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MACROELEMENTS IN RED CLOVER (*Trifolium Pratense* L.) RELATIVE TO COW REQUIREMENTS

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Abstract

Mineral contents of forages are very important for animal feeding. A number of inorganic elements are essential for normal growth and reproduction of animals. Therefore, it is important that rich forages in terms of mineral content are used in animal feeding. Among the forage plants, red clover is one of the best forage to meet the mineral demands of livestock. Mineral contents of forage species may show significant variations in aspecies. The aim of this study was to choose a superior cultivar in terms of macro-minerals contents for animal feeding among tetraploid and diploid cultivar of red clover. A field experiment was conducted in 2012 at Institute for forage crops in Kruševac (Serbia). The experiment was designed as a randomized complete design with three replications. The plants were harvested in the three stages of development during the spring. Higher content of N, P and K were found in the tetraploid cultivar of red clover (K-32) than in diploid cultivar (K-39), and decreased with growth and development. The content of Ca increased with plant development from 17.64 to 22.30 g kg^{-1} of DM in tetraploid cultivar of red clover, and from 19.50 to 28.0 g kg^{-1} of DM in diploid cultivar of red clover.

Keywords: *macro-minerals, plant-development, red clover*

Introduction

Red clover (*Trifolium pratense* L.) is commonly grown throughout the world. It is an important perennial forage legume because it is used in hay or as pasture in crop rotation (Famham and George, 1993) and have high productivity and protein content (Murray et al., 2007). It is especially, suitable for cattle breeding, because red clover has quality and nutritive hay (Acikgoz, 2001).

Mineral contents of forages are very important for animal feeding. A number of inorganic elements are essential for normal growth and reproduction of animals (NRC, 2001). Therefore, it is important that rich forages in terms of mineral content are used in animal feeding. Among the forage plants, red clover is one of the best forage plants to meet the mineral demands of livestock. Stage of forage maturity affects the content of a number of minerals in forages. A rapid uptake of minerals by plants usually occurs during the early stages of growth. With advancing maturity, the dry matter content of the plant generally increases more rapidly than mineral uptake causing concentrations of many minerals to decrease (Ammerman et al., 1982).

Mineral contents of forage species may show significant variations in a species. A large number of studies have shown important differences in term of mineral contents in a species (Jones et al., 1995; Lema et al., 2000; Yolcu et al., 2008; Sengul and Haliloglu, 2008). The aim of this study, was to choose a superior cultivar in terms of mineral contents for animal feeding among tetraploide and diploide cultivar of red clover in field conditions in the spring growth.

Material and methods

The experiment was designed as randomized block design in three replicates. Three stages of growth of red clover (*Trifolium pratense* L.) *cv* K-32 (4n) and *cv* K-39 (2n) were examined in the first cut. Soil type was with an organic matter content of approximately 3,5 % and a pH of 6,5. Samples were hand cut with scissors at 5 cm height. The first stage was cut after 62 days of vegetation, at the mid bud stage, another one after 76 days of vegetation (around 10-15% flowering), and a third one (around 50% flowering) after 84 days of vegetation. Dry matter was determined by drying out samples at 65ºC and grinding and sieving them to 1 mm particle size.

Forage was cut and a sample of the whole plants was collected. The concentrations of K, Ca and Mg were measured by atomic absorbance spectrophotometry. Samples for Ca and Mg analysis were prepared with $1gL^{-1}$ lanthanum. Phosphorus was measured colorimetrically, according to the ISO 6491 method. The amount of total nitrogen was measured by the Kjeldahl method on the TECATOR KJELTEC AUTO ANALYZER 1030.

The data were processed by the analysis of variance in a randomized block design. The significance of differences between arithmethic means was tested by Fisher test ($p< 0.01$).

Result and discussion

The development stage of plants and the form of red clover differentiated the concentration of mineral components in green forage (Table 1).

