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The influence of fertilization of red clover (*Trifolium pratense* L.) cultivars on productive and quality properties of forage

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ABSTRACT

Red clover ranks second only to alfalfa in terms of prevalence and significance in the production of bulky livestock fodder. Although alfalfa is considered more productive and of higher quality, large areas of land in Bosnia and Herzegovina are unsuitable for alfalfa cultivation (heavy, acidic soils). As a result, clover generally occupies larger areas than alfalfa. This crop thrives well in cool, moist, moderately heavy to heavy soils with slightly acidic to neutral reactions. Given that the quality of biomass and productivity of red clover largely depend on soil fertility, variety, environmental conditions and growth stage at harvest, this study aimed to determine the effect of fertilization (N_0, N_{20}, N_{40} and N_{60}) of red clover cultivars (Global and Una), in the agro-ecological conditions of Butmir, on the yield of dry matter, crude protein and the abundance and activity of nodule bacteria. The research results indicated that both the cultivar and the application of nitrogen fertilizer had a significant impact on the productivity and quality properties of red clover (dry matter yield, crude protein yield). The highest annual dry matter yield was achieved with the N_{60} variant for the Global cultivar (8.48 t/ha) and the N_{40} variant for the Una cultivar (8.50 t/ha). Similarly, the highest protein yield was recorded with the N_{60} variant for the Global cultivar (1,262.1 kg/ha) and with the N_{40} variant for the Una cultivar (1,073.9 kg/ha).

Keywords: red clover, cultivar, fertilization, dry matter, crude protein, nodulation

INTRODUCTION

Red clover is considered one of the most important perennial forage legumes in Bosnia and Herzegovina due to its excellent adaptation to a wide range of soil conditions, high yield potential, and good nutritional value. Considering global climate change and the development of organic agriculture, red clover is gaining importance as a valuable genetic resource in livestock feed production. Producing high-quality bulky fodder, which implies a high protein content, is one of the key objectives of livestock farming. Red clover is a forage crop, rich in protein, and has greater fiber digestibility compared to alfalfa (Leto et al., 2013). The quality of red clover fodder is influenced by numerous factors, including the plant growth stage, harvest timing, cultivar, soil fertility, and storage conditions. The growth stage of plants significantly affects the chemical composition and quality of red clover fodder (Makarenko and Pribytkov, 1989; Marković et al., 2008; Belonger and Tremblay, 2010). Different cultivars of red clover exhibit varying characteristics such as winter hardiness, regrowth rate, seed yield, and protein content, which impact the overall yield and forage quality. Under favorable conditions, red clover as a forage crop can achieve high dry matter yields. Under optimal conditions and with proper agricultural practices, red clover can achieve yields of 15 to 18 t/ha of dry matter. Numerous authors (Đukić et al., 2009; Bošnjak, 2013; Bezdrob et al., 2024, and others) have reported similar findings in their research. Red clover

is also significant in ecological production because it enriches the soil with nitrogen through symbiotic fixation by nodule bacteria, reducing the need for extensive use of mineral nitrogen (Frame, 2005). Microorganisms, as integral components of biogeocenosis, are important indicators of soil biological value and provide valuable insights into the success of plant cultivation on arable land. Govedarica et al. (1993) point out that the type of soil and its properties, among other factors, influence the abundance and activity of microorganisms in the soil. The presence of Rhizobium strains in acidic soils is low, which leads to a lack of nodulation and a reduction in dry matter yield (Jarak et al., 1999). Jarak et al. (2003) (2004) demonstrated that the application of inoculation with strains tolerant to low pH values resulted in an increase in red clover biomass yield by 10% to 15%. The population structure of symbiotic microorganisms varies depending on numerous factors, including soil type, cultivar, individual plants within the variety, and weather conditions. This research aimed to examine the influence of fertilization on the productivity (dry weight yield and crude protein yield) of red clover varieties, as well as on the number of symbiotic nodule bacteria.

