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THE PERFORMANCE OF PERENNIAL RYEGRASS IN BINARY MIXTURES WITH LUCERNE AND RED CLOVER UNDER N FERTILIZATION

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Abstract. Perennial ryegrass is a very important and widespread grass species used for livestock nutrition, in particularly ruminants. As a species that is most commonly used on grasslands, it is grown in mixtures with other types of grasses and legumes. The objective of the research was to investigate the performance of perennial ryegrass at various proportions in the mixtures with red clover and lucerne, and how different levels of N fertilization affect its competitiveness. Ryegrass achieved the highest yield with lucerne at seeding rate 50:50 and with red clover at seeding rate 70:30. Relative grass yield (RYg) of mixtures ranged from 1.01 to 1.55 respectively, which means that ryegrass in mixtures achieved 0.1-55% greater yield than pure ryegrass crop. N fertilization increased DMY and RYg, leaf : steam ratio, specific leaf area (SLA), leaf area ratio (LAR) and leaf area index (LAI) in both years thus increasing the competitive capability of perennial ryegrass.

Key words: perennial ryegrass, red clover, lucerne, performance, competitiveness

Introduction

Perennial ryegrass is a very important and widespread grass species used for livestock nutrition, particularly ruminants. As a plant of moderately warm conditions, with good supply of water, especially in conditions with irrigation, it realizes high yields of very good quality. However, due to the sensitivity to drought, it shows great yield reduction in summer months. As a species that is most commonly used on pastures, it is grown in mixtures with other types of grasses and legumes. Growing in the mixture is intended to achieve higher yields compared to growing in pure crops. This phenomenon can be explained by the ability of individual species to use in a different way available resources in space and time (*Ergon et al., 2016*). Thus, a mixture of two grasses and two legumes gives higher yield by 57% compared to the examined most productive monoculture (*Nyfeler et al., 2009*). In studies by *Elgersma and Schlepers (1997),* pure crop of perennial ryegrass has achieved significantly lower yields of dry weight of 1.7-2.0 t ha^{-1}, compared to its mixtures with white clover of 8.7 to 12.2 t ha^{-1}. Also, cultivation of perennial ryegrass and other grasses in mixtures, improves intra-annual yield stability *(Sanderson, 2010),* while legume component increases the nutritional value of herbage by increasing of protein content, digestibility, reducing fibres concentration and contributing to a better balance of feed *(Fraser and Kunelius, 1995).*

However, when combining species in mixtures characteristics of species should be considered and their competitive ability as a very important determinant of yield and stability. Maintaining of well-balanced grass-legumes mixtures is very important, given that the grasses are generally more competitive than legumes, especially in regard to the nutrient uptake. Thus meadow fescue (*Festuca pratensis* Huds.) is the least competitive, while tall fescue (*Festuca arundinacea* Schreb.) and orchardgrass (*Dactylis glomerata* L.) are very aggressive *(Frame et al., 1998).* According to *Laidlaw and Teuber (2001),* grass of temperate climatic areas are less competitive so that they are more suitable for the cultivation with forage legumes.

The goal of this research is to investigate the performance of perennial ryegrass at various proportions in the mixtures with red clover and lucerne, and how different levels of N fertilization affect its competitiveness.

Material and Methods

The experiment was conducted during 2014-2016 at the experimental field of the Institute for Animal Husbandry Zemun, Belgrade (44°49′N, 20°17′E, and elevation 96 masl). The study was performed in three replications using randomised complete block system. The soil was silty clay loam with pH of 7.08. Climate of this area could be characterized as the temperate continental with cold winters and hot summers. Temperature and precipitation for study period are presented in Figure 1.

