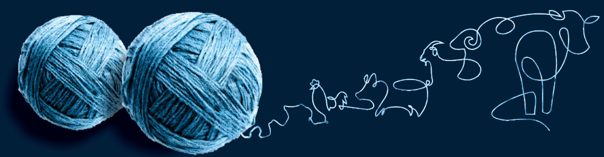
12th INTERNATIONAL SYMPOSIUM

MODERN TRENDS IN LIVESTOCK PRODUCTION



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9 -11 October 2019, Belgrade, Serbia

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MODERN TRENDS IN LIVESTOCK PRODUCTION

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Editor-in-Chief
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Director of the Institute for Animal Husbandry, Belgrade-Zemun

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Food safety, Veterinary Medicine Science

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Language editor

Olga Devečerski, grad.prof

Address of the Editor's office
Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080 Belgrade-Zemun, Republic of Serbia Tel. 381 11 2691 611, 2670 121; Fax 381 11 2670 164;
e-mail: biotechnology.izs@gmail.com; www.istocar.bg.ac.rs

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Institute for Animal Husbandry
Autoput 16, P. Box. 23,
11080, Belgrade-Zemun, Serbia
Tel: +381 11 2691 611; +381 11 2670 121;
+381 11 2670 541;
Fax: + 381 11 2670 164;

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Address:

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Autoput 16, P. Box 23,
11080, Belgrade-Zemun, Serbia

Tel:
+381 11 2691 611
+381 11 2670 121
+381 11 2670 541
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NITROGEN STATUS EVALUATION OF GRASS-LEGUME SWARDS UNDER FOUR N FERTILIZATION LEVELS

Zorica Bijelić[1], Violeta Mandić[1], Vesna Krnjaja[1], Dragana Ružić-Muslić[1], Aleksandar Simić[2], Zdenka Škrbić[1], Dušica Ostojić Andrić[1]

[1]Institute for Animal Husbandry, Belgrade-Zemun, Republic of Serbia
[2]University in Belgrade, Faculty of Agriculture, Belgrade-Zemun, Republic of Serbia
Corresponding author: Zorica Bijelić, zonesh@gmail.com
Invited paper

Abstract: Forage grass-legume mixture are highly productive ambience of different plant species intended for animal nutrition. The competitiveness of species in the mixtures is one of the important traits that significantly affect grassland management. The goal of the study was to evaluate nitrogen nutrition index of pure lucerne and their mixtures with grasses and sainfoin subjected to four different levels of nitrogen in three years duration. The experiment had a randomised block design with four replicates and eight treatments. Examined treatments were pure lucerne crop and mixtures of lucerne, orchard grass, tall fescue and sainfoin in the same proportion and four nitrogen fertilization rates (0, 70, 140, 210 kg ha^{-1}). Pure lucerne achieved higher yield by 12.8% than their mixtures. Forage production was the highest at 210 kgN ha^{-1} which was 14.6% more than treatments without nitrogen. Nitrogen uptake by the plant that were fertilized with N was significantly higher than the plants that were not fertilized. In all three experimental years control nitrogen treatment had satisfactory value of nitrogen nutrition index while other N treatments showed luxury consumption. Soil N reserves and nitrogen fixation in the treatment where N was not applied, were sufficient to ensure enough biomass production.

Key words: biomass production, grass-legume mixtures, nitrogen consumption

Introduction

In addition to water, nitrogen is one of the major limiting factors in production of forage biomass in the natural ecosystems. After mineralization of organic matter in the soil, N becomes available to plants in the form of nitrate and ammonium ions. The amount of available nitrogen in the soil varies from year to year, depending on a number of factors such as: climate, mineralization intensity and amount of organic matter. How much nitrogen will be used from the soil depends on plant species and of its yield potential. Different species in the grass-legumes mixtures have different capabilities for nitrogen accumulation and production of biomass, therefore, N assimilation in grass-leguminous mixtures strongly depends on the selection of species for mixtures. Plant requirements for nutrients are not constant during vegetative growth. After defoliation in the early stages of regrowth, larger quantities of N for the development of leaf mass and photosynthesis are required (*Marino et al., 2004*). Further, the structural composition of plant tissues is dominated by components with low nitrogen contents such as cell walls, which means that the N requirements per unit of dry matter decrease (*Lemaire and Salette, 1984*).