MATERIALS AND METHODS

The research was conducted at the Butmir experimental field not far from Sarajevo. The trial was established in late April 2023 using a randomized block design with four replications. The size of the basic plot was 5 m². Sowing was done at the end of April, manually in rows with a row spacing of 12.5 cm. The seeding rate for red clover was 15 kg/ha. During pre-sowing soil preparation, the mineral fertilizer (KAN 27%) was applied. The experiment examined two red clover cultivars, Global and Una, and three fertilization treatments: control (N_0), fertilization with 20 kg N/ha (N_{20}), 40 kg N/ha (N_{40}), and 60 kg N/ha (N_{60}), along with the abundance and activity of nodule bacteria. The total abundance of nodule bacteria was determined by the number of nodules formed on the main and lateral roots at the full bloom stage of the plants. Determination of the number of nodules in red clover cultivars was carried out at a depth of up to 12 cm. In the year of sowing, both swaths were analyzed. The total dry matter yield was determined based on the green mass yield measured at cutting, and the dry matter content (determined by drying samples to 14% moisture). The total nitrogen content in the plant biomass was determined using the Kjeldahl method. The crude protein content was calculated based on the total nitrogen content. The yield of crude protein was determined based on the dry mass yield and the content of crude protein. Weed control during the growing season was done manually. Cutting was performed at the flowering stage of red clover. The soil of the Butmir experimental field has an acidic reaction with a pH of 4.6 (in KCl) and pH 6.3 (in H_2O). The humus content was 2.9%, classifying the soil as moderately humus-rich. The phosphorus content was 8.5 mg/100 g of soil, placing it in the low-content class. According to the potassium content, the soil has a medium supply of this element (14 mg/100 g of soil). The weather conditions during the year of the study were quite favorable, characterized by high air temperatures and a uniform distribution of precipitation throughout the growing season. The total amount of precipitation in 2023 was 1408.7 l/m², which is 597.5 l/m² above the long-term average. The mean annual air temperature was 1.47 °C higher than the long-term average (9.63 °C). Statistical methods. All experimental measurements were statistically analysed by the ANOVA (analysis of variance) method at a level of significance set at 0.05. The statistical analyses were made using the SPSS 22.0 software program (IBM, Armonk, New York, USA).

RESULTS AND DISCUSSION

Dry matter yield

The dry matter yield of red clover cultivar Global in the first cutting (Table 1) ranged from 5.34 t/ha in the (N_{20}) variant to 7.04 t/ha in the (N_{60}) variant. The variant fertilized with 60 kg N/ha also achieved a statistically significantly higher dry matter yield compared to the other examined variants. The control variant achieved a significantly higher dry matter yield compared to the variants fertilized with 20 and 40 kg N/ha. The response of the variety Global to lower doses of nitrogen (20 and

Table 1. Dry matter yiled (t/ha) of red colover (cultivars Global and Una)

Variant	Variety „Global“		Total yield	Variety „Una“		Total yield
	I cutting	II cutting		I cutting	II cutting	
N_0	6.12^{b}	1.17^{ns}	7.29^{b}	6.0^{a}	2.32^{a}	8.32^{ab}
N_{20}	5.34^{c}	1.04^{ns}	6.38^{c}	5.56^{b}	2.1^{ab}	7.66^{bc}
N_{40}	5.44^{c}	1.06^{ns}	6.5^{c}	6.62^{a}	1.88^{b}	8.5^{a}
N_{60}	7.04^{a}	1.44^{ns}	8.48^{a}	6.38^{a}	1.8^{b}	8.18^{b}
Average	5.98	1.17	7.15^{b}	6.14	2.02	8.16^{a}

^{abc} Values marked with different letters differ significantly ($P < 0.05$); ^{ns} no significant difference

40 kg N/ha), which resulted in lower yields even than the control, can be attributed to the inhibition of nodulation - a phenomenon known in legumes when small amounts of mineral nitrogen are introduced at an early stage of development. The average dry matter yield of the first cutting of red clover cultivar Global was 5.98 t/ha, which is 4.81 t/ha higher than the second cutting. The dry matter yield of the second cutting ranged from 1.04 t/ha in the (N_{20}) variant to 1.44 t/ha in the (N_{60}) variant.

There were no statistically significant differences in dry matter yield between the variants of Global cultivar in the second cutting. Overall, the highest dry matter yield in the year of the study was achieved by the variant fertilized with 60 kg N/ha (8.48 t/ha), which also showed a statistically significantly higher dry matter yield compared to the other tested variants. The significantly lower dry matter yields were achieved by the N_{40} variant (6.5 t/ha) and the N_{20} variant (6.38 t/ha) which is in accordance with the research results (Valjevac et al., 2025). The dry matter yield of red clover (cultivar Una) in the first cutting ranged from 5.56 t/ha in the (N_{20}) variant to 6.62 t/ha in the (N_{40}) variant. The dry matter yield in the (N_{60}, N_{40}, and N_0) variants was significantly higher compared to the variant fertilized with 20 kg N/ha. In the second cutting, the dry matter yield ranged from 1.8 t/ha in the (N_{60}) variant to 2.32 t/ha in the control variant (N_0). Overall, the highest total dry matter yield was achieved by the variant fertilized with 40 kg N/ha (8.5 t/ha), which also had a statistically significantly higher dry matter yield compared to the N_{60} and N_{20} variants. The highest average annual dry matter yield in the year of sowing was achieved by the Una cultivar (8.16 t/ha), while the Global variety yielded 7.15 t/ha, which closely matches the results obtained in previous research (Bezdrob and Alibegović-Grbić, 2010; Leto et al., 2013). The highest annual dry matter yield was achieved by the N_{60} variant of the Global cultivar (8.48 t/ha), and the N_{40} variant of the Una cultivar (8.5 t/ha). The lowest annual dry matter yield was achieved by the N_{20} variant for both red clover varieties, with values ranging from 6.38 t/ha (Global cultivar) to 7.66 t/ha (Una cultivar). The cultivar had a significant impact on dry matter yield. The dry matter yield of the Una cultivar was statistically significantly higher compared to the Global variety (except in the N_{60} variant).