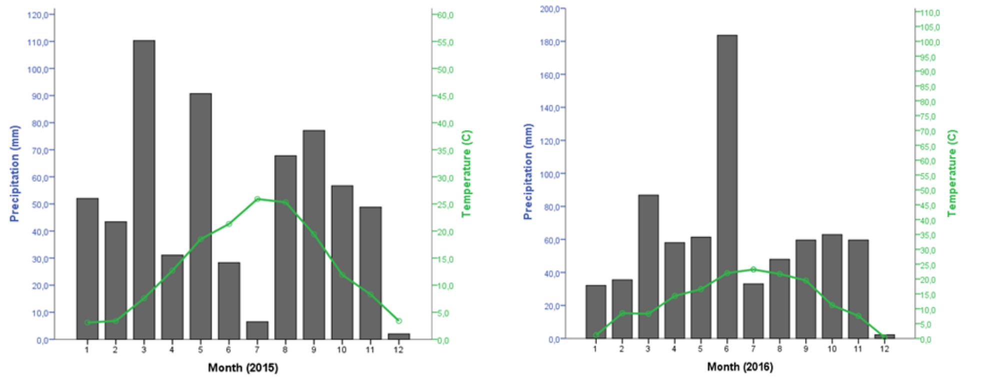

Figure 1. Average monthly temperature (°C) and sum of precipitation (mm) in two experimental year

In the spring of 2014., perennial ryegrass cultivar Calibra was sawn with lucerne cultivar NS Banat and red clover cultivar K-39 in grass-legumes mixtures with various proportions of species: 50% perennial ryegrass and 50% lucerne (50:50 PR+L); 50% perennial ryegrass and 50% red clover (50:50 PR+RC); 70% perennial ryegrass and 30% lucerne (70:30 PR+L); 70% perennial ryegrass and 30% red clover (70:30 PR+RC). Grass-legumes mixtures were sawn on field plots (2m x 5 m) with seed rate of 25 kg ha^{-1} for grass and 20 kg ha^{-1} for legumes. Nitrogen fertilizer in the form of ammonium nitrate was applied at the beginning of vegetation in amounts of 0, 50 and 100 kg N ha^{-1}.

Experimental measurements were done in the first and the second production year (2015-2016). All plots were cut three times in each studied year. Samples of herbage mass were collected from surface of 1 m^2, measured and after drying in the oven at 60 ° for 72 h, dry matter was determined. Also, another sample from 1m^2 surface, was collected and used for separation of species to determine botanical composition and for separation of leaves from steams to determine leaf : steam ratio. Leaf areas per plant were measured by using the software ImageJ and utilized for calculation of growth parameters: leaf area index (LAI), specific leaf area (SLA) and leaf area ratio (LAR).

LAI= leaf area per plant/No plant per m^2

SLA= leaf area per plant/leaf weight per plant (cm^2 g^{-1})

LAR= leaf area per plant/ weight per plant (cm^2 g^{-1})

Relative yields of the grass in the mixtures were calculated using the equation according to *De Wit (1960):*

RYg = DMYgl/DMYgg,

where DMYgl is the dry matter yield of the grass in the mixture and DMYgg is dry matter yield of the grass in the monoculture. RYg >1 indicated that grass in mixtures overyielded grass in monoculture which can be attributed to the level of nitrogen fertilization or efficiency of nitrogen fixation and transfer.

Data were analysed by the General linear model (SPSS 20.0) for ANOVA to detect differences of productive parameters of perennial ryegrass between mixtures at different level on nitrogen fertilization. Shapiro-Wilk test was used to determine whether or not the observations themselves are normally distributed and Levene's test for testing homogeneity of variances. Differences among means were detect with LSD at the probability level of 0.05.