Nitrogen originating from the soil and mostly deriving from mineralization is often not enough for plants to achieve potential yield. Grass-leguminous mixtures also provide N from symbiotic nitrogen fixation where the legumes fix the atmospheric N_2 and create for themselves as well as associated species, conditions with larger N supply. In that way, legumes and grasses meet their own N-demand deriving from symbiosis more than 80% or 40% of N, respectively (*Nyfeler et al., 2011*). The remaining part of the missing nitrogen is recovered from soil nitrogen and N from mineral fertilizers. If the amount of added nitrogen is not fully utilized for yield production, it leads to an increased risk of nitrogen leaching into deeper soil layers, which is a major problem for the environment (*Tripolskaja and Verbyliene, 2014, Marin et al., 2017*). In order to avoid these losses, the French have developed a mathematical model for diagnosis of N status during the vegetative cycle of plants that could be further used for N fertilization planning. This model is based on a critical concentration of nitrogen (Nc) in plants, i.e. a minimum amount of N necessary to achieve maximum yield (*Lemaire and Gastal, 2009*). According to *Lemaire and Gastal (1997)* the equation for calculating Nc for perennial grasses and legumes is:

$$Nc=4.8DM^{-0.32}$$

The content of nitrogen in plants during vegetation is not constant and it changes depending on the vegetative growth of the plant. *Lemaire et al. (1985)* showed that for grasses and lucerne the decline in plant N concentration (%N) is related to dry matter accumulation (DM) whatever the climatic or the species and genotype. Also, *Farruggia et al. (2004)* have concluded that values of Nc at the start of the growing period are high and decline during growth, in relation to dry matter accumulation.

Nitrogen nutrition index (NNI) is defined as the ratio between the actual concentration of nitrogen in the plants and Nc (*Lemaire and Meynard, 1997*):

$$NNI = Nm/Nc$$

According to *Duru et al*. (1997), when NNI is close or equal to 1(0.8-1), the N plant status is considered satisfactory. A deviation from this range, when the NNI is below 0.8, means that there is insufficient supply of N, or when NNI is above 1 there is an oversupply of N: a valueof 0.6 indicating that crop N availability was only 60% of the critical level (*Louarn et al., 2015*).
Today, due to increasing pollution of natural resources, water, air and soil, scientists place the environment protection as the priority, which undoubtedly limits agricultural production. Also, taking into account climate change and increased energy consumption for the production of mineral fertilizers, agriculture carries the global burden of humanity, whether to increase production in order to feed the growing human population or reduce the impact on the environment and warming of the atmosphere. For these reasons, the monitoring of the dynamics of nitrogen adoption during the vegetative cycle of plants and the assessment of the nutrition status is of great importance.
The aim of this study is to evaluate the nitrogen nutrition index of different grass-legume mixtures fertilized with four different levels of nitrogen and to predict their nutritional status through mathematical model.

Materials and Methods

The study was carried out on the experimental field of Institute for Animal Husbandry, Zemun, Belgrade (44°49′N, 20°17′E, and elevation 96 masl), during three year period. At the experimental site, the mean annual precipitation was 714.4 mm and mean annual temperature 16.7°C. The soil is a low carbonate chernozem with pH of 7.26 and well supplied with humus and nitrogen. Agrochemical characteristics of the topsoil arable (0–20 cm) layer, before the experiment, were: $CaCO_3$ – 0.33%, humus – 4.35%, total N- 1975 ppm, P_2O_5- 90.9 mg 100^{-1}, K_2O- 16.2 mg 100^{-1}.
The study plot was previously planted with maize. The experiment was arranged in a completely randomized block design with four replicates. The net plot size was 2 × 5 m. Sowing was done in the early spring after land preparation. Lucerne was sown in monoculture and in mixtures with grasses and sainfoin in a different ratio: lucerne 50% + orchardgrass 50%, lucerne 33.3% + orchardgrass 33.3% + tall fescue 33.3%, lucerne 25% + orchardgrass 25% + tall fescue 25% + sainfoin 25%. Four different nitrogen treatments of 0, 70, 140 and 210 kg ha^{-1} were applied, one

half, early in the spring at the begining of vegetation and the second half, after the first cut. The nitrogen source used was ammonium nitrate (AN) with a N concentration of 34%.
The swards were cut 4 times per year. In three year period, aboveground fresh biomass was measured by cutting the sward in each plot with a beginning of lucerne flowering at approximately 5 cm above ground. Samples of 1 kg were then randomly taken from the cut material and dried in the oven at 60°C for 72 h to determine the dry matter content and the total yield. These samples were used for analyses of N content by using the Kjeldahl method.
The critical N content (Nc) was estimated by applying matematical model developed by *Lemaire and Gastal (1997)*, for temperate grasses and lucerne, while nitrogen nutrition index was calculated as the ratio between the real concentration of N in the plants and Nc, according to *Lemaire and Meynard (1997)*.
Statistical analyses were performed using *Statistica 8 (2007)*. Analyses of variance (ANOVA) were used to test the effects of categorical factors on tested crop properties while differences between treatments means were estimated by the LSD test. The response of NNI to other examined properties, differed among nitrogen levels, is shown graphically on the scatterplot.

