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Estimation of red clover (*Trifolium pratense* L.) forage quality parameters depending on the cut, stage of growth and cultivar

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Summary

A field trial was conducted to determine the forage quality parameters of two red clover cultivars – Nike and K-39 and to quantify the effects of cultivar, cut and stage of growth on red clover forage quality. The experiment was conducted in a randomised block design with three replicates. The changes in chemical constituents of red clover were analysed by Weende system of analysis. The differences between forage quality of investigated red clover cultivars were significant for crude ash, crude protein, crude fibre and nitrogen free extract ($P< 0.05$). The results of this investigation indicated that the crude protein content of red clover declined with advancing maturity in the second and third cut (from 245.60 to 180.50 g kg^{-1} of DM and from 256.25 to 160.25 g kg^{-1} of DM, respectively). The achieved results show that *cv* K-39 has lower forage quality at the second cut, with lower crude protein and higher crude fibre than *cv* Nike.

Key words: red clover, forage quality, cut, stage of growth

Introduction

Animal breeding and obtaining high levels of production via healthy nurture are important for regions that deal with stockbreeding. Despite obtaining animals which are at high levels of production, there are still some problems in meeting the forage requirements of these animals. Therefore, forage crops with high production characteristics should be improved so that animals can be fed adequately and healthily during long winters.

Red clover (*Trifoium pratense* L.) is the second most important perennial forage legume, after alfalfa. It is grown on about 20 million hectares worldwide, while in Serbia it has been grown on around 120.000 ha annually in the last few years (Vasiljević et al., 2009). This forage crop is adapted to a wide range of

climatic conditions, soil types, fertility levels, use patterns and management. It is easy to establish, has high seedling vigour, is an excellent nitrogen fixer and is suitable for use in crop rotations. The yield potential of red clover is excellent and some red clover varieties can have higher fodder yields than alfalfa. Red clover is also of very good quality in the light of its nutritive value and ensiling (Taylor and Quesenberry, 1996; Hoffman and Broderick, 2001).

Forage quality is affected by many independent factors including maturity, crop species, harvest and storage, environment, soil fertility and variety. The stage of development considerably affects chemical composition and forage quality of red clover (Belonger, 2010; Marković et al., 2008; Makarenko and Pribytkov, 1989). In the early spring, young red clover plants have high proportion of leaves, high content of moisture, proteins and minerals and low content of fibres. During the growing season, under the effect of longer days and higher temperatures, the ageing plants undergo morphological changes: leaf growth becomes slower, the stem increases in length and proportion of dry matter increases. On the other hand, forage quality decreases drastically, especially digestibility and the contents of proteins and minerals. The optimum time for red clover cutting is the stage when 20-25% flowers are in bloom (Wiersma et al., 1998).

The main objectives of this study are to determine and compare forage quality of two red clover cultivars and to quantify the effects of cultivar, cut and stage of growth on red clover forage quality.

Materials and methods

The experiment was designed as three-factorial trial in a randomised block system with three replicates. Three stages of growth of red clover (*Trifolium pratense* L.) *cv* Nike and K-39 were examined in the second and third cut. Samples were hand cut with scissors at 5 cm height. The first stage was cut after 22 days of vegetation, at full boot stage, another one after 29 days of vegetation (around 40% flowering), and a third one in full flowering after 36 days of vegetation. Dry matter was determined by drying out samples at 65° C and grinding and sieving them to 1 mm particle size.

Crude ash was determined by ashing at 550° C. Crude proteins were computed indirectly from the amount of total nitrogen, measured by the Kjeldahl method modified by Bremner, multiplied by factor 6.25. Crude fibre was determined by being sequently refluxed in dilute base followed by dilute acid. Crude fat was determined by the method of Soxlet. The amount of nitrogen free extract in samples was determined as a difference between 1000 and amounts of crude ash, crude protein, crude fibre and crude fat.