Results and discussion

Dry matter yield and growth parameters of perennial ryegrass

Investigation of the effect of type of mixture and N fertilization on the dry matter yield and the relative yield of perennial ryegrass in the mixture, we have found that N fertilization had a significant impact on DMY and RYg in both experimental years, while the mixture, and the interaction of the two studied factors showed significant effect only in the first year of the study (Table 1 and Table 2). In the first production year, perennial ryegrass had significantly higher DM production in 70: 30 PR+RC mixture than in the other mixtures. In all mixtures perennial ryegrass yielded from 2.3 to 3.63 t ha^{-1} respectively. With lucerne ryegrass yielded more at seeding rate 50:50 and with red clover at seeding rate 70:30. In second production year, although there is no significance, red clover in mixtures reduced yield of ryegrass generally. The highest yield, ryegrass achieved in mixture with lucerne 50:50 PR+L. *Gierus et al. (2012)* concluded that ryegrass in mixtures with legumes achieved the highest yield in mixture with white clover, and the lowest in mixture with grazing-type of lucerne. Mixtures with red clover had yield of ryegrass greater then mixture with hay-type lucerne, but the differences were not statistically significance.

Table 1. Impact of N fertilization and mixture type on DMY and RYg of perennial ryegrass in 2015

Mixtures	DMY (t ha^{-1})			Average mixtures	RYg			Average mixtures
	Level of N				Level of N			
	0	50	100		0	50	100	
50:50 PR+L	1.50	2.11	4.84	2.82^{b}	0.66	0.93	1.94	1.18^{b}
50:50 PR+RC	0.96	2.10	3.85	2.30^{b}	0.45	1.02	1.79	1.08^{b}
70:30 PR+L	1.43	2.78	3.28	2.50^{b}	0.35	1.24	1.33	0.98^{b}
70:30 PR+RC	2.92	4.12	3.86	3.63^{a}	1.29	1.86	1.49	1.55^{a}
Average fertilization	1.70^{c}	2.78^{b}	3.96^{a}		0.69^{c}	1.26^{b}	1.64^{a}	
level of significance								
N fertilization	**				**			
Mixture type	**				**			
N x mixture	*				**			

PR-perennial ryegrass, L-lucerne, RC-red clover, DMY-dry matter yield, RYg-relative grass yield; ns- non significant; *- significant at p≤0.05; **-significant at p≤0.01.

The mean RY of the grass component in the mixtures are presented in Table 1. And Table 2. The values of RYg in both years were greater than 1, indicating that DMY of ryegrass were higher than that in pure stand. Only mixture 70:30 PR+L showed less values than 1. RYg of all other mixtures ranged from 1.01 to 1.55 respectively, which means that in this mixtures ryegrass achieved 0.1-55% higher yield than pure ryegrass crop. Ryegrass in mixtures benefited from nitrogen fixation and mostly yielded more than ryegrass monoculture. Researching forage production of *Dactylis glomerata* and *Trifolium subterraneum* mixtures, *Kyriazopoulos et al. (2013)* founded that RY of orchard grass ranged between 0.47 and 1.17. Same as in our study mixture with grass-legume ratio, 75:25 had RY less than 1. Other mixtures with greater proportion of legume had RYg>1 which indicated better interspecific competition of grass as high presence of legume favours its productivity.

Table 2. Impact of N fertilization and mixture type on DMY and RYg of perennial ryegrass in 2016

Mixtures	DMY (t ha^{-1})			Average mixtures	RYg			Average mixtures
	Level of N				Level of N			
	0	50	100		0	50	100	
50:50 PR+L	2.09	3.09	2.94	2.93	1.03	1.37	1.43	1.27^{a}
50:50 PR+RC	1.91	2.51	4.37	2.71	0.87	1.05	2.16	1.36^{a}
70:30 PR+L	2.00	0.93	2.89	2.09	0.89	0.37	1.40	0.89^{b}
70:30 PR+RC	1.51	2.35	2.36	1.94	0.69	1.03	1.31	1.01^{ab}
Average fertilization	1.88^{b}	2.23^{b}	3.14^{a}		0.87^{b}	0.95^{b}	1.57^{a}	
level of significance								
N fertilization	**				**			
Mixture type	ns				*			
N x mixture	ns				ns			

PR-perennial ryegrass, L-lucerne, RC-red clover, DMY-dry matter yield, RYg-relative yield grass; ns- non significant; *- significant at p≤0.05; **-significant at p≤0.01.