Results and Discussion

Total dry matter production was significantly higher for pure lucerne sward and fertilized treatment. In the first study year lucerne mixture with orchardgrass achieved the highiest yied compered to pure lucerne crop and other lucerne mixtures. In further years, the yield of pure lucerne crop was predominant. Our results are in agreement with *Foster et al. (2014)* whose monoculture lucerne crop shows higher DMY than most grass-lucerne mixtures. On the other hand, there are studies where the lucerne mixtures have achieved higher yields than pure lucerne crop (*Malhi et al., 2002*), as well as study which proves that increasing the number of species in the mixture increases the yield (*Papadopoulos et al., 2012*). In agricultural ecosystems, grass–legume mixtures have the potential to increase productivity, herbage nutritive value and resource efficiency (*Peyraud et al., 2009*). Recent results of a great European experiment, with two grasses and two legumes at thirty-one sites, have demonstrated strong positive mixing effects (*Finn et al., 2012*). These findings are based on the complementary utilization of natural resources such as light, water, or nutrients. However, there are studies showing the presence of species in mixtures that are well adapted to the agronomic environment (soils, climate, and management), highly productive and can have major effects on productivity rather than species richness. So, the relationship between biomass productivity and species richness can vary depending on the presence or absence of certain species (*Picasso et al., 2008*). This may have been the case in our study where set of climatic conditions and pedological characteristics have acted more favorably on the development of lucerne compared to the examined grasses.

The forage DM yield of pure lucerne and grass-legume mixtures was significantly affected by N fertilization in all three study year (Table 1). The DM yield increased with increasing N rates. The highest yields were achieved under 210 kgN ha^{-1} while the lowest without N fertilization. *Tomić et al. (2011)* and *Bijelić et al. (2017)* also state that N fertilization significalntly favores the yield of grass-legume mixtures. On the other hand, in some studies, fertilization shows no effect on grass-legume dry matter yields (*Yolcu et al., 2010*). These differences could be explained by differences in climate, soil conditions, supply of N as species characteristics.

There were no statistically significant differences between the crops in N concentration, but only in the first year of testing. In other years, the pure crop of lucerne had significantly higher N content. Concentration of N was in the range 23.9-26.9 g kg $^{-1}$ DM.

According to *Fairey (1991)*, herbage productivity and quality are more influenced by crop-management factors like harvesting frequency and N fertilizer supply than by the species composition of the seeded mixture. In our research fertilization significantly increased N content in forages in every study year. Also, in the study of *Sartor et al. (2014)*, pasture biomass nitrogen content is significantly affected by the addition of nitrogen fertilizers. Addition of 200 and 400 kgN ha^{-1} increases N content by 29.4% and 35.2% respectively. Compared to this, in our experiment, lower doses of nitrogen resulted in a larger N content increase. So, the level of 210 kgN ha^{-1} increased on average 58.8% compared to control. Species in grass-legumes mixtures respond differently to N fertilization (*Martin et al., 2017*). The lack of N response from legumes is due to their N-fixing ability. The N concentration of legumes was unaffected by increasing N-fertiliser rate, whereas in grasses and herbs it increased.

Table 1. Dry matter yield (DM), N concentration and nitrogen nutrition index (NNI) of pure and muxtured crop at different level of added N in three years period.

