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## Determining the content of nitrogen and some macroelements in the dry mass of perennial leguminous plants

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#### **Abstract**

The aim of this study was to determine the content of elements N, P, K, Ca, and Mg in the dry mass of perennial fodder legumes, mowed in the optimal growth phase. The experiment with perennial legumes of alfalfa (Medicago sativa), red clover (Trifolium pretense) and bird's-foot trefoil (Lotus corniculatus L.) was set up as a random block system in four replicates on valley-brown soil and was conducted for two years. The green mass of perennial legumes in the first and second mowing was harvested at the end of budding/ beginning of flowering phase. In the third and fourth regrowth, mowing of the biomass was done after 5 weeks. Immediately after each mowing, representative sample of green biomass was taken from each replicate of each legume tested. The content of nitrogen, phosphorus, potassium, calcium, and magnesium in the dry plant material was determined. The content of nitrogen and potassium in the fodder of alfalfa and red clover was higher in the first year of the study, which was not the case for bird's-foot trefoil. The dry matter of alfalfa on average had the highest content of nitrogen (39.8 g kg<sup>-1</sup> DM) and calcium (19.6 g kg<sup>-1</sup> DM), bird's-foot trefoil of phosphorus (10.6 g kg<sup>-1</sup> DM) and potassium (26.1 g kg<sup>-1</sup> DM), and red clover of magnesium (3.7 g kg<sup>-1</sup> DM). In the dry mass of bird's-foot trefoil the average content of nitrogen was 39.0 g kg<sup>-1</sup> DM, phosphorus 10.6 g kg<sup>-1</sup> DM, potassium 26.1 g kg<sup>-1</sup> DM, calcium 15.7 g kg<sup>-1</sup> DM, and magnesium 3.5 g kg<sup>-1</sup> DM.

Key words: nitrogen, macroelements, alfalfa, red clover, bird's-foot trefoil

#### Introduction

Perennial leguminous fodder plants are grown on a substantial area of arable land in Bosnia and Herzegovina and are of great importance for the livestock nutrition. This type of feed contains nitrogen and other macro and microelements, which are not easy to supplement animals with, due to difficulties in regulating their consumption, utilization, and toxicity (Marković et al., 2007). The content of macro and microelements in pasture forage is especially important for ruminant breeders (Durek et al., 2020). Deficiency of one or several macroelements can cause physiological and metabolic disorders in animals. In order to ensure high livestock productivity, in addition to a sufficient amount of fodder, it is important to provide minerals essential for physiological processes. Animals mostly meet their needs for macroelements from fodder (green mass, hay, haylage, silage) produced on the farm. The content of macroelements in plant fodder depends on plant species, soil characteristics, and applied agrotechnical measures (Živkov-Baloš et al., 1999). Perennial leguminous fodder plants differ in the content of macroelements, their concentration depending on the type and method of fertilization. Alfalfa, red clover, and bird's-foot trefoil have different ability to accumulate certain macroelements in the green mass. Fodder plants grown on soils with a high content of heavy metals can absorb them in larger quantities and accumulate in productive organs. Such fodder should not be used in the livestock diet because its products (meat, milk, eggs) would not be suitable for human consumption (Lakić et al., 2020). The mineral content in the plant leaves slightly changes during vegetation, while mineral content in the plant stems changes significantly with aging (Åman, 1985). Alfalfa and red clover leaves have 2-3 times higher nitrogen content compared to the stems (Wilman and Altimimi, 1984). The phosphorus content in the green fodder of alfalfa, red clover, and bird's-foot trefoil is highest in the early stage of plant development, prior to flowering stage. The concentration of potassium, which is important in the livestock diet, is highest in young plants (Vučković, 2004). With the aging of fodder plants, the potassium content in the fodder decreases. Green mass obtained from the plants in the early stage of development has the highest content of calcium and magnesium, elements important for animal bones. Leguminous plants, other than lupins (*Lupinus* spp.), have increased calcium requirements (Heris et al., 1968). Macroelements phosphorus and calcium are important for the tissue structure, osmotic pressure maintenance and they also affect the acidity, while the state of colloids is affected by potassium, calcium and magnesium (Pavlović-Trajković et al., 1996).

The aim of this study was to determine the content of macronutrients N, P, K, Ca, and Mg in the feed prepared from perennial forage legumes, harvested in the optimal stage of development.

### Material and Methods

The experiment with perennial legumes of alfalfa, red clover, and bird's-foot trefoil was set up as a random block system in four replicates on valley-brown soil on the alluvial substrate of the Vrbas river, Bosnia and Herzegovina. The tests were performed in the experimental field Delibašino selo and in the laboratories of the PI Agricultural Institute of Republic of Srpska, Banja Luka, in the period 2011-2013. The green mass of perennial legumes in the first and second cut was mowed at the end of budding/beginning of flowering phase. In the third and fourth regrowth, fodder crops were mowed every 5 weeks. Alfalfa and red clover were mowed four times in both years of testing, and bird's-foot trefoil three times. Immediately after mowing, an average sample of green biomass was taken, from each replicate of every legume tested. After weighing, biomass samples were dried in an oven at 60°C. After drying, the samples were ground in a hammer mill, packed in bags, and then used for determination of the macroelements content.