Regarding nitrogen fertilization, treatment with 100 kgN ha^{-1} had higher yield of ryegrass then other two treatments. In first production year mixture ryegrass with 100N achieved 3.96 t ha^{-1} and in second year 3.14 t ha^{-1}.Treatment with 50 kgN ha^{-1} also increase yield by 64% in first either 19% in second year compared to zero fertilization.

The average RYg values in both years were under production of pure stand for treatment without N fertilization. Yield of PR in mixtures in treatment with 50kgN ha^{-1}outyielded pure grass in first year by 26% and treatment of 100 kgN ha^{-1} by 64 and 57% respectively. This result indicate that N fertilization increased competitive ability of ryegrass what is in accordance with *Vasquez et al. (2008).*

The growth parameters of ryegrass plants in mixtures are presented In Table 3.

Table 3. LAR, leaf : steam ratio, LAI and SLA of perennial ryegrass in mixtures with legumes and fertilized with N in second investigation year

Mixture	LAR (cm^2 g^{-1})	Leaf : steam ratio	LAI	SLA (cm^2 g^{-1})
50:50 PR+L	95.26^{bc}	0.56^{c}	1.82	295.37^{b}
50:50 PR+RC	119.44^{ab}	0.78^{b}	1.89	322.57^{a}
70:30 PR+L	137.67^{a}	1.18^{a}	1.78	290.20^{b}
70:30 PR+RC	85.48^{c}	0.58^{c}	1.66	251.05^{c}
level of significance	**	**	ns	**
N fertilization				
0	103.74	0.61^{c}	0.83^{c}	265.65^{b}
50	117.90	0.77^{b}	2.07^{b}	277.30^{b}
100	106.75	0.96^{a}	2.46^{a}	326.45^{a}
Level of significance	ns	**	**	**

PR-perennial ryegrass; L-lucerne; RC-red clover;ns- non significant; *- significant at p≤0.05; **-significant at p≤0.01.

Mixture type significantly affected LAR, SLA and leaf : steam ratio. Mixture 70:30 PR+RC had the lowest value of all three parameters while leaf area ratio and leaf: steam ratio were the highest in 70:30 PR+L and SLA in 50:50 PR+RC.

N fertilization did not affect LAR. In research of *Lopes et al. (2011)* abundant N fertilization, significantly reduced LAR what they explained as the result of lower investment in the area of light capture with N fertilization at the expense of investment in the structural components of the plant, as the canopy becomes heavier. In our experiment low doses of N increased LAR, while higher level of N decreased LAR.

Added nitrogen increase proportion of leaves toward steam in range from 0.61 (0 kgN) to 0.96 (100kgN) or leaf proportion increase for 0.0035 for each kg of N, what is very desirable for better quality and digestibility of forages. In research of *Salvador et al. (2016),* N fertilization also increase leaf : steam ratio at the level of

100 kgN ha^{-1}. Further addition of N decline this ratio what authors explained as the result of change the use of this nutrient mostly for inflorescence formation and stem elongation.

Likewise, N fertilization impact on SLA, raising values from 265.65 cm^2 g^{-1} (0kgN) to 326.45 cm^2 g^{-1} (100kgN) respectively.

There was no significant difference in ryegrass LAI between mixtures. Only N fertilization showed significant impact on LAI value. Hence, LAI increase with N fertilization in the range from 0.83 (0 kgN) to 2.46 (100 kgN), respectively. Researching specific leaf area of three dominant perennial grass species in a long term nitrogen fertilization experiment, *Knops and Reinhart (2000)* also concluded that SLA and LAI increased with increasing levels of nitrogen fertilization.