Years	2010			2011			2012		
Factors	DM (tha^{-1})	N ($gkg^{-1}DM$)	NNI	DM ($t\ ha^{-1}$)	N ($gkg^{-1}DM$)	NNI	DM (tha^{-1})	N (gkg^{-1} DM)	NNI
Mixtures									
M	15.6^{a}	25.1	1.25	16.0^{a}	25.7^{a}	1.28	16.4^{a}	26.9^{a}	1.31
I	16.1^{a}	24.2	1.21	14.1^{c}	24.8^{b}	1.21	14.1^{bc}	25.2^{c}	1.24
II	14.7^{b}	23.9	1.19	14.2^{c}	24.1^{b}	1.19	14.2^{b}	25.7^{bc}	1.28
III	14.6^{b}	24.3	1.19	14.8^{b}	24.9^{b}	1.23	13.3^{c}	26.6^{ab}	1.29
F Prob.	**	NS	NS	**	**	NS	**	*	NS
N fertilization									
0	14.8^{b}	17.3^{c}	0.88^{c}	13.3^{d}	17.9^{d}	0.87^{d}	13.3^{c}	19.6^{c}	0.95^{c}
70	15.3^{b}	25.7^{b}	1.26^{b}	14.2^{c}	24.5^{c}	1.19^{c}	14.9^{b}	26.0^{b}	1.26^{b}
140	14.7^{b}	26.9^{a}	1.32^{ab}	15.0^{b}	27.2^{b}	1.34^{b}	14.1^{b}	29.5^{a}	1.41^{a}
210	16.1^{a}	27.6^{a}	1.39^{a}	16.5^{a}	29.8^{a}	1.51^{a}	15.7^{a}	29.5^{a}	1.51^{a}
F Prob.	**	**	**	**	**	**	**	**	**
Interaction of two factors									
F Prob.	**	NS	NS	**	NS	NS	*	NS	NS

M-pure lucerne, I-lucerne+orchardgrass, II-lucerne+orchardgrass+tall fescue, III-lucerne+orchardgrass+tall fescue+ sainfoin
* F statistic significant at the 0.05 probability level,
** F statistic significant at the 0.01 probability level,
NS, means nonsignificant.

Type of mixtures showed no significant impact of NNI values of crops. Pure lucerne sward had higher values of NNI compared to its mixtures. Also mixtures with high contribution of legumes had greater values of NNI. This fact could be attributed to nitrogen fixation. Also, *Razec and Razec (2006)*, show in their research that values of NNI increase with the increase of legume content in the mixture. Lucerne like other examined legumes has great positive influence on the improvement of sward N supply (*Kadžiuliene and Kadžiulis, 2007*). However, lucerne-based swards without N fertilization reache indices close to 1.0.

Nitrogen nutrition index of grass-legumes mixtures were generally significantly influenced by N fertilization across all investigation years. Values of the NNI from 0.8 to equal to 1.0 indicate that the crop is in the situation of nonlimited N supply. Treatment without N fertilization had NNI values from 0.88-0.95 which is considered optimum for supply of N. After successive nitrogen application from 70-210 kgN ha^{-1} lucerne and their mixtures start to show luxury consumption (Figure 1.).