Crude protein yield

The application of higher doses of nitrogen affected the total yield of crude proteins, especially in the Global cultivar under the N_{60} variant, and the Una variety under the N_{40} variant, which was the result of an increased yield of dry matter. Similar findings were reported by Alibegović-Grbić et al. (2010). Additionally, a significantly higher crude protein yield was achieved in the control variant without applied nitrogen, as a result of better nodulation. Higher doses of nitrogen fertilizers can reduce the activity of Rhizobium bacteria, leading to a decrease in the number of nodules. The crude protein yield in the year of study (Table 2) for the Global cultivar ranged from 724 kg/ha in the (N_{40}) variant to 1,016.5 kg/ha in the (N_{60}) variant.

Table 2. Crude protein yield (kg/ha) of red clover (cultivars Global and Una)

Variant	Variety „Global"		Total yield	Variety „Una"		Total yield
	I cutting	II cutting		I cutting	II cutting	
N_0	914.3^{b}	202.5^{b}	1.116,8^{b}	652,8^{b}	393,0^{a}	1.045,8^{a}
N_{20}	760.9^{c}	198.9^{bc}	959,8^{c}	573,1^{c}	341,2^{b}	914,3^{b}
N_{40}	724,0^{c}	178.1^{c}	902,1^{c}	761,3^{a}	312,6^{b}	1.073,9^{a}
N_{60}	1.016,5^{a}	245.6^{a}	1.262,1^{a}	669,9^{b}	259,2^{c}	929,1^{b}
Average	853.9	206.2	1.060,1^{a}	664.2	326.5	990,7^{b}

^{abc} Values marked with different letters differ significantly ($P < 0.05$); ^{ns} no significant difference

The crude protein yield in the (N_{60}) variant was significantly higher compared to the other tested variants. The average crude protein yield in the second cutting was 206.2 kg/ha, which was 647.7 kg/ha lower than the average yield of the first cutting (853.9 kg/ha).

The highest crude protein yield in the second cutting was achieved in the N_{60} variant (245.6 kg/ha), which was statistically significantly higher compared to other variants. The significantly lower protein yield in the second cutting was observed in the N_{40} variant (178.1 kg/ha). The overall average annual protein yield was 1,060.1 kg/ha. The highest total annual protein yield was achieved by the N_{60} variant (1,262.1 kg/ha). The N_{60} variant produced a statistically significantly higher protein yield compared to the other tested variants. The crude protein yield in the first cutting of red clover (Una cultivar) ranged from 573.1 kg/ha in the (N_{20}) variant to 761.3 kg/ha in the (N_{40}) variant. In the second cutting, the lowest crude protein yield was recorded in the N_{60} variant (259.2 kg/ha), while the highest was achieved in the control variant N_0 at 393 kg/ha. The overall average annual protein yield for the Una variety was 990.7 kg/ha. The highest total annual protein yield was observed in the N_{40} variant (1,073.9 kg/ha). The N_{40} variant and the control variant (N_0) produced statistically significantly higher protein yields compared to the N_{20} and N_{60} variants. The comparative yield of crude protein is characterized by the fact that the Global cultivar achieved higher yields compared to the Una cultivar. The maximum crude protein yield for red clover was recorded in the N_{60} variant (1,262.1 kg/ha) for the Global cultivar, and in the N_{40} variant (1,073.9 kg/ha) for the Una cultivar. The lowest annual crude protein yield for the Global cultivar was observed in the variant fertilized with 40 kg N/ha (902.1 kg/ha), while for the Una variety, it was recorded in the variant fertilized with 20 kg N/ha (914.3 kg/ha). The variety had a significant impact on protein yield. The crude protein yield of the red clover cultivar Global was statistically significantly higher compared to the protein yield of the Una cultivar (except for the N_{40} variant). Research by (Valjevac et al., 2025). indicates that the red clover variety has a significant impact on the total crude protein yield.