The data were processed by the analysis of variance in a randomised block design. Effects were considered different based on significant ($P< 0.05$) F ratio. The significance of differences between arithmetic means was tested by LSD test.

Results and discussion

The results of analyses of variance (Table 1) revealed statistically significant effects of the cut sequence on forage quality. The cut was an important source of variability for all qualitative parameters in this trial. The analyses of

variance also revealed significant differences between stages of growth for all investigated parameters except crude fat and significant differences between investigated cultivars of red clover for all forage quality parameters.

Overall, as red clover matured, the content of crude ash decreased from the first to the third stage of growth in both investigated cuts and in both investigated cultivars. The interactions cut x stage of growth, cut x cultivar and stage of growth x cultivar were also significant ($P< 0.05$). The higher content of crude ash was found in the third cut at the first and second stage of growth. *Cv* Nike had higher content of crude ash in the second cut.

Studying the quality characteristics of dry matter at different stages of development of red clover cultivars, Vasiljević et al. (2005) found that the average ash content in the two stages was 8.9%. Dinić et al. (1990, 1994) reported the ash content of 10.3% in red clover at the stage of early bloom.

Forage quality of red clover depends primarily on the plant development, the height of cut and environmental conditions. Taylor and Quesenberry (1996) state the two most authoritative quality parameters are crude proteins and in vitro dry matter digestibility. These levels decline with age, for all perennial legumes as a result of reduction of leaf to stem proportion and the process of lignification. The decrease in digestibility after budding occurs as a result of increased lignin content and increased proportion of polysaccharide.

Growth stages and plant age are important factors affecting the chemical composition and forage quality of red clover (Ignjatovic et al. 2001, Markovic et al. 2010). In the early spring, young plants of red clover have a large proportion of leaves, high moisture content, proteins and minerals, and low content of fibres. During the period of vegetation under the influence of longer days and higher temperatures with ageing, plants undergo morphological changes: growth of leaves is slower, the stems are extended, dry matter yield increases and quality decreases dramatically, particularly digestibility, proteins and minerals.

The analyses showed significant ($P< 0.05$) cut x stage of growth, cut x cultivar, stage of growth x cultivar and cut x stage of growth x cultivar interactions for crude proteins and crude fibres. The results of the trial are presented in Table 1.

The results of this trial indicated that crude protein concentration of both red clover cultivars declined with advancing maturity. In the second cut, *cv* Nike contained more crude protein than *cv* k-39 at all sampling dates, crude protein concentration in *cv* K-39 was higher in the first development stage than in *cv* Nike (in the third cut). Along with plant growth and development, crude protein content decreased from first to third stage of growth by 22.3% in the second and by 36.6% in the third cut (*cv* Nike), and 30.7% in the second and 38.3% in the third cut (*cv* K-39).

During maturation, crude fibre concentration increased from 171.5 to 237.2 g kg^{-1} of DM in the second and from 139.6 to 197.6 g kg^{-1} of DM in the third cut (*cv* Nike), and from 195.0 to 267.0 g kg^{-1} of DM in the second and from 123.3 to 206.2 g kg^{-1} of DM in the third cut (*cv* K-39).

Tab. 1. Forage quality parameters of red clover (*Trifolium pratense* L.) *cv* Nike and K-39 (g kg^{-1} of DM)
Parametri kvaliteta crvene djeteline (*Trifolium pratense* L.) *cv* Nike i K-39 (g kg^{-1} DM)

