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CHEMICAL ATTRIBUTES AND QUALITY IMPROVEMENT OF FORAGE LEGUMES

S. Vasiljević, D. Milić, A. Mikić

Institute of Field and Vegetable Crops, 21000, Novi Sad, Republic of Serbia
Corresponding author: sanjava@ifvcns.ns.ac.yu
Invited paper

Abstract: Fresh, dried or preserved, forage legumes are highly suitable for use as roughage in the animal diet because of their richness in protein, vitamins, and mineral matter. Previous work on forage legumes breeding for improved quality has mostly focused on increasing the crude protein content of these crops. Another major parameter of forage legume quality, especially from the point of view of ruminant nutrition, is the *in vitro* digestibility of dry matter. Values of both these parameters decrease with age in all forage legumes due to a reduced leat to stem ratio and lignification. The decrease in digestibility after budding comes as a result of an increased lignin content and a rise in the proportion of starch polysaccharides. In the context of forage quality, especially important from the point of view of ruminant nutrition is the relation between structural and non-structural carbohydrates. Structural carbohydrates include neutral detergent fiber (NDF) and acid detergent fiber (ADF), while non-structural ones are mostly starch. The latest efforts in forage legume breeding should encompass certain specific traits in order to completely fulfill the needs for safe feed in animal husbandry.

Key words: forage legumes, chemical attributes, quality improvement

Introduction

Fresh, dried or preserved, forage legumes are highly suitable for use as roughage in the animal diet because of their richness in protein, vitamins, and mineral matter. As roughage, they are also characterized by an increased fiber content (over 18% crude cellulose). The proportion of available (digestible) energy per unit weight or volume is lower in these feeds than in the concentrate, and most of the energy is in the form of cellulose or hemicellulose *(Grubić and Adamović, 2003)*.

Along with grasses of the family *Poaceae,* forage legumes of the family *Fabaceae* are the primary type of roughage in the diet of ruminants. Since legumes are rich in protein (15-23%), especially in the leaves (over 25%), they are the most important source of crude protein in roughage *(Vasiljevic et al., 2005b)*. Because of this fact, previous work on forage legumes at the Institute of Field and Vegetable Crops in Novi Sad has mostly focused on increasing the crude protein content of these crops *(Vasiljevic et al., 2003)*. The fibers of forage legumes are found for the most part in the stem. In contrast to the grasses, the fibers of which are less lignified and hence more digestible, forage legumes have fibers that are characterized by high levels of soluble nutrients. This compensates for the somewhat lower digestibility of their fiber, resulting in an increased nutritive value. In addition, it has been established that legume fibers ferment more rapidly in the rumen *(Hinders, 1995)*, which is why ruminants can consume larger amounts of legumes than grasses.

Improvement of quality of the major forage legumes (lucerne, red clover, and annual legumes such as pea, vetch, lupines, and broad bean) requires that there is sufficient genetic variability when it comes to the main quality parameters (protein, fiber) and the levels of antinutritional and toxic substances in order to enable the development of new, higher-quality varieties. The latest efforts in forage legume breeding should encompass certain specific traits depending on the species in order to completely fulfill the needs for safe feed in animal husbandry.

Lucerne

Lucerne (*Medicago sativa* L.) is the most important forage legume in the temperate climate *(Michaud et al., 1988)*. It is grown on over 30 million hectares globally and (as a pure crop) on about 200,000 ha in Serbia *(Đukić, 2005)*.

Along with selection for increased yield, breeding for improved forage quality is one of the most important focal points in the development of new lucerne varieties. Increased leaf contribution to yield leads to an increase in lucerne quality due to a higher crude protein content and greater digestibility and hence results in an increased nutritional value of the crop *(Julier et al., 2001; Rotili et al., 2001)*. Lucerne is bred for a higher nutritional value by the way of developing varieties with an increased leaf proportion in dry matter yield *(Katić et al., 2005b)*. Lucerne has more crude protein, vitamins and carotene in the leaves than in the stem *(Kalu and Fick, 1981)*, and lucerne leaves are also more digestible than the stems *(Kirilov, 2001)*. The proportion of leaves in dry matter yield also depends on genetic factors, i.e. variety *(Katic et al., 2005b)*, as well as on stand density and the phonological stage of plant development *(Lamb et al., 2006)*. Many studies have shown that lucerne quality depends the most on phenological stage and decreases with plant age *(Rotili et al., 2001; Katic et al., 2005 a)*. The highest proportions of leaves and crude protein are found in the early vegetative stages of lucerne growth and development *(Kalu and Fick, 1981; Katic et al., 2001)*. From the early vegetative phase to the start of flowering, the crude protein content of lucerne decreased by an average of 3.81 g /

phenological stage in the leaves and 5.55 g/ phenological stage in the stem *(Katic et al., 2005a)* (Table 1).