The results of this investigation showed that stage of harvest is an indicator of forage quality. With growth and development the nitrogen content decreased from 29.50 to 23.80 g kg^{-1} (4n) ($p< 0.01$), and from 25.60 to 21.60 g kg^{-1} DM (2n). The higher nitrogen content in dry matter of the tetraploid *cv* of red clover might be explained by higher leaf:stem ratio, because leaf tissue is almost always the highest quality part of the forage, and tetraploid cultivar usually have higher leaf:stem ratio.

The concentration of phosphorus ranged between 2.36 and 1.88 g kg^{-1} DM, with more phosphorus found in DM of cv K 32 (4n). The significant difference between the cultivars of red clover were determined only in the first stage of development. These results are in agreement with Bieniaszewski and Fordonski (1996) who found out that tetraploid forms contained more phosphorus and potassium than diploid ones. It was reported that forages for cattle and sheep should contain P between 1.7 and 3.9 g kg^{-1} DM (NRC, 2001).

In our experiment, the content of potassium decreased with growth and development from 21.70 to 14.40 g kg^{-1} DM in cv K 32, and from 19.10 to 10.90 g kg^{-1} DM in cv K 39.

Table 1. Concentration of macro-minerals in red clover, g kg^{-1} DM

		b_1	b_2	b_3	$\overline{X}_A$
N	a_1	**29.50**^{A,a}	**26.40**^{NS,b}	**23.80**^{NS,c}	26.60^{a}
	a_2	**25.60**^{B,a}	**24.10**^{NS,a}	**21.60**^{NS,b}	23.80^{b}
	$\overline{X}_B$	27.55^{a}	25.25^{b}	22.70^{c}	
P	a_1	**2.52**^{A,a}	**2.12**^{NS,b}	**1.94**^{NS,b}	2.19^{a}
	a_2	**2.19**^{B,a}	**1.99**^{NS,ab}	**1.82**^{NS,b}	2.00^{b}
	$\overline{X}_B$	2.36^{a}	2.06^{b}	1.88^{c}	
K	a_1	**21.70**^{A,a}	**15.70**^{NS,b}	**14.40**^{A,b}	17.30^{a}
	a_2	**19.10**^{B,a}	**15.20**^{NS,b}	**10.90**^{B,c}	15.10^{b}

	$\overline{X}_B$	20.40[a]	15.50[b]	12.70[c]	
Ca	a_1	**17.60**[B,b]	**18.90**[A,b]	**22.30**[B,a]	19.60[b]
	a_2	**19.50**[A,b]	**16.80**[B,c]	**28.00**[A,a]	21.40[a]
	$\overline{X}_B$	18.60[b]	17.90[c]	25.20[a]	
Mg	a_1	**18.00**[A,a]	**14.90**[B,c]	**16.30**[NS,b]	16.40[b]
	a_2	**16.20**[B,ns]	**16.20**[A,ns]	**17.30**[NS,ns]	16.60[a]
	$\overline{X}_B$	17.10[ns]	15.60[ns]	16.80[ns]	

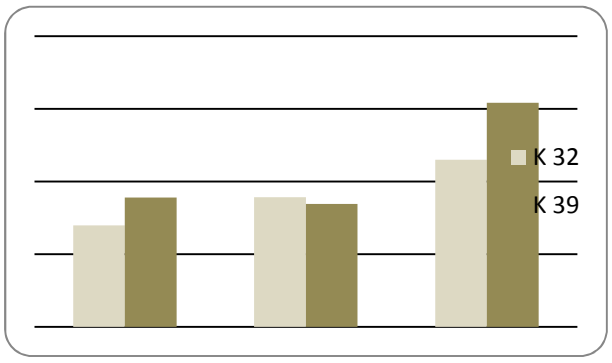
Different letters (A, B) denote significant differences ($p< 0.01$) between red clover cultivars, and different letters (a, b, c) denote significant differences between different stages of development ($p< 0.01$); NS, ns – non significance

When analyzing the concentration of Ca in three stages of development of tetraploid and diploid cv of red clover, it was demonstrated that K 32 (4n) contained more amount of Ca only at the beginning of flowering ($p< 0.01$). Considering the nutritional usability of forages, the concentration of Ca in red clover was twice as high as required by animals (Plaza et al., 2009). Although, forages are generally high in Ca, the availability of calcium may be low because of the presence of calcium oxalate.