The content of nitrogen, phosphorus, potassium, calcium, and magnesium in the dry plant material was determined. The amount of N was determined by the Kjeldahl method - Bremner modification (1960). Total P was determined spectrophotometrically, using standard ISO 6491 method with molybdenum - vanadate reagent. Potassium was determined by measuring the emission intensity at a wavelength of 766 nm, using an AAS (atomic absorption spectrometer). The macroelements Ca and Mg were determined by AAS with the addition of lanthanum salt for ionization control, at 422.7 and 202.6 nm.

The obtained results were processed by analysis of variance, and the significance of the differences between the mean values was determined using the LSD test.

#### **Results and Discussion**

The results presented in Table 1 show that the dry mass of the examined perennial legumes had the highest content of nitrogen, followed by potassium and calcium. The phosphorus and magnesium content in the forage of legumes was significantly lower. Also, content of all tested macroelements in the feed varied during the experiment.

Tab. 1. The analysed elements content of perennial legumes (g kg<sup>-1</sup> DM) by years of testing

Perennial legume	Elements	N (g kg <sup>-1</sup> DM)		P (g kg <sup>-1</sup> DM)		K (g kg <sup>-1</sup> DM)		Ca (g kg <sup>-1</sup> DM)		Mg (g kg <sup>-1</sup> DM)		
	Year/ cut	I year	II year	I year	II year	I year	II year	I year	II year	I year	II year	
Alfalfa	Average of the I cut	40.1	39.2	10.0	9.0	23.0	22.0	21.0	22.0	3.0	2.4	
	Average of the II cut	39.1	38.1	11.0	13.0	23.0	23.0	20.0	16.0	3.2	3.1	
	Average of the III cut	41.2	38.8	7.6	9.0	33.0	21.0	17.0	19.0	2.9	3.2	
	Average of the IV cut	42.3	39.5	6.4	10.0	30.0	18.0	18.0	24.0	3.0	3.5	
	Average by years	40.7	38.9	8.8	10.3	27.3	21.0	19.0	20.3	3.0	3.1	
	Average	39.8		9.5		24.2		19.6		3.05		
LSI	LSD 0.05 0.01		1.9 2.8		2.5 3.6		8.4 12.5		4.6 6.8		0.6 0.9	
Red clover	Average of the I cut	33.9	26.0	8.0	9.0	26.0	21.0	17.0	13.0	3.8	2.5	
	Average of the II cut	35.8	36.2	9.0	12.0	20.0	23.0	18.0	15.0	4.2	3.6	
	Average of the III cut	36.6	34.7	11.0	10.0	27.0	18.0	18.0	15.0	3.7	4.2	
	Average of the IV cut	40.1	36.0	8.0	9.0	26.0	20.0	14.0	14.0	3.6	3.9	
	Average by years	36.6	33.2	9.0	10.0	24.8	20.5	16.8	14.8	3.8	3.6	
	Average	34.9		9.5		22.6		15.8		3.7		
LSD 0.05 0.01		4.2 6.2		1.9 2.9		6.1 9.0		2.2 3.2		1.0 1.5		
Bird's-foot trefoil	Average of the I cut	38.9	42.9	9.0	12.0	26.0	28.0	18.0	11.5	3.7	2.8	
	Average of the II cut	35.6	40.1	10.0	12.0	26.0	27.0	17.0	15.0	3.8	3.1	
	Average of the III cut	39.5	37.4	11.0	10.0	24.0	26.0	20.0	12.0	4.0	3.4	
	Average by years	38.0	40.1	10.0	11.3	25.3	27.0**	18.6**	12.8	3.9**	3.1	
Average		39.0		10.6		26.1		15.7		3.5		
LSD 0.05 0.01		3.2 4.8		1.8 2.6		0.6 0.8		1.5 2.3		1.5 2.2		

There was no statistically significant difference in the content of the tested macronutrients in the fodder of alfalfa and red clover during two years of the experiment (Table 1). However, content of K, Ca, and Mg in the dry mass of bird's-foot trefoil was significantly different in the first year of the study compared to the second year. The ratio of calcium and phosphorus in the dry mass of alfalfa was 2:1, while in red clover and bird's-foot trefoil was lower and ranged from 1.5-1.7:1.

