FORAGE QUALITY AND ENERGY CONTENT OF PERENNIAL LEGUME-GRASS MIXTURES AT THREE LEVEL OF N FERTILIZATION

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Abstract: The aim of this study was to investigate fodder quality and nutritive value of different grass-legumes mixtures influenced by various level of N fertilization. Studied factors had an impact only on the content of crude protein (CP), crude protein yield (CPY) and nitrate content in the forage. The level of N fertilizer showed a highly significant and positive impact on the CP and nitrate content. Treatment with 210 kg N ha^{-1} is characterized by the highest content of CP and nitrate of 189.7 g kg^{-1} DM and 2524 ppm, respectively, and the highest protein yield of 1.95 t ha^{-1}. The value of nitrate in the forage does not exceed the limit that is considered hazardous to the health of animals. Energy value of forage obtained from the grasslands of ME ≈7.75 and NEL≈4.32 MJ kg^{-1} DM is lower than values obtained in other studies.

Key words: lucern,grass, mixture, quality, energy content

Introduction

Lucerne is one of the most commonly used legumes for animal feeding in Serbia because of its high yielding, good nutritional quality, resistance to drought, uniform yield during the growing season and ability to fix nitrogen. Total area on which it is cultivated is 176 178 ha, of which 128 495 ha in Central Serbia, and 47 683 ha in Vojvodina. Total dry matter production, in four or five swaths per year, ranges evenn over 18 tonnes per hectare. The resulting hay, depending on the time of cutting and way of preserving, is of excellent quality with high crude protein content (18-22%), excellent digestibility, favorable amino acid composition with a high content of minerals, especially calcium and phosphorus, carotene and vitamins. Grass-legume mixtures are rarely used in production, mainly as a mixture of field peas or vetch with grains (*Avena sativa* L.). The reason for this is absence

of knowledge of the benefits of growing mixtures as compared to pure crop. Mixtures offer several adventages over pure crops. These adventage include the possibility of good fermentation and preparation of high quality silage (*Dinić et al., 1996*), the successful grazing without the risk of bloat occurrence, prolonged stand longevity, the control of erosion, weed control (*Casler and Walgenbach, 1990*) and reduced use of N fertilizer which is useful for solving the problem of N surplus (*Danso et al., 1991*). N fixation of legume species can be enhanced with competition from nonlegume species (*Pirhofer-Walzl et al., 2012*). Lucern usually grown with grasses similar morpho physiological traits like cocksfoot, tall fescue, perennial ryegrass, meadow fescue. In the research of *Albayrak et al. (2011),* mixture of lucerne and sainfoin with grasses gave fodder of high quality, that were not significantly different from pure legume crops in regard to the content of ADF, NDF and TDN. The crude protein content in the mixture of lucerne and grasses was not significantly different (15.42 to 16.50%) from the content of CP in pure crop of sainfoin (16.30 to 17.45%). Quality of feed produced from grassland is primarily influenced by the components, species and the cultivares, the proportion of their participation in the mixture and the level of nitrogen fertilization (*Samuil et al., 2012*).

The aim of this study was to investigate fodder quality and nutritive value of different grass-legumes mixtures influenced by various level of N fertilization.

Matherials and methods

The experiment was conducted in 2006 and 2007 and located on the experimental field of Institute for Animal Husbandry (44° 49′ 10″ N, 20° 18′ 45″ E) with an average annual rainfall of 693 mm. The soil type was low carbonate chernozem, with pH 7.18, humus 3.25% and organic mater 6.44%. The experiment was arranged in split plot design, in four repetitions, with a plot size of 2 m x 5 m. The main experimental factor was the type of fodder mixture and the second different level of nitrogen fertilization. Seeds of the lucerne *Medicago sativa* L. (cv. K-28) was sown individually and in the mixture with perennial grass species *Dactylis glomerata* L. (cv. K-40), *Festuca arundinacea* Schreb. (cv. K-20) and with perennial legume species *Onobrychis sativa* L. (cv. Krajina).

Table 1. Studied fodder mixture sructure

Species	Mixture structure %			
	L	L+C	L+C+F	L+C+F+S
Medicago sativa L.	100	50	33.3	25
Dactylis glomerata L.	-	50	33.3	25
Festuca arundinacea Schreb.	-	-	33.3	25
Onobrychis sativa L.	-	-	-	25

L-lucern; C-cocksfoot; F-tall fescue, S-sainfoin.

The plots were sown in the spring, 2005, with sowing rate of 20 kg ha^{-1} lucern, 30 kg ha^{-1} cocksfoot, 25 kg ha^{-1} tall fescue and 140 kg ha^{-1} sainfoin.

There were four levels of nitrogen fertilization: control with no nitrogen, 70 kgN ha^{-1}, 140 kgN ha^{-1} and 210 kgN ha^{-1}. Nitrogen fertilizers were applied twice, half the amount at the beginning of vegetation and half after first cut.