Table 1. Daily decrease g/(kg/day) of crude protein during different phenological stages in lucerne

	to Mid-veg. (1)	to Late veget. (2)	to Early bud. (3)	to Late bud. (4)	Mean
CP con. g kg/day leaf	6.02	2.42	4.86	1.94	3.81
CP con. g kg/day stem	8.8	5.7	4.98	2.74	5.55

However, the leaf proportion and hence the crude protein content of lucerne are negatively correlated with green forage and hay yields *(Julier et al., 2001; Katic et al., 2005b)*. An indirect increase of leaf contribution to yield and an increase of lucerne quality and nutritional value are achieved by developing varieties tolerant of early cutting *(Rotili et al., 2001; Lamb et al., 2006)*.

The digestibility of lucerne depends on its fibre content. *Riday et al., (2002)* argue that the genetic variation of fibre content is one of the main reasons of forage quality variation. Variation in lucerne forage digestibility and forage intake is correlated with the variation in the cell wall content. The previous results suggest the presence of significant genetic variation in the fiber (structural carbohydrate) content of lucerne *(Katic et al., 2008)*. Knowing the genetic and seasonal variation of the fibre content of lucerne forage is an important part of the strategy for developing new, higher-quality cultivars of this crop.

The seasonal variation of fibre, NDF and ADF levels was under the influence of higher air temperatures and lower soil moisture. However, part of the variation may also have been a result of the faster growth and maturity of lucerne plants in the summer. Hemicellulose content is at its lowest in the autumn, possibly due to a drop in temperature and a shortening of day length and illumination *(Katic et al., 2008* - Table 2*)*.

Table 2. Seasonal variation of fibre content in lucerne (g kg $^{-1}$) during 2006

Cut	CF	ADF	NDF	Hemicellulose (NDF-ADF)
1	331.3	320.9	411.9	72.7
2	396.3	401.6	499.3	77.1
3	413.6	409.3	508.3	78.0
4	390.1	396.7	478.2	64.3
CV%	7.9	7.9	5.6	92.2
0.05	10.0	12.0	12.0	6.0
LSD 0.01	14.0	16.0	16.0	8.0

Ideas on how to improve lucerne quality change with changes in our knowledge of the nutritional value of the forage. Protein content is a hereditory trait and can be increased by breeding *(Riday and Brummer 2002; Lamb et al., 2006; Rotili et al., 2001; Julier et al., 2000; Julier et al., 2001; Katić et al., 2001; Katić et al., 2008)*. It is important that the proteins are resistant to degradation in the rumen and that losses of ammonia nitrogen are reduced. Alternatively, increasing carbohydrate content increases the energy needed for microbial development in the rumen, and microbial activity reduces ammonia nitrogen losses. Another way to increase the available carbohydrate content is to increase fiber digestibility by selection for a higher ratio of digestible to indigestible fibers. Research conducted in the U.S. has shown that a small increase in fiber digestibility results in a significant rise in milk production by milk cows.

Red clover

Alongside lucerne, red clover (*Trifolium pratense* L) is the most important perennial forage legume. 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.

An increase in forage quality, most importantly the crude protein content, is one of the main goals of red clover breeding *(Vasiljevic et al., 1999)*. The breding program on improved forage quality of the Institute of Field and Vegetable Crops in Novi Sad has so far produced two varieties (Kolubara - *Vasiljevic et al., 2001* i Avala - *Vasiljevic et al., 2008b*) with an increased crude protein content relative to the standard.

Table 3. Chemical composition of red clover by development stage

Variety	Phenological phase					
	Budding		Beginning of flowering		Full flowering	
	CP	CF	CP	CF	CP	CF
Junior	21.11	21.35	18.41	25.85	16.39	27.56
Diana	20.82	21.73	19.05	25.86	16.72	27.60
Milvus	21.05	21.31	18.01	26.76	16.28	28.11
K-17	21.15	21.14	19.70	28.02	16.43	29.13
Kolubara	20.59	20.50	18.49	25.26	16.39	27.21
Prosek	20.94	21.20	18.73	26.35	16.44	27.92
LSD 0.05	1.46	2.42	2.51	3.61	1.70	3.02
LSD 0.01	2.13	3.52	3.65	5.26	2.48	4.40

Stage of development, i.e. plant age, is an important factor affecting the chemical composition and quality of red clover forage *(Ignjatović et al., 2001)*. In a study of the major forage quality parameters in divergent red clover genotypes in the second year of plant life, significant differences were found among the different phenological stages *(Vasiljevic et al., 2005a* - Table 3*)*. Most researchers *(Fairey 1988; Wiersma et al., 1998)* report that the optimal stage for red clover cutting both in terms of yield and quality is when about 20-25% of the flowers appear. At this stage, dry matter digestibility ranges between 65 and 70%, and after that it declines.