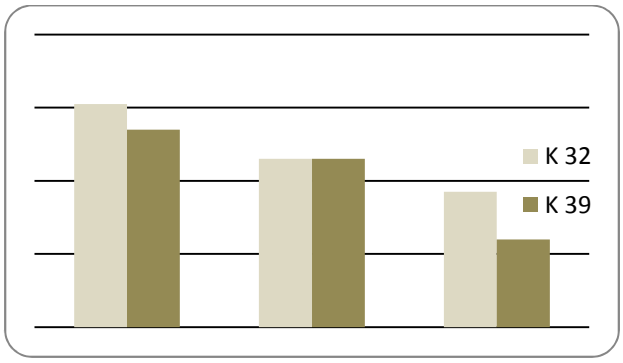
The concentrations of magnesium were not shaped analogously, and the tetraploid cv of red clover contained more of this element only at the first stage of plant development. In this cultivar, concentration of Mg decreased from bud stage to beginning of flowering, and after that increased to 50% of flowering ($p< 0.01$). This, however, does not undermine the finding that the content of magnesium fully covered the nutritional demands of animals, especially dairy cows, which according to the INRA (1988) – demand 1.5 to 2.0 g Mg kg^{-1} of DM.

Mineral imbalances, deficiencies or excess and low bioavailability of essential minerals result in negative economic impacts when animal performance and health are compromised (Van Soest, 1983). The results of this study affect the existence of variation among the populations regarding their Ca:P and K:(Ca+Mg) ratios. Calcium is associated with phosphorus metabolism in animals (Onal Asci, 2012). In this current study the Ca:P ratio was between 6.98 and 11,50 in dry matter of tetraploide cultivar and between 8.90 and 15.40 in dry matter of a diploid cultivar of red clover. These results are in agreement with Onal Asci (2012) (Ca:P ratio of red clover was between 4.53 and 11.45). Although the generally recommended ratio of Ca:P is 2:1, ranges in dietary 1:1 and 7:1 resulted in similar performance of ruminant livestock (Buxton and Fales, 1994) without unfavourable effects if adequate vitamin D is available (Barnes et al., 1990). Legumes used sources of calcium, but not phosphorus (Schroeder, 2004). Low P and high Ca content resulted in this wide Ca:P ratios (Basaran et al., 2011).

Graf. 1. Ca:P



Graf. 2. K:(Ca+Mg)



The ratios of Ca:P and K:(Ca+Mg) in di- and tetraploid red clover cultivars

Magnesium concentrations are important, but must be considered in relation to K and Ca in herbage. The ratio tended to decline as the growing season progressed. It is recommended that K:(Ca+Mg) ratio should be below 2.20 (Kidambi et al., 1989). K:(Ca+Mg) ratio of both cultivar of red clover were lower than the critical value. As Saba et al. (2000) claim, high content of potassium, due to the antagonostic metabolic effect of this element on magnesium, may constitute an additional factor which depresses the bioavailability of magnesium.

Conclusions

The concentration of nitrogen, phosphorus and potassium was higher in the tetraploid cultivar of red clover (K 32), unlike the content of Ca, which was lower except at the beginning of flowering, and Mg which was also lower, except at the mid bud stage. The Ca:P ratios were a product of greater concentrations of Ca combined with lesser amounts of P in the tissue. These cultivars can be successfully used to meet a part of mineral requirement of livestock and to improve new quality red clover cultivars for animal feeding.

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