Cutting in the experiment was done in phase of one third of blooming of lucerne flowers. Samples of 1 kg for chemical analysis were taken after cutting, and were dried at a temperature of 105°C.

To determine the chemical composition of the fodder the following methods of analysis were used: the Kjeldahl method to determined crude protein content, the Wende method for crude fiber analysis and nitrate with colorimetric method. Metabolizable energy (ME) and net energy for lactation (NEL) were calculated by formula acording Obračević (1990):

$GE(MJ\ kg^{-1}) = (0.02414\ x\ CP) + (0.03657\ x\ Cf) + (0.02092\ x\ CF) + (0.01699\ x\ NFE)$

$ME(MJ\ kg^{-1}) = (0.01715\ x\ dCP) + (0.03766\ x\ dCf) + (0.0138\ x\ dCF) + (0.01464\ x\ dNFE)$

$NEL(MJ\ kg^{-1}) = ME\ x\ kl$

$kl = 0.6\ x\ (1 + 0.004\ x\ (q-57))\ x\ 0.9752$

$q(\%) = (ME\ /\ GE)\ x\ 100$

CP - crude protein (g kg^{-1});
Cf - crude fat (g kg^{-1});
CF – crude fibre (g kg^{-1});
NFE – nitrogen free extracts (g kg^{-1});
d – digestible
q - metabolizability coefficient

The experimental data were processed by the method of analysis of variance (two factor experimental design), applying the programme ANOVA and means were compared using Fisher's protected least significant difference (LSD) test. A linear regression was also carried out between the content of CP, nitrate and level of N fertilization. Results of the variance analysis are presented in tables as averages over two years of research by individual factors, and regression analysis are presented graphically.

Results and discussion

The crude protein content of the fodder significantly increased, as the aplication of the N fertilizer increased. The highest CP was recorded in treatment with the highest N rate (210 kg ha^{-1}) of 189.7 g kg^{-1} and the lowest in control treatment of 170.2 g kg^{-1}. *Komarek et al. (2007)* and *Tomić et al. (2012)* have

proved in their research that the crude protein content in forage of grass - legume mixtures progressively increases with the addition of N fertilizers. Although the type of the fodder mixture did not have a significant influence on the content of crude protein, the degradation of the contents of the CP according to the share of legumes in the mixture is observed, which is in agreement with the research of *Mika et al. (2004).* Protein yield changed significantly compared to the tested factors and their interaction. Pure lucerne crop had significantly higher protein yield as compared to lucerne mixtures. The two-component mixture of lucerne and cocksfoot stands out in regard to the yield of protein of 1.62 t ha^{-1}. Contrary to the present study, in the research of *Lättemäe and Tamm (1997),* CPY did not show statistically significant differences between individual species and mixtures thereof. The highest yield of CP, has been realized by mixture of white clover and grasses of 1.54 t ha, which is less compared to the average yield of CP of examined grasslands. The mixture of white clover and grass with 1.54 t ha of CP stands out. CPY increased progressively with the addition of N fertilizer. Treatment with 210 kg N ha^{-1} gave a significantly higher CP yield than the control and fertilization with lower quantities of N.

Table 2. Content of crude protein (g kg^{-1}), yield of crude protein (t ha^{-1}) depending on the type of fodder mixture and N fertilization

	CP (g kg^{-1})				Mean	CPY (t ha^{-1})				Mean
	0	70	140	210		0	70	140	210	
L	175.2	183.4	191.5	194.8	186.2	1.62	1.98	2.00	2.30	1.97[a]
L+C	170.1	173.7	174.1	191.4	177.3	1.48	1.51	1.54	1.94	1.62[b]
L+C+F	163.4	169.3	175.2	184.7	173.2	1.35	1.42	1.55	1.92	1.57[bc]
L+C+F+S	172.2	175.4	183.2	187.9	179.7	1.32	1.59	1.65	1.62	1.55[c]
Mean	170.2[b]	175.4[b]	181.0[ab]	189.7[a]		1.44[c]	1.63[b]	1.69[b]	1.95[a]	
Level of significance										
Mixture	ns					**				
Level of N	**					**				
Mixture x Level of N	ns					**				

L-lucern; C-cocksfoot; F-tall fescue, S-sainfoin; CP- crude protein; CPY- crude protein yield; ns- non significant, *- significant at p≤0.05; **-significant at p≤0.01.