Parameter		A_1				A_2			
		B_1	B_2	B_3	χ (AC)	B_1	B_2	B_3	χ (AC)
Crude ash	C_1	106.90^a	97.70^b	91.70^c	**98.77^a**	106.80^a	91.50^b	80.80^c	**93.03^b**
	C_2	99.20^a	89.90^b	83.70^c	**90.93^b**	106.10^a	97.80^b	79.70^c	**94.53^a**
	χ (AB)	**103.05^a**	**93.80^b**	**87.70^c**		**106.45^a**	**94.65^b**	**80.25^c**	
	χ (A)		**94.85^a**				**93.78^b**		
	χ (BC)	**106.85^a**	**94.60^b**	**86.25^c**		**102.65^a**	**93.85^b**	**81.70^c**	
	χ (B)		**104.75^a**		**94.22^b**			**83.97^c**	
	χ (C)		**95.90^a**				**92.73^b**		
Crude protein	C_1	246.60^a	211.10^b	191.50^c	**216.40^a**	252.90^a	195.10^b	160.40^c	**202.80^b**
	C_2	244.60^a	189.30^b	169.50^c	**201.13^b**	259.60^a	194.50^b	160.10^c	**204.73^a**
	χ (AB)	**245.60^a**	**200.20^b**	**180.50^c**		**256.25^a**	**194.80^b**	**160.25^c**	
	χ (A)		**208.76^a**				**203.76^b**		
	χ (BC)	**249.75^a**	**203.10^b**	**175.95^c**		**252.10^a**	**191.90^b**	**164.80^c**	
	χ (B)		**250.92^a**		**197.50^b**			**170.37^c**	
	χ (C)		**209.60^a**				**202.93^b**		
Crude fiber	C_1	171.50^c	190.90^b	237.20^a	**199.86^b**	139.60^b	197.60^a	197.60^a	**178.26^a**
	C_2	195.00^c	231.70^b	267.00^a	**231.23^a**	123.30^c	155.70^b	206.20^a	**161.73^b**
	χ (AB)	**183.25^c**	**211.30^b**	**252.10^a**		**131.45^c**	**176.65^b**	**201.90^a**	
	χ (A)		**215.55^a**				**170.00^b**		
	χ (BC)	**155.55^c**	**194.25^b**	**217.40^a**		**159.15^c**	**193.70^b**	**236.60^a**	
	χ (B)		**157.35^c**		**193.97^b**			**227.00^a**	
	χ (C)		**189.06^b**				**196.48^a**		
Crude fat	C_1	33.30^c	44.80^a	41.00^b	**39.70^a**	33.80^a	17.80^c	29.80^b	**27.13^a**
	C_2	26.90^a	25.70^b	17.70^c	**23.43^b**	31.60^a	17.80^b	17.50^c	**22.30^b**
	χ (AB)	**30.10^b**	**35.25^a**	**29.35^b**		**32.70^a**	**17.80^c**	**23.65^b**	
	χ (A)		**31.56^a**				**24.71^b**		
	χ (BC)	**33.55^b**	**31.30^b**	**35.40^a**		**29.25^a**	**21.75^b**	**17.60^c**	
	χ (B)		**31.40^a**		**26.52^b**			**26.50^b**	
	χ (C)		**33.41^a**				**22.86^b**		
Nitrogen free extract	C_1	441.70^b	455.50^a	438.60^c	**445.26^b**	466.90^c	498.00^b	531.40^a	**498.76^b**
	C_2	434.30^c	463.40^a	462.10^b	**453.26^a**	479.40^c	534.20^b	536.50^a	**516.70^a**
	χ (AB)	**438.00^c**	**459.45^a**	**450.35^b**		**473.15^c**	**516.10^b**	**533.95^a**	
	χ (A)		**449.26^b**				**507.73^a**		
	χ (BC)	**454.30^c**	**476.75^b**	**485.00^a**		**456.85^c**	**498.80^b**	**499.30^a**	
	χ (B)		**455.57^c**		**487.77^b**			**492.15^a**	
	χ (C)		**472.00^b**				**484.98^a**		

A_1 – second cut, A_2 – third cut; B_1 – first stage, B_2 – second stage, B_3 – third stage of growth; C_1 – *cv* Nike, C_2 – *cv* K-39; Different letters denote significantly different means ($P< 0.05$)

Red clover had more crude fibre content in the second than in the third cut. *Cv* K-39 contained 1.58 times more crude fibre content at the first stage of plant

development, 1.48 times more crude fibre at the second and 1.29 times more crude fibre at the third stage of growth in the second than in the third cut. However, *cv* Nike contained 1.23 times more crude fibre content at the first stage of plant development in the second than in the third cut, and 1.20 times more crude fibre content at the third stage of growth.