The decrease in digestibility after red clover budding comes as a result of of an increased lignin content and a drop in the digestibility of non-starch polysaccharides. In this context, especially important from the point of view of ruminant nutrition is the relation between structural and non-structural carbohydrates.

Vasiljević et al. (2008a) studied the levels of individual structural carbohydrate fractions (NDF, ADF, hemicellulose) in red clover varieties during 2004-2006 and found statistically significant differences among the different cuttings (Table 4) and no differences among the varieties. Both fractions of cellulose fiber (NDF, ADF) had higher levels in the summer due to increased temperatures and accelerated plant aging.

Table 4. Seasonal variation of structural carbohydrate content in forage dry matter (g kg $^{-1}$) of red clover during 2004-2005

Cut	Date of cutting	NDF	ADF	Hemicellulose (NDF-ADF)
I	21.07.2004.	356.6	293.6	62.7
II	11.09.2004.	329.4	238.5	91.0
I	13.05.2005.	352.1	289.6	62.5
II	23.06.2005.	416.5	298.2	118.3
III	01.08.2005.	391.7	325.4	66.2
LSD 0.05		32.1	21.8	35.2
LSD 0.01		44.3	30.1	48.5

According to *Taylor and Qusenberry (1996)*, stress caused by unfavorable environmental conditions as well as seasonal changes can significantly decrease red clover forage quality due to a change in the leaves:stem ratio in favor of the stem and because of accelerated aging. However, research carried out by *Buxton et al (1985)* showed that aging does not cause such a drastic drop in quality in red clover as in some other perennial legumes such as lucerne or thanks to a higher leaf proportion and lower cell wall and lignin contents *(Buxton and Hornstein, 1986)*.

In line with the current trends in the production of high-quality animal feed in the world (U.S., Canada, Great Britain, the Netherlands, Sweden) and in Serbia, our future breeding work for improved forage quality needs to identify genetic variability within the species *Trifolium pratense* L. related to the insufficiently studied quality parameters such as ADF, NDF, PPO, pectin polysaccharides, and phytoestrogens with the aim to develop improved varieties with respect to certain traits. Previous research has shown that in the course of silaging there is about 90% less protein degradation in red clover as compared to lucerne *(McKersie, 1983)*. The considerably lower level of protein degradation in silaged red clover is due to the presence of the polyphenol oxidase enzyme (polyphenol oxidase enzyme system - PPO), which inhibits plant protease activity in the silage *(Jones et al., 1995)*. This important trait should be used for on the one hand for developing red clover varieties with increased levels of polyphenol oxidase activity *(Fothergill and Rees, 2005; Eickler et al., 2008)* and on the other for intergenetic hybridization (transgenic lucerne plants - *Sullivan et al, 2004*). Some red clover genotypes have high phytoestrogen levels that disrupt the normal reproductive cycle in sheep. Formononetin is regarded as the most important phytoestrogenic compound in red clover *(Morley et al., 1966, 1968)*. Selection for lower formononetin levels in this crop is directed towards the development of genotypes suitable for sheep nutrition for the purposes of improved fertility *(McDonald et al., 1994)*.

Annual forage legumes

Annual forage legumes represent one of the highest quality solutions to the constant demand for plant protein in animal husbandry. Among the most important annual forage legume species are pea (*Pisum sativum* L.), common vetch (*Vicia sativa* L.), Hungarian vetch (*Vicia pannonica* Crantz), hairy vetch (*Vicia villosa* Roth), bitter vetch (*Vicia ervilia* (L.) Willd.), Narbonne vetch (*Vicia narbonensis* L.), faba bean (*Vicia faba* L.), grass pea (*Lthyrus sativus* L.), white lupin (*Lupinus albus* L.) and soybean (*Glycine max* (L.) Merr.). All these species may be used as green forage, forage dry matter, forage meal, silage and haylage *(Mikić et al., 2003)*, while vetches may be used for grazing as well.

Pea. Forage pea is certainly one of the most important annual forage legumes, with both winter and spring cultivars cultivated in temperate regions of Europe, Asia and North America. On average, forage yields in pea are about 35 t ha^{-1} of green forage in winter and up to 31 t ha^{-1} in spring cultivars *(Mihailović et al., 2007b)*, with up to 9 t ha^{-1} of forage dry matter in both winter and spring cultivars. The average crude protein content of forage dry matter is about 180 g kg^{-1} *(Mikić et al., 2006)*, with a rather high lysine content of forage dry matter of about 10 g kg^{-1} *(Mihailović et al., 2007a)*.