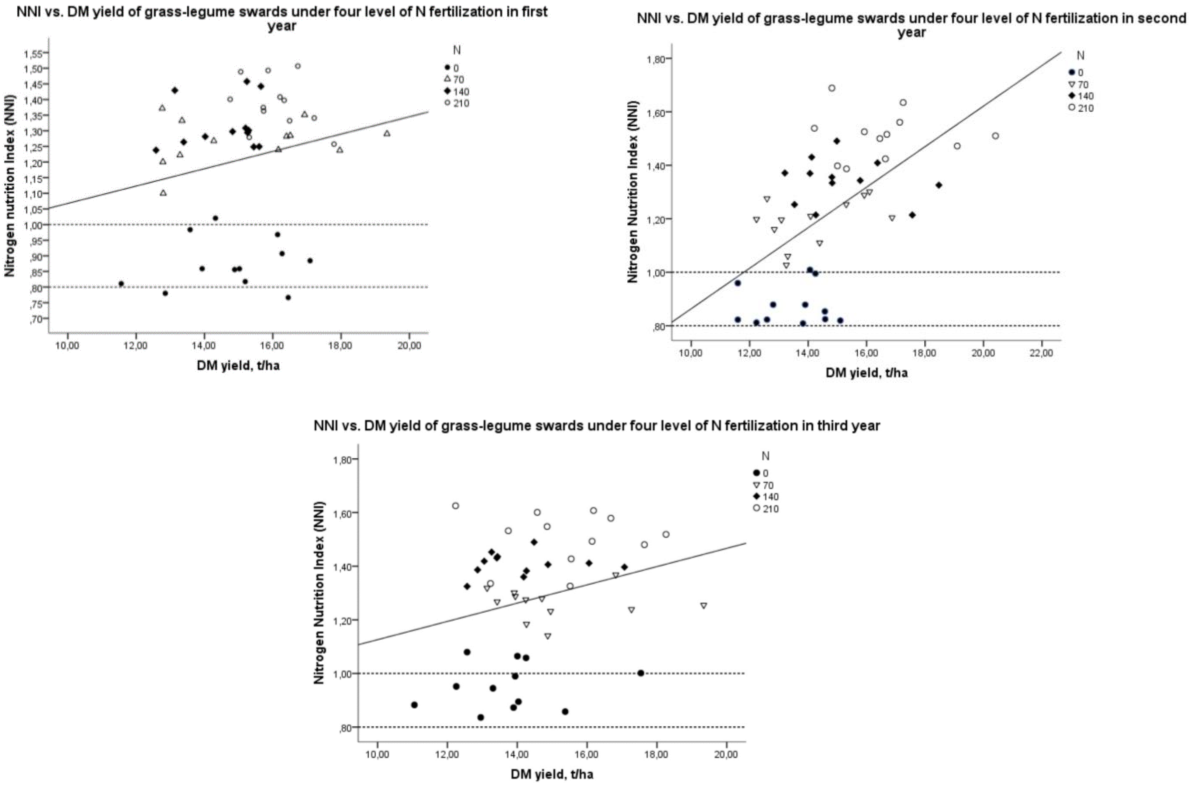

Figure 1. NNI vs. DM yield of grass-legume swards under four different levels of N fertilization in three years period.

This situation showed that the swards were grown in a good soil conditions of nitrogen supply which together with the nitrogen fixation provides optimal environment for growth and development of plants. Also, applied fertilizer can contribute to accelerating the process of decomposition of organic matter, the release of nutrients and the increase of their availability for the plants. Similar results is reported by *Sartor et al. (2014)* where natural pasture of *Urochloa plantaginea* under fertilization with two N levels of 200 and 400 kg ha^{-1}, shows consumption beyond what would be nedeed for its growth. On the other hand, 50 years old sward, with dominant species of *Agrostis* spp., *Holcus lanatus* and *Lolium perenne*, have not identified excessive values of the NNI for any fertilization level (*Farrugia et al., 2004*). The NNI observed for the 120 and 60 kgN ha^{-1} treatments were between 1.0 and 0.8.

Conclusion

In given agroecological conditions, the pure lucerne and the two-component mixture of alfalfa and orchardgrass yielded more than the other lucerne swards. Also, pure lucerne crop had significantly higher N content in relation to its mixtures.
N fertilization significantly increased DM yield of pure lucerne and grass-legume mixtures as well as the content of N in plants.
In grasslands, NNI has proved to be a useful diagnostic tool of the N status. Pure lucerne sward had higher values of NNI compared to their mixtures. Also mixtures with high contribution of legumes had greater values of NNI. NNI values were significantly influenced by N fertilization. Treatment without N fertilization had NNI values from 0.88-0.95 which indicated that the crop was in the situation of nonlimited N supply. Nitrogen treatment of 70, 140 and 210 kgN ha^{-1} showed luxury consumption.
We can generally conclude that the sward crops were grown under conditions of good nitrogen supply. Possibly, the addition of a small quantities of N at the initial growth stages would only be justified.

Evaluacija azotnog statusa travno-leguminoznih smeša pod uticajem četiri nivoa đubrenja

Zorica Bijelić, Violeta Mandić, Vesna Krnjaja, Dragana Ružić-Muslić, Aleksandar Simić, Zdenka Škrbić, Dušica Ostojić-Andrić

Rezime

Smeša krmnih trava i mahunarki je visoko produktivna sredina različitih biljnih vrsta namenjenih za ishranu životinja. Konkurentnost vrsta u smešama je jedna od važnih osobina koje značajno utiču na upravljanje travnjacima. Cilj studije bio je da se proceni indeks ishrane azotom čiste lucerke i njenih smeša sa travama i esparzetom pod uticajem četiri različita nivoa azota u trajanju od tri godine. Eksperiment je imao randomizirani blok dizajn sa četiri ponavljanja i osam tretmana. Ispitivani tretmani bili su čist usev lucerke i smeše lucerke, ježevice, visokog vijuka i esparzete u istom odnosu i četiri doze azotnog đubrenja (0, 70, 140, 210 kg ha-1). Čista lucerka je postigla veći prinos za 12,8% u odnosu na njene smeše. Proizvodnja krme bila je najveća sa 210 kg ha-1, što je za 14,6% više od tretmana bez azota. Uzimanje azota od strane biljke koja je đubrena N je bila značajno viša od biljaka koje nisu bile đubrene. U sve tri eksperimentalne godine kontrola je imala zadovoljavajuću vrednost indeksa ishrane azotom, dok su ostali N tretmani pokazali preteranu potrošnju. Rezerve N u zemljištu i fiksacija azota u tretmanu gde N nije primenjen, bili su dovoljni da osiguraju dovoljnu proizvodnju biomase.

Ključne reči: produkcija biomase, travno-leguminozne smeše, potrošnja azota

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