The content of crude fibre was the highest at the second cut, which was also confirmed by the results by Wiersma et al. (1998). Griffin et al. (1994) supposed that this is owing to a slower reduction of leaf to stem ratio in the summer as compared to the spring.

Forage producers have special interest in quality improvements so as to partially offset the decline in nutritive value associated with advancing maturity of the crop when harvest is delayed. Some farmers harvest second growth earlier to obtain higher protein and lower fibre concentrations during mid-summer when quality values are often the lowest of the three harvests. The protein and fibre concentrations observed for both cultivars of red clover in this early second harvest may have been excessive for normal dietary requirements in lactating dairy cattle (NRC, 2000).

An adequate fibre level is necessary in the rumen in order to promote a healthy population of cellulolytic species important to maintain normal lipid metabolism and milk fat level, and to enhance microbial protein output to the lower digestive tract (Van Soest et al., 1991). The need for early harvest in order to increase crude protein concentration may sacrifice forage yield and provide insufficient fibre concentration.

During growth and development, none of the investigated cultivars of red clover showed a consistent trend of crude fat and nitrogen free extract content. Overall, maturation and a higher concentration of crude fat in both cuts were found in *cv* Nike. Despite the crude fat, *cv* K-39 contained more nitrogen free extract than *cv* Nike at all sampling dates, except at the first stage of development in the second cut.

Conclusion

The general observation that decrease in content of crude proteins coincides with plant ageing was confirmed in this study. As plant growth advanced, there was greater accumulation of cellulose, while the content of crude proteins decreased. In the second cut, *cv* K-39 had less crude proteins than *cv* Nike, while in the third cut both cultivars were similar in terms of crude proteins content. At the same time, *cv* K-39 had higher crude fibre content than *cv* Nike. The highest content of crude fibres was recorded at the third stage of plant development (*cv* K-39) in the second cut. There were significant interactions between treatments for most forage quality parameters. Generally, *cv* Nike had better forage quality than *cv* K-39 in this period of investigation.

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Procjena parametara kvaliteta crvene djeteline (*Trifolium pratense* L.) u zavisnosti od kosidbe, stadijuma rasta i kultivara

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Apstrakt

Sproveden je poljski ogled da bi se odredili parametri kvaliteta krmnog bilja za dva kultivara crvene djeteline – Nike i K-39 i da bi se izmjerili uticaji kultivara, kosidbe i stadijuma rasta na kvalitet crvene djeteline. Eksperiment je obavljen kao nasumični blok dizajn sa tri ponavljanja**.** Ispitivane su promjene hemijskih sastojaka kod crvene djeteline korištenjem Weende sistema analize. Razlike između kvaliteta krmnog bilja kod analiziranih kultivara crvene djeteline bile su značajne na sirovi pepeo, sirove bjelančevine, sirova vlakna i BEM sadržaj ($P< 0.05$). Rezultati ovog ispitivanja ukazuju da se sadržaj sirovih bjelančevina kod crvene djeteline smanjivao sa odmaklim zrenjem u toku druge i treće kosidbe (od 245.60 do 180.50 g kg^{-1} DM i od 256.25 do 160.25 g kg^{-1} DM). Postignuti rezultati pokazuju da je *cv* K-39 imao niži kvalitet tokom druge kosidbe sa nižim sadržajem sirovih bjelančevina i višim sadržajem sirovih vlakana nego *cv* Nike.

Ključne riječi: crvena djetelina, kvalitet, kosidba, stadijum rasta

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