Vetches. Along with pea, vetches represent one of the most widespread annual forage legumes and are often cultivated in mixtures with cereals. Common vetch is the most significant on a world scale, while other species play a more regional role. In the conditions of Serbia, common, bitter and hairy vetches usually produce more than 30 t ha^{-1} of green forage and between 6 t ha^{-1} and 7 t ha^{-1} of dry matter *(Mihailović et al., 2007b)*. In comparison to the other annual legumes, vetches have a higher crude protein content of forage dry matter, which is often higher than 200 g kg^{-1} (Table 5), and, together with hairy and Hungarian vetches, they are considered to be of slightly better quality than common vetch. Vetches are richer in lysin content of forage dry matter in comparison to other annual legumes, especially pea, reaching 13 g kg in common vetch and 14 g kg^{-1} in hairy vetch g kg^{-1} *(Prokof'eva, 1985)*. Both wild and cultivated species of vetches represent a source of nitrogen, suitable for animal feeding and green manure. Species such as narrow-leafed vetch (*Vicia angustifolia*), large-flowered vetch (*Vicia grandiflora*) and hairy vetch are especially rich in this element (Table 6). As for the calcium content, it is cow vetch (*Vicia cracca*) that is characterised by its high content.

Table 5. Crude protein content of forage dry matter in three vetch species *(Mihailović et al., 2006b; Mihailović et al., 2007a)*

Species	Crude protein content (g kg^{-1})
Common vetch	203
Hairy vetch	215
Hungarian vetch	218

Table 6. Nitrogen and calcium content of dry matter in different *Vicia* species (mg 100 g^{-1}) *(Krstić et al., 2007)*

Species	Nitrogen			Calcium		
	Leaves	Stem	Green pod	Leaves	Stem	Green pod
V. hirsuta	3836	1767	2286	2587	1293	1250
V. cracca	3378	1718	4107	3680	833	546
V. angustifolia	5550	2245	3814	1150	517	505
V. sativa	3864	1454	4615	3453	1040	710
V. grandiflora	4907	2179	4029	1023	650	1010
V. villosa	4826	2178	3533	837	420	398
V. pannonica	3794	1268	3470	3507	867	1680
V. striata	4245	1335	2994	3007	773	980

Faba bean. Although most widely grown for grain, faba bean may be cultivated for both forage and biomass. Its average forage yields may reach more than 45 t ha^{-1} of green forage and nearly 13 t ha^{-1} of forage dry matter *(Mihailović et al., 2006a)*. The forage crude protein yields in spring faba bean cultivars may reach 2600 kg ha^{-1} *(Mikić et al., 2007b)*.

White lupin. In comparison to other annual legumes, white lupin may have lower forage yields but has a great potential for forage crude protein yields and forage nitrogen yields exceeding 2100 kg ha^{-1} and 300 kg ha^{-1}, respectively *(Mihailović et al., 2007b)*.

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Hemijska svojstva i poboljšanje kvaliteta krmnih leguminoza

S. Vasiljević, D. Milić, A. Mikić

Rezime

Krmne leguminoze kao kabasta stočna hrana u svežem stanju, osušene ili konzervisane su izvanrednog kvaliteta za ishranu stoke jer su bogate proteinima, vitaminima i mineralnim materijama. Dosadašnji pravci u oplemenjivanju krmnih leguminoza na bolji kvalitet su prevashodno bili usmereni na veći sadržaj proteina. Drugi važan parametar kvaliteta krmnih leguminoza, naročito s aspekta ishrane preživara je *in vitro* svarljivost suve materije. Vrednost oba ova parametra kvaliteta opada sa starenjem kod svih krmnih leguminoza kao rezultat smanjenja udela lista u odnosu na stabljiku i procesa lignifikacije. Opadanje svarljivosti nakon faze butonizacije se javlja kao posledica povećanog sadržaja lignina i povećanog udela strukturnih polisaharida. Posebno mesto u ovom domenu kvaliteta krme, s aspekta ishrane preživara zauzima odnos strukturnih i nestrukturnih ugljenih hidrata. U strukturne spadaju neutralna deterdžentska vlakna (NDF - neutral detergent fiber) i kisela deterdžentska vlakna (ADF - acid detergent fiber), dok nestrukturne pretežno čini skrob. Novije pravce u oplemenjivanju krmnih leguminoza na kvalitet trebalo bi usmeriti i na neka specifična svojstva kako bi se u potpunosti za potrebe stočarstva obezbedila zdravstveno-bezbedna hrana.

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