Number of nodules on red clover roots

The number and mass of nodules serve as indicators of nitrogen fixation efficiency (Gwata et al., 2004). The obtained research results for the number of nodules per plant varied significantly in different variants of nitrogen fertilization (Table 3). The highest number of nodules was recorded in the control variant N_0 for both red clover cultivars (Global: 28.6; Una: 27.8). The lowest average number of nodules, across two cuts, was observed in the N_{60} variant (Global: 23.5; Una: 22.4), which was statistically significantly lower compared to the control variant and the variants with lower nitrogen doses (N_{40} and N_{20}). The nitrogen fertilizer affected the reduction in the number and activity of symbiotic nitrogen-fixing bacteria, which is consistent with the findings of Stevanović et al. (2016).

Table 3. Impact of Nitrogen fertilizers on root length, root mass, and number of nodules per plant

Variant	Cultivar „Global“			Cultivar „Una“		
	Total number of nodules	Root length (cm)	Root mass (g)	Total number of nodules	Root length (cm)	Root mass (g)
N_0	28.6^{a}	14.5^{b}	1.0^{a}	27.8^{a}	16.0^{b}	0.7^{ns}
N_{20}	26.8^{b}	12.4^{c}	0.9^{b}	26.0^{b}	17.3^{a}	0.6^{ns}
N_{40}	25.4^{c}	16.0^{a}	2.3^{a}	24.4^{c}	15.4^{b}	0.6^{ns}
N_{60}	23.5^{d}	14.3^{b}	0.7^{b}	22.4^{d}	18.0^{a}	0.8^{ns}
Average	26.1	14.3	1.2	25.1	16.7	0.7

^{abcd} Values marked with different letters differ significantly ($P < 0.05$); ^{ns} no significant difference

Similar conclusions were made by Sørensen and Jensen (1991), according to which excessive availability of mineral nitrogen inhibits root development due to a reduced need of the plant for symbiotic nitrogen fixation. Zahran (1999) also points out that the efficiency of the symbiosis between plants and Rhizobium spp. is optimal at lower concentrations of mineral nitrogen in the soil, which further explains the lower root mass in treatments with higher N doses (N_{60}). The average root length of the Una cultivar was 16.7 cm, which is 2.4 cm longer than the Global cultivar (14.3 cm).

The longest root length (18 cm) for Una was achieved in the N_{60} variant, while the Global cultivar had the longest root length (16 cm) in the N_{40} variant. The root mass of the Global cultivar was greatest in the N_{40} variant (2.3 g), which was significantly higher than the root mass in the variants fertilized with 20 and 60 kg N/ha. The root mass of Una cultivar was greatest in the N_{60} fertilization variant (0.8 g). Moderate nitrogen application (40 kg N/ha) favored root development of the Global cultivar, while the Una cultivar showed a more limited response in terms of increasing root mass to increased nitrogen intake. These differences may be due to genetically determined efficiency of nutrient uptake and root system development, but also to different tolerance to high concentrations of available nitrogen in the soil.

CONCLUSION

The results of this research showed that the application of higher doses of nitrogen had a positive effect on the total dry matter yield and crude protein yield. The variety had a significant effect on the dry matter and protein yields. The dry matter yield of the Una cultivar was significantly higher compared to the dry matter yield of the Global cultivar (except in the N_{60} variant). The impact of nitrogen fertilizer on the nodulation of red clover depended on the application rate. The total number of nodules decreased with increasing fertilizer dosage for both varieties. Excessive application of nitrogen fertilizers can reduce the activity of Rhizobium bacteria, which results in a decrease in the number of nodules. Moderate application of nitrogen fertilizers can improve the overall yield and quality of red clover without negatively affecting nodulation. Therefore, it is important to conduct research that will help determine the optimal conditions for growing red clover in different agroecological conditions.

REFERENCES

Alibegović-Grbić, S., Bezdrob, M., Čivic, H. (2010) Dry matter and protein yields of red clover, Italian ryegrass and their mixtures. Grassland in a Changing World,15, 160-162.

Belonger, G., Tremblay, G. F. (2010) Fodder quality of legume-based pastures. NJF Seminar 432. The Potential of Forage Legumes to Sustain a High Agricultural Productivity - A Nordic Perspective. NJF Report, Hvanneyri, Iceland, 20-22 June, 6 (3), 97-112.

