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THE EFFECT OF DEVELOPMENT STAGE ON CHEMICAL COMPOSITION OF ALFALFA LEAF AND STEM**

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Abstract: Content of crude protein and cell wall constituents in leaves and stems of alfalfa (Medicago sativa L.) cv K-22 with advancing maturity was investigated. Sampling was done in 7-day intervals in second cut. During growth and development, crude protein content decreased in leaves and stems, from 332,6 to 328,8 gkg^{-1}DM and from 138,6 to 122,0 gkg^{-1}DM, respectively. From first to third development stage, content of ADF in leaves and stems increased, from 352,0 to 476,7 gkg^{-1}DM and from 592,8 to 867,1 gkg^{-1}DM, respectively. Highest contents of ADF and hemicellulose was stated in plant parts in third development stage, in leaves 187,4 and 289,3 gkg^{-1}DM, and in stems 499,4 and 367,7 gkg^{-1}DM. The highest changes of lignin contents are in stems, while content of lignin in leaves increased from 42,0 to 49,0 gkg^{-1}DM.

Key words: alfalfa, growth stage, crude protein, NDF, ADF, lignin

Introduction

Various forage legumes, alfalfa foremost, provide ruminants with a crucial source of proteins and other nutrients. A number of studies so far have demonstrated how the quality of alfalfa plants decreases with plant age as the proportion of stem increases at the expense of leaves in total forage yield (*Buxton et al.*,1985, *Sanderson et al.*, 1989). Alfalfa leaves, compared to the stem, are richer in proteins, essential amino acids and vitamins. In a study conducted by *Dinić et al.* (1996), crude proteins content in leaves was 2.1-fold higher than in the stem in a second cutting (258.6 : 121.1g/kg DM). The nutrient value of leaves changes insignificantly during plant development. The quality of stem, on the other hand, changes more dramatically with age as contents of cellulose and lignin increase and those of proteins, sugar, vitamins and other nutrients decrease (*Ocokoljić*, 1975). Old forage is reluctantly consumed and insufficiently utilised by animals due to high content of crude cellulose, which reduces the utilisation of other nutrients. Early mowing can help keep down the fraction of fibres in forage, and thus improve its digestibility and increase the proportion of its net energy (*Grubić et al.*, 1997).

Analytical methods for estimation of the quality of fibrous feed have changed considerably over the past decades. The standard Weende procedure of chemical analysis of fibres, known as crude fibre, updated by Henneberg and Stohman (1860), is hardly adequate enough to measure fibre ratios in forage as hemicellulose and lignin get partially lost in the process of analysis. A more up-to-date detergent method has therefore been developed to determine fibre components (*Van Soest*, 1963, *Van Soest and Wine*, 1967). Commercial standards for nutrient determination proposed by the American Forage Grassland Council (AFGC) use acid detergent fibre (ADF) for determining the digestibility of forage by ruminant animals, and neutral detergent fibre (NDF) for determining consumption. The quality of forage can only be measured based on these two parameters.

Lignin is built up in the cell wall during the formation and hardening of the secondary cell wall (*Jung*, 1989). It is readily synthesized and its hydrocarbon chains incrusted in all available cell compartments, especially the cellulose crystal lattice. The amount of lignin generally increases from one stage of development to another both in leaves and stalk (*Åman*, 1984).

This study was conducted in order to investigate the changes that take place in nutrient values of the leaves and stem of alfalfa plants in different stages of growth.

Material and methods

Three stages of growth of alfalfa (*Medicago sativa* L.) cv K-22 were examined after second cutting. Samples were hand cut with scissors at 5 cm height. The first stage was cut on June 17th, at full boot stage, another one on June 24th (around 40% flowering), and a third one in full flowering on July 1st. Dry matter was determined by drying out samples at 65ºC and grinding and sieving them to 1 mm particle size.

Crude proteins were computed indirectly from the amount of total nitrogen, measured by the Kjeldahl method modified by Bremner (1960), multiplied by factor 6.25. Neutral detergent fibre was determined as the insoluble part of a neutral detergent solution (*Van Soest and Wine*, 1967). Acid detergent fibre was determined as the fraction of forage insoluble in acid detergent solution (*Van Soest*, 1963). The amount of hemicellulose in samples was determined as a difference between the amounts of NDF and ADF (*Van Soest and Wine*, 1967). Lignin content in ADF was determined as lignin insoluble in 72% H_2SO_4, applying the method of *Van Soest* (1963).

