Variant	Cutting regime	
	Earing	Flowering
Control (N <sub>0</sub> )	789.18c	767.11c
Fertilization (N <sub>50</sub> )	1071.30b	1125.18b
Fertilization (N <sub>70</sub> )	1072.80b	1079.35b
Fertilization (N <sub>90</sub> )	1504.32a	1152.95a
Average	1109.40	1031.14

<sup>abc</sup>Values marked with different letters differ significantly (P<0,05) <sup>ns</sup> no significant difference

The cutting regime had a significant impact on the yield of crude protein. The cutting regime in grass earing phase achieved a statistically significantly higher yield of crude protein compared to the grass flowering phase in the control variant (N<sub>0</sub>) and variant (N<sub>90</sub>), while there was no statistically significant difference between other nitrogen fertilized variants. The average yield of crude proteins was higher at the earlier cutting (grass earing phase) compared to the average yield at the later cutting (grass flowering phase), which is consistent with the research by Alibegović-Grbić *et al.* (2004), in which it was also determined that the cutting of grasslands at the grass earing phase had a

positive impact on the content of crude protein in dry matter as well as on the total protein yield per unit area.

### Soil microbiological activity

The examined parameter of soil microbiological activity and the number of ammonifiers showed the dependence on the plant phenophase and the amount of applied N fertilizer. The application of a smaller amount of N fertilizer in the grass earing phase increased the number of ammonifiers, whereas high amounts of N fertilizer had an inhibitory effect, which is consistent with the results of research (Đukić i Mandić, 1997; Mandić, 2001). The number of ammonifiers (Table 3) was the largest in the variant fertilized with 50 kg N ha<sup>-1</sup> (10.8; 10.4).

Table 3. Total number of bacteria in the soil under the grassland fertilized with different doses of nitrogen in the grass earing phase

Variant	Actinomycetes CFU x 10 <sup>3</sup> /g	Fungi CFU x 10 <sup>2</sup> /g	Total number of bacteria CFU x 10 <sup>5</sup> /g	Total number of ammonifiers CFU x 10 <sup>4</sup> /g
Control (N <sub>0</sub> )	7.2	404.4	915.5	9.4
Control (N <sub>0</sub> )	11.1	373.3	922.2	8.9
Fertilization (N <sub>50</sub> )	7.5	231.1	862.2	10.4
Fertilization (N <sub>50</sub> )	7.5	211.1	675.5	10.8
Fertilization (N <sub>70</sub> )	13.3	202.2	568.8	6.7
Fertilization (N <sub>70</sub> )	15.5	198.8	604.4	6.6
Fertilization (N <sub>90</sub> )	18.3	182.2	553.3	4.0
Fertilization (N <sub>90</sub> )	20.5	173.3	462.2	3.8

By further increasing the amount of nitrogen fertilizer, the number of ammonifiers decreased. The lowest total number of bacteria was found in variant N<sub>90</sub> (462.2; 553.3), and the highest in the control variant N<sub>0</sub> (922.2; 915.5). The comparison of the variants fertilized with nitrogen shows that the largest total number of bacteria was found in the variant that was fertilized with 50 kg N ha<sup>-1</sup> (862.2; 675.5). In the grass earing phase, the largest number of fungi was found in the control variant N<sub>0</sub> (404.4; 373.3), and the smallest in the variant fertilized with 90 kg N ha<sup>-1</sup> (173.3; 182.2). Of the variants

fertilized with nitrogen fertilizers, the largest number of fungi was found in the variant fertilized with 50 kg N ha<sup>-1</sup> (211.1; 231.1). The number of actinomycetes depended on the amount of nitrogen fertilizer applied. The largest number of this group of microorganisms was recorded in variant N<sub>90</sub> (20.5; 18.3).

The number of fungi in the flowering phase was smaller compared to the earing phase (Table 4). The highest number was found in the control variant N<sub>0</sub> (255.5; 242.2) without the application of nitrogen fertilizer, and the lowest in the variant fertilized with 90 kg N ha<sup>-1</sup> (70.7; 60.8). In contrast to the number of fungi, the number of bacteria in the grass flowering phase was larger in all variants, compared to the earing phase. The highest number of bacteria was recorded in the control variant (1280.5; 1244.4), and when the fertilized variants are concerned, the highest number was found when applying 50 kg N ha<sup>-1</sup> (995.5; 977.7). In the flowering phase, the number of actinomycetes increased compared to the earing phase.