Bezdrob, M., Alibegović-Grbić, S. (2010) Comparative value of dry matter yield of red clover (*Trifolium pratense* L.), italian ryegrass (*Lolium multiflorum* Lam.) and their mixtures. Biotechnology in Animal Husbandry, 26 (Spec. Issue), 423-428.

Bezdrob, M., Rakita, N., Hamidović, Saud., Gavrić, T., Simić, A., Omerović, Z., Dokso, A. (2024) The influence of the vegetation cycle and the mixture (grasses and legumes) on the height of the plants on sown grasslands. Journal of Central European Agriculture, 25 (1), 154-162. DOI: https://doi.org/10.5513/JCEA01/25.1.4136

Bošnjak, K. (2013) Produktivnost binarnih smjesa crvene djeteline i trava ovisno o stadiju zrelosti u trenutku košnje. Glasnik zaštite bilja, 36 (4), 38-45.

Celić, M. (2013) Uporedba lucerne i crvene djeteline u proizvodnji voluminozne krme. Završni (bachelor) rad. Zagreb: Sveučilište u Zagrebu Agronomski fakultet.

Đukić, D., Stevović, V., Janjić V. (2009) Proizvodnja stočne hrane na oranicama i travnjacima. Udžbenik, Novi Sad, Kragujevac.

Frame, J. (2005) Forage legumes for temperate grasslands. Food and Agriculture Organization of the United Nations and Science Publishers, Inc., USA: pp. 200-210.
DOI: https://doi.org/10.1017/S0021859606226054

Govedarica, M., Jarak, M., Milošević, N. (1993) Mikrobiološke karakteristike zemljišta Vojvodine. Poglavlje u monografiji „Teški metali i pesticidi u zemljištu – Teški metali i pesticidi u zemljištima Vojvodine". Novi Sad. Poljoprivredni fakultet, pp. 259 - 268

Jarak M., Đukic, D., Govedarica, M., Milošević, N., Jeličić, Z., Đurić, S. (2003) Production of lucerne as affected by bacterization and liming. Grassland Science in Europe, 8, 641-644.

Jarak, M., Đukić, D., Đurić, S., Stevović, V., Đalović. I. (2004) Aktiviranje mikrobioloških procesa zemljišta s ciljem povećanja prinosa krmnih leguminoza. Acta Agriculturae Serbica, 17, 221-228.

Jarak, M., Govedarica, M., Milošević, N., Đurić, S., Petrov, S. (1999) Uticaj teških metala na kvržice bakterije lucerke. Zbornik radova, Naučni institut za ratarstvo i povrtlarstvo, Novi Sad., 32, 247-252.

Leto, J., Perčulija, G., Bošnjak, K., Kutnjak, H., Vranić, M., Čačić, M. (2013) Utjecaj bakterizacije, kultivara i stadija zrelosti na prinos i kemijski sastav crvene djeteline. Mljekarstvo, 63 (2), 98-108.

Makarenko, M. A., Pribytkov, T. F. (1989) Metody sozdanija selekcionnovo materiala klevera lugonovo, ljucerny s povyšennoj kormovoj cennostju. Sbornik Naučnih Trudov Vsesojuznogo *Naučno-Issledovatelskogo Instituta Kormov*, 42, 37- 41.

Marković, J., Diniü, B., Lugiü, Z., Štrbanoviü, R., Stanisavljeviü, R. (2008) Chemical constituents of red clover (*Trifolium pratense* L.) at different stages of maturity. Journal of Mountain Agriculture on the Balkan, 11 (5), 853-865.

Sørensen, J., Jensen, E. S. (1991) Beneficial effects of Rhizobium on growth of non-legumes. FEMS Microbiology Letters, 85 (4), 381–386.

Stevanović, P., Popović, V., Glamočlija, Đ., Tatić, M., Spalević, V., Jovović, Z., Simić, D., Maksimović, L. (2016) Uticaj azotnih hraniva na nodulaciju soje (*Glicine* max.) na černozemu i pseudogleju. Radovi sa XXX savetovanja agronoma, veterinara, tehnologa i agroekonomista, 22 (1-2), 67-77.

Valjevac, M., Bezdrob, M., Hamidović, S., Rakita, N., Karahmet, E., Gavrić, T., Imamović, B. (2025) The effect of foliar biofertilizer and variety on the productivity and nodulation of red clover. 3rd International symposium on biotechnology, Faculty of Agronomy in Čačak Proceedings, pp. 61-67.
DOI: https://doi.org/10.46793/SBT30.08MV

Zahran, H. H. (1999) Rhizobium–legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiology and Molecular Biology Reviews, 63 (4), 968–989.