The results of crude fiber (CF) are presented in Table 3. The examined factors had no significant impact on the content of CF. In spite of this, CF content had a slight tendency of increase with the increase in the share of grasses in the mixture. The minimum CF content was recorded for lucerne monoculture of 290.0 g kg^{-1}, and the highest in cocksfoot and lucerne mixture of 300.1 g kg^{-1}. Fertilization also had a significant impact, but there are some differences between the control and treatment with the least amount of nitrogen and the other two treatments. Large amounts of N fertilization decreased the content of CF in monoculture and mixture. Also *Tomić et al. (2012)* concluded that type of fodder crop and level of N fertilization didn′t have significant influence on the CF content.

Nitrogen from the soil is taken up by plant in the form of nitrate. Plants convert nitrate (NO_3) to nitrite (NO_2) which converted to ammonia and then to amino acids and proteins. However, in certain conditions like prolonged cool temperatures, shade, disease and high levels of soil nitrogen, the roots will accumulate nitrate faster than the plant can convert the nitrate to protein. That nitrate can cause noninfectious disease called nitrite poisoning. According to *ARC (1980),* if the values of nitrate in the forage exceed 3000 ppm, the forage is considered potentially dangerous and should be avoided in certain categories of animals. The nitrate content in the observed mixtures increased proportionately relative to the quantity of added N fertilizer (Table 3). The control treatment had the lowest nitrate content of 956 ppm, the highest content of 2524 ppm treatment with 210 kgN ha^{-1}. Although fertilization increases the content of nitrate up to 164%, the value of nitrates does not exceed values that are regarded as hazardous to animal nutrition.

Table 3. Content of crude fibre (g kg^{-1}) and nitrates (ppm) depending on the type of fodder mixture and N fertilization

	CF (g kg^{-1})				Mean	Nitrate (ppm)				Mean
	0	70	140	210		0	70	140	210	
L	283.7	282.1	276.7	282.8	290.0	869	1180	1640	2150	1460
L+C	286.8	298.7	294.0	297.6	300.1	999	1289	1636	2694	1655
L+C+F	289.5	288.3	287.8	287.1	299.0	988	1400	1618	2590	1649
L+C+F+S	297.8	290.6	288.9	285.0	299.6	969	1287	1847	2663	1691
Mean	289.4	289.9	286.9	288.1		956[c]	1289[bc]	1685[b]	2524[a]	
Level of significance										
Mixture	ns					ns				
Level of N	ns					**				
Mixture x Level of N	ns					ns				

L-lucern; C-cocksfoot; F-tall fescue, S-sainfoin; CF- crude fibre; ns- non significant, *- significant at p≤0.05; **-significant at p≤0.01.

The content of metabolizable energy (ME) was fairly uniform according to individual factors of observation. It ranged from 7.73 to 7.80 MJ kg^{-1}, depending on the type of the mixture and from 7.65 to 7.86 MJ kg^{-1}, depending on the level of fertilization. Net energy for lactation (NEL) also showed no significant variation with respect to the investigated factors. Values ranged from 4.22 MJ kg^{-1} in lucerne without fertilization to 4.43 MJ kg^{-1} in lucern and cocksfoot mixture, fertilized with 210 kgN ha^{-1}. Energy value of the tested mixture was lower than the value obtained by other researchers, for instance *Lättemäe and Tamm (1997),* reporting the ME content of grass-legume mixtures on average 10.6 MJ kg^{-1} DM, and *Mika et al. (2004)* reporting the content of NEL of 5.85 - 6.10 MJ kg^{-1} DM. According to *NRC (2001),* the ME requirements of pregnant heifers (450 kg mature weight) are 87.4 - 109.2 MJ day^{-1}, and cows in early lactation, which give 30 liters of milk with 4%

milk fat, NEL 125 MJ day $^{-1}$. From the results we can conclude that pregnant heifers must consume 11-14 kg day^{-1} of DM (12-16.7 kg hay at 15% moisture) to meet the daily requirements for ME and cows in early lactation 29 kg day $^{-1}$ of DM (34 kg hay at 15% moisture) to meet the NEL needs.

Table 4. The content of metabolizable energy (MJ kg^{-1}) and net energy for lactation (MJ kg^{-1}) depending on the type of fodder mixture and N fertilization

	ME (MJ kg^{-1})				Mean	NEL (MJ kg^{-1})				Mean
	0	70	140	210		0	70	140	210	
L	7.58	7.76	7.76	7.82	7.73	4.22	4.33	4.33	4.36	4.31
L+C	7.60	7.67	7.73	7.94	7.73	4.24	4.27	4.31	4.43	4.31
L+C+F	7.64	7.71	7.73	7.87	7.74	4.26	4.30	4.31	4.39	4.31
L+C+F+S	7.77	7.78	7.85	7.81	7.80	4.33	4.34	4.38	4.36	4.35
Mean	7.65	7.73	7.77	7.86		4.26	4.31	4.33	4.38	
Level of significance										
Mixture	ns					ns				
Level of N	ns					ns				
Mixture x Level of N	ns					ns				

L-lucern; C-cocksfoot; F-tall fescue, S-sainfoin; ME- metabolizable energy; NEL- net energy for lactation; ns- non significant, *- significant at p≤0.05; **-significant at p≤0.01.