The highest content of nitrogen and calcium was found in alfalfa dry mass; bird's-foot trefoil had the highest content of phosphorus and potassium, while red clover had the highest amount of magnesium. The lowest content of calcium in the dry mass of alfalfa was in the budding phase, while the highest was in the phenological phase of full flowering (Erić et al., 1996). The concentration of magnesium in alfalfa fodder varied, depending on the phenological phase at mowing, from 2.7 to 3.5 g kg<sup>-1</sup> DM, similar to red clover fodder (2.1-3.8 g kg<sup>-1</sup> DM) (Marković et al., 2007), which is consistent with our results. Hav obtained from alfalfa foliar fertilized with Se. Zn. and Cu during the vegetation had a P content of 2.2-2.4 g kg<sup>-1</sup> DM, K 14.2 g kg<sup>-1</sup> DM, Ca 2.36-3.46 g kg<sup>-1</sup> DM, and Mg 2.7-3.2 g kg<sup>-1</sup> DM (Petković et al., 2019). It was reported that N, K, and Ca were present in the highest concentration of alfalfa dry matter, while S, Mg, and P were present to a much lesser extent (Ferreira et al., 2015), which is in accordance with the results of our study. Red clover hay reportedly contained 0.9-4.5 g kg<sup>-1</sup> DM of phosphorus, 5.7-26.7 g kg<sup>-1</sup> DM of potassium, 9.7-22.9 g kg<sup>-1</sup> DM of calcium, and 0.01-1.85 g kg<sup>-1</sup> DM of magnesium (Miller, 1958). The content of P and Mg was significantly higher in our analysed red clover samples (9.5 g kg<sup>-1</sup> DM and 3.7 g kg<sup>-1</sup> DM, respectively). In dry feed bird's-foot trefoil the average nitrogen content was 25.17 g kg<sup>-1</sup> DM, phosphorus 1.26 g kg<sup>-1</sup> DM, potassium 7.35 g kg<sup>-1</sup> DM, calcium 15.2 g kg<sup>-1</sup> DM, and magnesium 2.94 g kg<sup>-1</sup> DM (Ocokoljić et al., 1983). The N, P, K contents in bird's-foot trefoil dry feed obtained in our experiments were much higher, with average values of 39.0, 10.6 and 26.1 g kg<sup>-1</sup> DM, respectively.

Proper nutrition of the livestock is ensured with the balanced potassium content in relation to the total amount of calcium and magnesium in animal feed. If the magnesium content is below 2.0 g kg<sup>-1</sup> DM, and potassium content is higher than 25.0 g kg<sup>-1</sup> DM, with the ratio between potassium and the total calcium and magnesium content higher than 2.2, then tetany disease in cattle occurs (Gross, 1973).

#### Conclusion

The content of analysed elements in the feed of perennial legumes is of great importance, because the livestock meets most of its needs by consuming it.

The highest nitrogen content in the dry matter was determined in alfalfa, in both years of study. The dry mass of alfalfa had also the highest content of calcium. The ratio between calcium and phosphorus in alfalfa fodder was favourable. The highest content of magnesium was determined in the dry mass of red clover. The dry mass of bird's-foot trefoil stood out for its phosphorus content. Unlike for alfalfa and red clover, the year of study had a highly significant effect on the content of potassium, calcium and magnesium in the dry mass for bird's-foot trefoil

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# Одређивање садржаја азота и неких макроелемената у сувој маси вишегодишњих легуминозних биљака

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#### Сажетак

Циљ истраживања је био да се утврди садржај елемената N. P. K. Са и Мд у сувој маси вишегодишњих крмних легуминоза, покошених у оптималној фази развоја биљака. Оглед са вишегодишњим легуминозама луцерком (Medicago sativa), црвеном дјетелином (Trifolium pretense) и смиљкитом (Lotus corniculatus L.) постављен је по методи случајног блок система у четири понављања на долинско-смеђем земљишту у току двије године. Зелена маса вишегодишњих легуминоза у првом и другом откосу покошена је у фази крај пупања/почетак цвјетања. У трећем и четвртом порасту кошење биомасе обављено је након 5 седмице. Непосредно након сваког кошења узиман је просјечан узорак зелене биомасе са сваког понављања од сваке испитиване легуминозе. У сувој маси биљног материјала анализиран је садржај азота, фосфора, калијума, калцијума и магнезијума. Садржај азота и калијума у крми луцерке и црвене дјетелине био је већи у првој години испитивања, док је код смиљките било обрнуто. Сува материја луцерке у просјеку је имала највећи садржај азота (39,8 g kg-1 СМ) и калцијума (19,6 g kg<sup>-1</sup> СМ), смиљките фосфора (10,6 g kg<sup>-1</sup> СМ) и калијума (26,1 g kg<sup>-1</sup> CM), а црвене дјетелине магнезијума (3,7 g kg<sup>-1</sup> CM). У сувој маси смиљките просјечан садржај азота био је 39,0 g kg<sup>-1</sup> СМ, фосфора 10,6 g kg<sup>-1</sup> CM, калијума 26,1 g kg<sup>-1</sup> CM, калцијума 15,7 g kg<sup>-1</sup> CM и магнезијума 3,5 g kg<sup>-1</sup> СМ.

*Кључне ријечи*: азот, макроелементи, луцерка, црвена дјетелина, смиљкита.

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