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LIGNIFICATION AS THE MAJOR FACTOR LIMITING RED CLOVER DM AND NDF DIGESTIBILITY

Jordan Marković[1], Nedeljko Racić[1], Filip Bekčić[1], Đorđe Lazarević[1], Tanja Vasić[2], Sanja Živković[2], Bogdan Cekić[3]

Abstract: NDF is a good indicator of fiber content in forages, but on the other hand NDF digestibility gives more accurate estimates of total digestible nutrients. The aim of this study was to evaluate two red clover cultivars for NDF digestibility and DM digestibility depending on the stage of the development - mid bud stage, early bloom and mid bloom, harvesting in three different cuts. Results of this study showed that lignin content increased with plant maturation in all three cuts. The highest lignin content was determined in the third cut ranged from 5.87 to 5.92% and 5.56 to 6.53% in DM of K 39 and K 32 red clover cultivars. Intensive lignifications of cell wall in the third cut influenced the highest decreasing of NDF digestibility.

Keywords: digestibility, lignin, red clover, stage of development

Introduction

Red clover is one of the most important leguminous plants grown in the temperate climate zone. It grows well on different types of soil, has the ability to fix atmospheric nitrogen and it is characterized by high nutritive value for ruminants (Leto et al., 2004).

There are many factors that impact and affect forage nutritive value. Plant maturity is the largest factor in limiting fiber digestibility (Buxton, 1996). This is due to the rapid accumulation of lignin in cell walls, as the plant matures (Jung, 1989). The cell wall is made up of different components whose interactions determine its structure and function. The main element that plays a leading role in regulating cell wall degradation is lignin with additional support from the cell wall components, due to the particular linkages that occur between cell wall constituents (Moore and Jung, 2001). However, harvesting forages at optimum stage of maturity is important to maximize both yield and quality including NDF (Neutral Detergent Fiber) digestibility. The indigestible fraction of NDF is

[1]Institute for forage crops Kruševac, 37251 Globoder, Serbia (jordan.markovic@ikbks.com)
[2]University of Niš, Faculty of Agriculture, Kosančićeva 4, 37000 Kruševac, Serbia
[3]Institute for animal husbandry, Autoput 16 P. BOX 23, 11080 Belgrade – Zemun, Serbia

a major factor affecting the utilization of carbohydrate sources as it varies greatly and may exceed more than one half of the total NDF in the rumen (Varga, 2006). Therefore, knowing information regarding forage digestibility and NDF digestibility is critical as it allows producers the opportunity to allocate higher digestible forages to higher producing cows and accordingly plan harvesting.

The aim of this study was to evaluate two red clover cultivars for NDF digestibility and DM digestibility depending on the stage of the development harvesting in three different cuts.

Materials and methods

This experiment was carried out at the experimental field of Institute for forage crops Kruševac, situated in central Serbia. Two cultivars of red clover - diploid *cv* K 39 (2n) and tetraploid *cv* K 32 (4n) were evaluated at three stages of development in the first, second and the third cut. The first studied stage of development was mid bud (MB), the second was early bloom (EBL) and the third was mid bloom (MBL).

NDF was determined according to the method by Mertens (2002). Lignin was determined as the residue insoluble in 72% (w/w) sulphuric acid, applying the method of Van Soest and Robertson (1980). The proportion of lignin in total NDF (L, % of NDF) was calculated as the percentage of lignin in the total NDF content. Two stage pepsin-cellulase method was used for *in vitro* DMD (Dry Matter Digestibility) according to the method by De Boevar et al. (1986). *In vitro* NDFD (NDF Digestibility) was determined according to the method by Riveros and Argamentaria (1987).

Experiment was established as a randomized complete block design in three replications, with factorial arrangements of two main factors (2 red clover cultivars x 3 stages of development) in all three cuts, separately. The data were processed by the analysis of variance in a randomized block design (ANOVA, Stat. Soft. STATISTICA 6). The significance of differences between arithmetic means was tested by Tukey test ($p< 0.05$).

Results and discussion

The content of NDF and lignin, as well as NDFD and DMD of two red clover cultivars at three stages of development harvested in three cuts are presented in the Table 1.

The NDF content in forages is the best single indicator of the feed intake potential in ruminants and a useful and reliable tool in the meals formulation for high-producing dairy cows. The results of this study showed that significantly changes in NDF content was observed in dry matter of diploid red clover cultivars, especially during the second and the third cuts. The content of NDF in dry matter of diplid red clover ranged from 40.36 to 49.54% of DM in the second cut and from 41.11 to 46.22% of DM in the third cut ($p< 0.05$), respectively. The results obtained in the investigation conducted by Buxton et al. (1985) showed that with advancing red clover development there was not a significant decline in quality as well as some other perennial legumes. Similar results were obtained in this study in dry matter of tetraploid red clover cultivar, we assume due to higher leaves proportion. Van Soest (1994) indicated that during the spring, high temperatures in interactions with the advancing of plant development lead to a much faster decline in quality than during the summer. Plants grown in spring can be characterized by very high quality if they are harvested early, but delaying harvesting can have a very negative effect on quality. In the late summer temperatures do not increase linearly and the advancing in plant development leads to a slow decline in quality (Buxton, 1996), which was confirmed in this study.