The results of regression analysis are shown in Figure 1 and Figure 2. The level of N fertilization is in a positive linear relationship with both quality parameters, the content of CP and NO_3 in the forage.
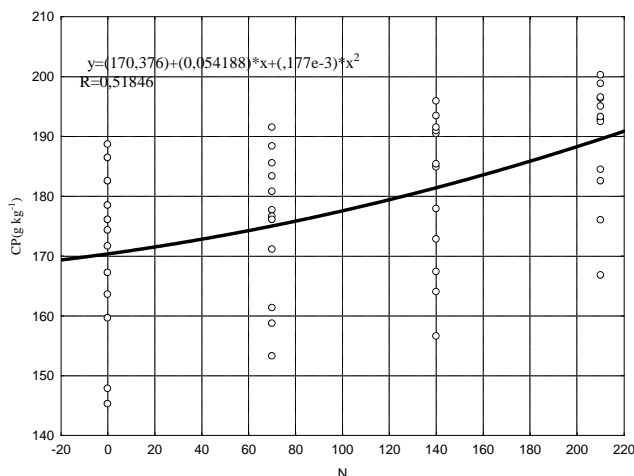

Figure 1. Regression analysis of crude protein content (g kg^{-1} DM) relative to N fertilization
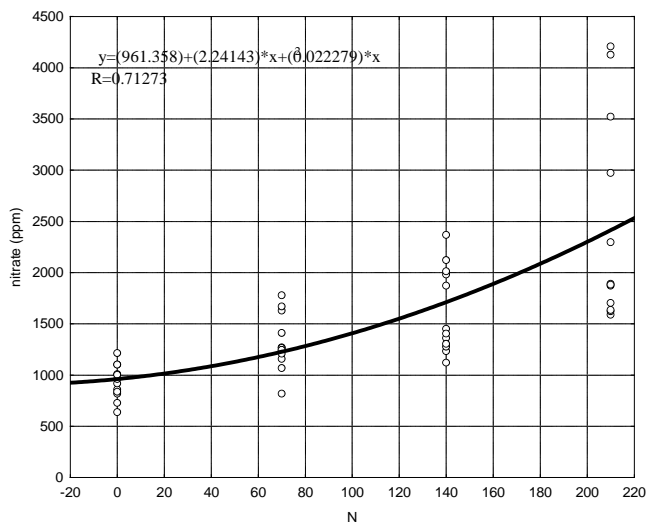
Figure 2. Regression analysis of nitrate content (ppm) relative to N fertilization

The changes in the content of crude protein with a coefficient of determination of $i^2 = 26.88\%$ are explained by changes in the utilization of N mineral fertilizer. A medium positive dependence of 0.51846 (Figure 1) is observed between the studied parameters. The changes in the content of nitrate with $i^2 = 50.80\%$ are explained by changes in the utilization of N mineral fertilizers, and a medium positive dependence of 0.71273 (Figure 2) is determined between the investigated parameters.

Conclusion

Examining the impact of the structure of forage mixture and the level of N fertilization on the quality and energy value of feed, we can conclude that the studied factors had impact only on the CP content, protein yield and nitrate content in the forage. The level of N fertilizer showed a highly significant and positive impact on changes of the CP and nitrate content. Nitrate levels in the forage did not exceed the critical limit considered harmful to the health of certain categories of animals.

Reducing the share of lucerne in the mixture and increasing levels of N fertilizer increased the yield of protein.

Energy value of the tested mixtures was relatively low, so in order to meet the energy requirements of animals it is necessary, in addition to lucerne hay and its mixtures, to include concentrated feed and silage of better energy performance in the animal diet.

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Rezime

Cilj ovog istraživanja je bio da se ispita kvalitet stočne hrane i hranljiva vrednost različitih travno-leguminoznih smeša pod uticajem različitih nivoa N đubrenja. Ispitivani faktori imali su utisaja samo na sadržaj sirovih proteina (CP), prinos proteina (CPY) i sadržaj nitrata u krmi. Nivo N đubriva ispoljio je visoko značajan i pozitivan uticaj na promunu sadržaja CP i nitrata. Tretman sa 210 kgN ha^{-1} karakteriše se najvećim sadržajem CP i nitrata od 189,7 g kg^{-1} DM odnosno 2524 ppm kao i najvećim prinosom proteina od 1,95 t ha^{-1}. Vrednost nitrata u krmi ne prelazi limit koji se smatra opasnim za zdravstveno stanje zivotinja. Energetska vrednost krme dobijene sa travnjaka je niži u odnosu na druga istraživanja i iznosi za ME ≈7,75 i NEL≈4,32 MJ kg^{-1} DM.

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