Proportion of perennial ryegrass in mixtures

Botanical composition of the mixtures are presented in Figure 2. In both year proportion of perennial ryegrass was lower than the sown proportions. This fact could be explained with greater competitive ability of red clover and lucerne for light and nutrients in existing agro-climatic conditions.

In first production year proportion of ryegrass was higher in mixtures with lucerne in regard to red clover mixtures. Seeding rate of 50:50 had lower proportion of ryegrass, 22-40%, than seeding rate of 70:30, 42-48%. In second production year share of ryegrass in all mixtures were relatively constant from 22 to 29%. Nitrogen fertilization increased proportion of ryegrass in the mixtures, in the first place treatments with 50N. However, 50N increased ryegrass proportion to 45% in first and to 32% in second year. Increased within 100N is slightly less and varies from 43-26%. *Nyfeler et al. (2009)* also confirmed the fact that grass species proportion in mixtures are positively affected by N fertilization. In research of *Leto et al. (2008)*, N150 treatment increase 29% DM yield of *Dactylis glomerata* and *Poa pratensis* and 9% their contribution to total DM yield in comparison with N0.

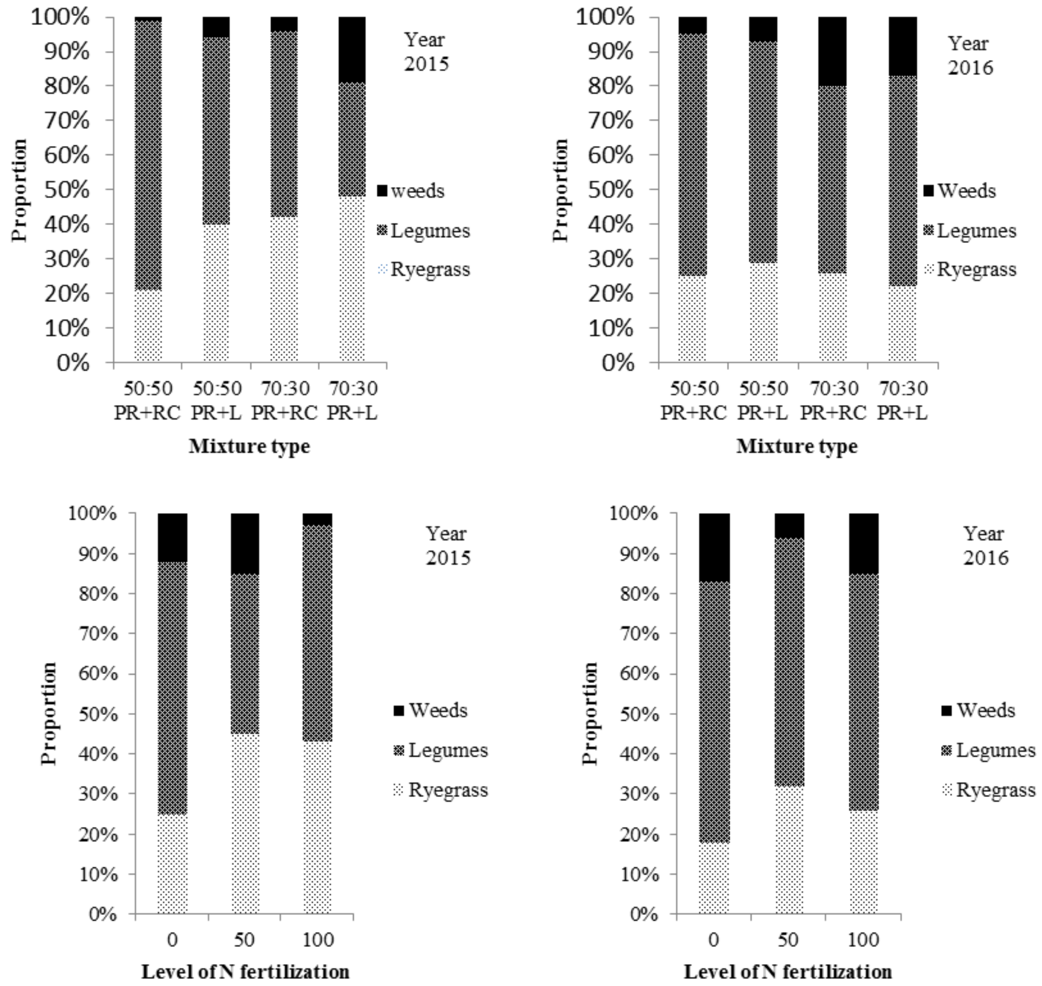

Figure 2. Proportional composition of mixtures of perennial ryegrass, red clover and lucerne, with different level of N fertilization, displayed for two cutting years

Dry matter yield of ryegrass in mixtures with legumes vs. legume proportion

In Figure 3. and Figure 4 were presented ryegrass DM yield relative to legume proportion in mixtures. Distinguishing between levels of N fertilization (Figure3) in 2015, the highest peak of the DMY line, shifts with increasing the proportion of legumes. Increased N fertilization reduced legume proportion to achieve the highest yield *(Nyfeler et al., 2009).* So, in treatment with 100 kgN ha^{-1} the highest yield of ryegrass were achieved when the proportion of legume were from 35-40%. Treatment with 50N gave highest yield with 40-50% legumes proportion and 0N with 60-65% of legumes. In 2016 treatment N treatment gave highest yield when legume proportion were 40-50% and 0 treatment with 60% of legumes.

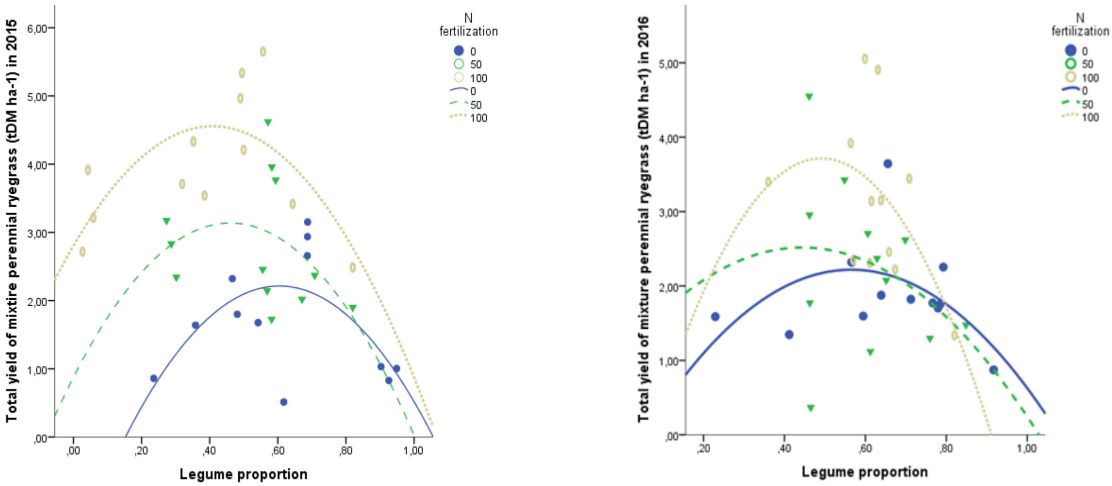

Figure 3. Dry matter yield of perennial ryegrass in mixtures fertilized with different levels on N vs. legume proportion