Table 4. Total number of bacteria in the soil under the grassland fertilized with different doses of nitrogen in the grass flowering phase

<b>Variant</b>	<b>Actinomycetes CFU x 10<sup>3</sup>/g</b>	<b>Fungi CFU x 10<sup>2</sup>/g</b>	<b>Total number of bacteria CFU x 10<sup>5</sup>/g</b>	<b>Total number of ammonifiers CFU x 10<sup>4</sup>/g</b>
Control (N <sub>0</sub> )	20.3	255.5	1244.4	10.6
Control (N <sub>0</sub> )	20.8	242.2	1280.5	10.2
Fertilization (N <sub>50</sub> )	22.2	120.1	977.7	12.7
Fertilization (N <sub>50</sub> )	24.4	107.7	995.5	13.1
Fertilization (N <sub>70</sub> )	17.7	98.2	623.3	9.5
Fertilization (N <sub>70</sub> )	20.6	89.4	686.4	8.8
Fertilization (N <sub>90</sub> )	22.5	70.7	584.1	6.3
Fertilization (N <sub>90</sub> )	26.6	60.8	575.5	5.9

## CONCLUSION

Based on the results of research on the effects of the application of different doses of nitrogen and the phase of plant development at cutting on the yield and forage value of grasslands, the following conclusions can be made.

Dry mass yield in the earing and flowering phases was the highest in the variants that were fertilized with 90 kg N ha<sup>-1</sup>, and the lowest in the control variant. A statistically significantly higher dry mass yield was achieved when cutting was performed in the grass flowering phase compared to the grass earing phase (except for the control variant N<sub>0</sub>). A statistically significantly higher crude protein yield was achieved in variants (N<sub>0</sub> and N<sub>90</sub>) compared to the yield achieved with the cutting regime in the grass flowering phase. The application of nitrogen on grassland had a positive impact on increasing the yield of dry mass and crude proteins, particularly the applied doses of 70 to 90 kg N ha<sup>-1</sup>. The examined parameter of soil microbial activity showed dependence on the development stage of plant and the amount of N fertilizer applied. A higher number of bacteria and actinomycetes was found in the flowering stage compared to the earing stage, while the number of fungi was higher in the earing stage compared to the flowering stage in all variants of the experiment.

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## **EFEKAT PRIMJENE RAZLIČITIH DOZA AZOTA I FAZE RAZVOJA BILJAKA PRI KOSIDBI NA PRINOS I KRMNU VRIJEDNOST TRAVNJAKA**

### **Rezime**

Travnjaci u Bosni i Hercegovini zauzimaju preko 50% ukupnih poljoprivrednih površina. Međutim, iako travnjaci zauzimaju velike površine nisu optimalno iskorišteni. Neadekvatan način korištenja bez upotrebe i primjene azotnog đubriva kao i kosidba u kasnijim fazama razvoja biljaka imaju negativan uticaj za dobijanja kvalitetne krme i postizanja visokih i stabilnih prinosa. S obzirom da kvalitet biljne mase i produktivnost travnjaka u velikoj mjeri zavise od plodnosti zemljišta, botaničkog sastava i faze razvoja biljaka pri kosidbi, cilj provedenih istraživanja je bio utvrđivanje uticaja različitih doza gnojidbe sa azotom i faze razvoja biljaka pri kosidbi na prinos suhe mase, sirovih proteina i na mikrobiološku aktivnost sa travnjaka.

Rezultati istraživanja su pokazali, da faza razvoja biljaka pri kosidbi, te primjena različitih doza azotnog đubriva imaju značajan uticaj na prinos suhe mase i prinos sirovih proteina. Prinos suhe mase se kretao od 7,54 t ha<sup>-1</sup> u varijanti N<sub>0</sub> (faza klasanja trava) do 13,19 t ha<sup>-1</sup> u varijanti N<sub>90</sub> (faza cvatnje trava). Kosidbom u fazi klasanja trava, te primjenom 70 do 90 kg ha<sup>-1</sup> azota može se dobiti znatno više kvalitetne krmne mase sa travnjaka.

Ključne riječi: *travnjaci, azot, faza razvoja biljaka, prinos suha mase, prinos sirovog proteina.*