Table 1. Content of NDF, lignin, NDFD and DMD of red clover cultivars

Cut		K 39			K 32		
		MB	EBL	MBL	MB	EBL	MBL
I	NDF, %	38.48^{e}	45.54^{c}	44.54^{b}	40.09^{de}	41.00^{cd}	47.09^{a}
	Lignn, %	3.50^{c}	5.21^{a}	5.43^{a}	4.46^{b}	4.48^{b}	5.59^{a}
	L, % NDF	9.12^{b}	12.26^{a}	12.19^{a}	11.13^{a}	10.93^{a}	11.89^{a}
	NDFD,%	62.77^{a}	53.37^{b}	44.89^{c}	54.11^{b}	54.78^{b}	41.70^{d}
	DMD, %	83.55^{a}	79.56^{b}	76.74^{c}	80.60^{b}	80.21^{b}	74.30^{d}
II	NDF, %	40.36^{c}	44.62^{b}	49.54^{a}	45.28^{b}	44.15^{b}	45.27^{b}
	Lignn, %	4.36^{c}	4.86^{bc}	5.81^{a}	5.11^{abc}	5.39^{ab}	5.61^{ab}
	L, % NDF	10.80	10.92	11.73	11.29	12.20	12.39
	NDFD,%	57.88^{a}	49.77^{b}	51.32^{b}	51.72^{b}	36.78^{d}	47.27^{c}
	DMD, %	81.12	79.87	78.73	79.95	74.97	79.85
III	NDF, %	41.11^{c}	43.17^{b}	46.22^{a}	43.36^{b}	40.16^{c}	43.31^{b}
	Lignn, %	5.87^{ab}	5.92^{ab}	5.97^{ab}	5.56^{b}	6.08^{ab}	6.53^{a}
	L, % NDF	14.28^{ab}	13.71^{ab}	12.90^{b}	12.84^{b}	15.15^{a}	15.07^{a}
	NDFD,%	60.07^{a}	50.58^{b}	40.72^{c}	60.52^{a}	51.70^{b}	35.22^{d}
	DMD, %	84.77^{b}	82.05^{c}	78.44^{d}	87.90^{a}	83.08^{c}	75.80^{e}

NDF – Neutral Detergent Fiber; L – Lignin; NDFD – Neutral Detergent Fiber Digestibility; DMD – Dry Matter Digestibility; MB – Mid Bud stage of development; EBL – Early Bloom stage of

development; MBL – Mid Bloom stage of development; Different letters in the rows denote significantly different means ($p< 0.05$) between the stages of development and cultivars

With red clover growth and development, lignin content increased in both investigated red clover cultivars. The highest value of lignin content was recorded in the MBL stage of development in all three cuts (Table 1). The least intensive changes with the plant growth and development were determined in the third cut of diploid red clover cultivar, and in the second cut of tetraploid red clover cultivar. In addition to being undegradable itself, lignin makes it difficult to break down the cellulose and hemicelluloses that could be used. This is the main reason why animals use less energy from the plants harvested in later stages of development. The main factors affecting the NDF digestibility is the degree of structural carbohydrates lignifications – the proportion of lignin in the total NDF. The results obtained in this study showed that proportion of lignin in total NDF increased with plants growth and development, except in the third cut of diploid red clover cultivar (Table 1). Although, the highest degree of fibre lignifications was found in the third cut of both investigated red clover cultivars. The degree of NDF lignifications in the third cut ranged from 14.28 to 12.90% of NDF in the DM of diploid red clover cultivar, and from 12.84 to 15.07% of NDF in the DM of tetraploid red clover cultivar.

As the result of intensive lignifications process NDF digestibility declined in both red clover cultivars harvested during all three cuts. The highest decreasing of NDF digestibility was observed in the third cut by 32.22% (relatively) in the diploid red clover cultivar and by 41.81% (relatively) in the tetraploid red clover cultivar, respectively regarding to the advancement of stage of red clover development in each cuts. On the other hand, the smallest decreasing of NDF digestibility was observed in the second cut (Table 1). DM digestibility decreasing with red clover growth and development due to increased concentration of the cell wall in the stem and due to decline and reduction of leaf-to-stem ratio was confirmed in this study. The results showed that the highest DM digestibility was in the third cut, although this period of vegetation was very dry and warm. Red clover harvested in the second cut was characterized by the similar values of DMD (ranged from 81.12 to 78.73% in diploid red clover cultivar and from 79.95 to 79.85% in tetraploid red clover cultivar). Lower DMD determined in the second cut than in the two others might be explain by a favourable conditions for intensive biosynthesis of structural carbohydrates and lignin and probably an unfavourable leaf-to-stem ratio. Taylor and Quesenberry (1996) pointed out that tha two most significant quality parameters are crude protein concentration and DMD. The value of

both these parameters generally decrease with growth and development in all perennial legumes as a result of the leaf-to-stem ratio declining and intensive lignifications process.

Conclusion

Ruminants require forage NDF in the diet to maintain rumen function and maximize productivity, but also NDF digestibility is a key determinant of the nutritive value of a diet. The highest increasing of NDF content was observed in the second cut of diploid red clover cultivar, from 40.36 to 49.54% of DM. Lignin content increased in all cuts of both red clover cultivars, whereas the highest values were in the third cut. At the same time, the lowest NDF digestibility was observed at the mid bloom stage of development in the third cut for *cv* K 39 and *cv* K 32, 40.72 and 35.22%, respectively. Based on the obtained results we can recommend the use of red clover for ruminant nutrition at the mid bud stage of development.

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