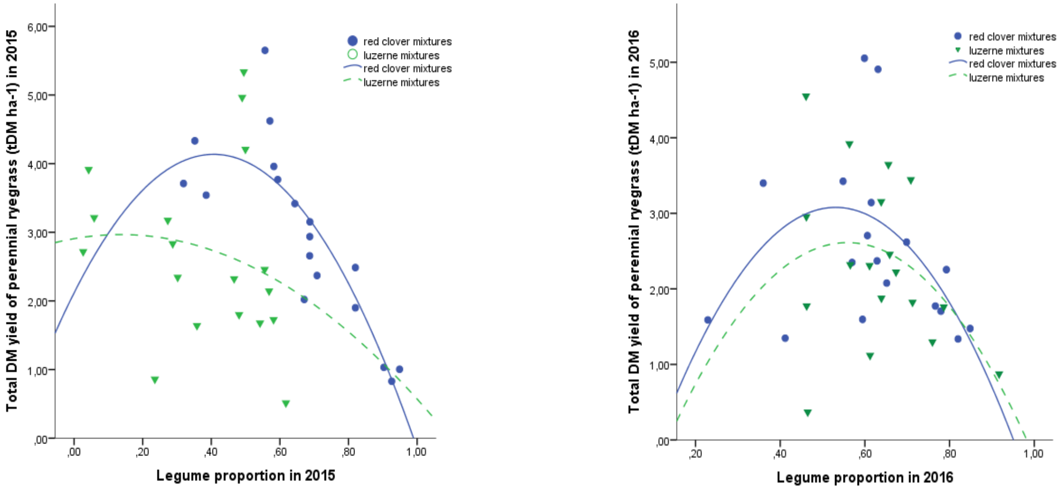

Figure 4. Dry matter yield of perennial ryegrass in mixtures with red clover and lucerne vs. legume proportion

In 2015 ryegrass in mixture with lucerne had highest DMY when the share of lucerne was lower and in 2016 the highest yield was achieved with lucerne proportion of 50-60%. Proportion of 45 and 55 % of red clover in both years effected the greatest DMY of ryegrass. Increase of legume share in mixture will decrease ryegrass abundance, competitiveness and it will have implications in poor utilization of available resources.

Conclusion

In first production year ryegrass achieved the highest yield with lucerne at seeding rate 50:50 and with red clover at seeding rate 70:30. N fertilization increase DMY and RYg in both years thus increasing the competitive capability of perennial ryegrass. Added nitrogen also increase proportion of leaves toward steam, SLA, LAR and LAI. Even though the proportion of grass in the mixture were below the level of sowing rate, fertilization increased the proportion of grasses and particularly doses of 50N. Fertilization rate of 100N achieved the highest yield of ryegrass when the proportion of legume were from 35-40%, rate 50N with legume proportion 40-50% and 0N with 60-65% of legumes. Dry matter of ryegrass was higher in mixture with red clover than with lucerne particularly with red clover proportion of 45-55%. This grass can be grown successfully with red clover and lucerne with the addition of lower doses of nitrogen in order to maintain the legume component in the mixture.

Proizvodni rezultati višegodišnjeg ljulja u binarnim smešama sa lucerkom i crvenom detelinom u uslovima đubrenja azotom

Zorica Bijelić, Violeta Mandić, Vesna Krnjaja, Dragana Ružić-Muslić, Aleksandar Simić, Bogdan Cekić, Violeta Caro-Petrović

Rezime

Višegodišnji ljulj je veoma važna i rasprostranjena vrsta trava koja se koristi za ishranu stoke, naročito preživara. Kao vrsta koja se najčešće koristi na travnjacima, ona se uzgaja u mešavinama sa drugim vrstama trava i mahunarki. Cilj istraživanja bio je istražiti proizvodne rezultate višegodišnjeg ljulja u različitim proporcijama u smešama sa crvenom detelinom i lucerkom, kao i kako različiti nivoi N đubrenja utiču na njegovu konkurentnost. Ljulj je postigao najveći prinos sa lucerkom pri razmerisetvenoj of 50:50 i sa crvenom detelinom, 70:30. Relativni prinos trava (RYg) smeša se kretao od 1,01 do 1,55, što znači da je ljulj u smešama postigao 0.1-55% veći prinos od čistog useva. N đubrenje povećalo je DMY i

RYIg, odnos lista i stabljike, specifičnu lisnu površinu (SLA), odnos lisnih površina (LAR) i indeks lisnih površina (LAI) u obe godine, čime se povećava konkurentna sposobnost višegodišnjeg ljulja.

Ključne reči: višegodišnji ljulj, crvena detelina, lucerka, performanse, konkurentnost

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References

DE WIT C.T. (1960): On competition. Verslag Landbouwkundig Onderzoek, 66, 1-82.

ELGERSMA A., SCHLEPERS H. (1997): Performance of white clover/perennial ryegrass mixtures under cutting. Grass and Forage Science, 52, 134-146.

ERGON A., KIRWAN L., BLEKEN M. A., SKJELVAG A.O., COLLINS R.P., ROGNLI O.A. (2016): Species interactions in a grassland mixture under low nitrogen fertilization and two cutting frequencies: 1. dry-matter yield and dynamics of species composition. Grass and Forage Science, 71, 667-682.

FRAME J., CHARLTON J.F.L., LAIDLAW A.S. (1998): Temperate Forage Legumes. Wallingford: CAB International.

FRASER J., KUNELIUS H.T. (1995): Herbage yield and composition of white/clover associations in Atlantic Canada. Journal of Agricultural Science, 125, 371-377.

GIERUS M., KLEEN J., LOGES R., TAUBE F. (2012): Forage legume species determine the nutritional quality of binary mixtures with perennial ryegrass in the first production year. Animal Feed Science and Technology 172, 150– 161.

KNOPS J., REINHART K. (2000): Specific Leaf Area Along a Nitrogen Fertilization Gradient. The American Midland Naturalist 144 (2), 265-272.

KYRIAZOPOULOS A.P., ABRAHAM E.M., PARISSI Z.M., KOUKOURA Z., NASTIS A.S. (2013): Forage production and nutritive value of Dactylis glomerata and Trifolium subterraneum mixtures under different shading treatments. Grass and forage Science, 68, 72-82.

LAIDLAW A.S., TEUBER N. (2001): Temperate forage grass-legume mixtures: advances and perspectives. Proceedings XIX International Grassland Congress. Brazil, 85-92.

LETO J., KNEŽEVIĆ M., BOŠNJAK K., VRANIĆ M., GUNJAČA G. (2008): Changes in grassland yield and botanical composition under contrasting managements. VII. Alps-Adria Scientific Workshop, Stara Lesna, Slovakia, 867-870.

LOPES M. N., POMPEU R. C. F. F., CÂNDIDO M. J. D., LACERDA C. F. S., RODRIGO G., FERNANDES F. R. B. (2011): Growth index in massai grass under different levels of nitrogen fertilization. Revista Brasileira de Zootecnia, 40 (12), 2666-2672.

NYFELER D., OLIVIER HUGUENIN-ELIE O., SUTER M., FROSSARD E., CONNOLLY J., LÜSCHER A. (2009): Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. Journal of Applied Ecology, 46, 683-691.

SALVADOR P. R., PÖTTER L. R.OCHA M. G., HUNDERTMARCK A. P., SICHONANY M. J. O., AMARAL NETO, L. G., NEGRINI M., MOTERLE P. H. (2016): Sward structure and nutritive value of Alexandergrass fertilized with nitrogen. Anais da Academia Brasileira de Ciências, 88(1), 385-395.

SANDERSON M. (2010): Stability of production and plant species diversity in managed grasslands: A retrospective study. Basic and Applied Ecology, 11, 216-224.

VASQUEZ E., SHELEY R., SVEJCAR T. (2008): Nitrogen Enhances the Competitive Ability of Cheatgrass (*Bromus tectorum*) Relative to Native Grasses. Invasive Plant Science and Management 1(3):287-295.

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