Data were processed by the analysis of variance in a randomized block design. The significance of differences between arithmetic means was tested by LSD test.

Results and discussion

As accumulation of nutrients in plants varies in their different stages of growth, so does their chemical composition over the vegetation period, as well as the chemical composition of different plant organs.

General observation that a decrease in contents of crude proteins coincides with plant aging was confirmed in this study (Table 1).

Table 1. Chemical composition of leaves and stems of alfalfa in different stages of maturity ($gkg^{-1}DM$)

	Plant parts Delovi biljke	I stage I faza	II stage II faza	III stage III faza	LSD-0.05	LSD-0.01
Crude protein Sirovi proteini	Leaf - List Stem - Stablo	332.6 138.6	330.9 133.1	328.8 122.0	2.025 4.179	3.068 6.332
NDF	Leaf - List Stem - Stablo	352.0 592.8	435.9 814.0	476.7 867.1	18.097 27.092	27.420 41.048
ADF	Leaf - List Stem - Stablo	165.8 426.2	176.5 471.9	187.4 499.4	6.249 7.548	9.469 11.437
Hemicelulosa Hemiceluloza	Leaf - List Stem - Stablo	186.2 166.6	259.4 342.1	289.3 367.7	22.271 29.414	33.744 44.567
Lignin	Leaf - List Stem - Stablo	42.0 84.4	46.4 102.6	49.0 119.6	1.926 8.973	2.919 13.596

Much higher contents of crude proteins (from 332.6 $gkg^{-1}DM$ in the first stage to 328.8 $gkg^{-1}DM$ in the third) were found in the leaf than in the stem (highest value 138.6 $gkg^{-1}DM$ in the first stage, and lowest 122.0 $gkg^{-1}DM$ in the third). It suggests an importance of harvesting the plants in their early stage of growth and shows the great nutrient value of leaves. The decrease in protein contents in the leaf with plant aging is closely related to an increase in the proportion of old leaves in later stages of growth and translocation of nutrients from leaf to root and generative plant organs. As plant growth advances, there is a greater accumulation of cellulose and lignin in the stem, while the content of crude proteins decreases (*Ocokoljić*, 1975).

The results of this study show that contents of cell walls increase differently in plant organs of this legume species as plants age (Table 1). The highest content of NDF and ADF was recorded in stems in the third stage of development (867.1gkg^{-1}DM and 499.4gkg^{-1}DM), while NDF was 1.8- and ADF 2.6-fold lower in the leaf at the same stage of development. The data collected are consistent with a report by *Ignjatović et al.*(1998), who found a higher content of NDF and ADF in the stem. Analysing NDF content in different stem segments and leaves of several leguminous plants, *Buxton and Hornstein*, 1986, found the highest amount of NDF in the stem base of alfalfa and lowest in its top leaves. Based on various studies, *Minson* (1990) concluded that the content of cell walls in leaves increased with plant age only up to 10%, so that declining quality of fibre feeds is closely related with a decreasing proportion of leaves in total plant biomass and reduced quality of stems.

During growth and development, the content of hemicelulose was found to increase in the plant organs examined. In the first stage of development, a higher content of hemicellulose was found in the leaf (186.2 gkg^{-1}DM) than in stem (166.6gkg^{-1}DM), but changes were greater in the stem with plant aging. Such trend in hemicellulose contents is not consistent with findings reported by some other authors. *Sanders and Wedin* (1988) reported an initial decrease and then an increase in hemicellulose content in alfalfa stem.

Our findings show that lignin content increases with the progress of plant development both in leaves and stem (Table 1). From the first to the third stage of develoment, lignin content in leaves increased by 16%, and as much as 41% in stem. This is consistent with the findings reported by *Aman* (1984), who found 20-60gkg^{-1} lignin in leaves and 50-120 gkg^{-1}DM in stalk. *Buxton and Hornstein* (1986) specified that lignin content was highest in the stalk base and lowest at the top.

The composition of forage plants is changeable and results from interactions among the plant itself, its stages of development and various environmental factors. Changes in the quality of forage plants should be considered in the context of animal feeding requirements. A priority is to define optimal amounts regarding each specific parameter of quality for any particular species and category of domestic animal. Data on the feed quality during vegetation can be a basis for determining an optimal date for harvesting or grazing. Harvest time can therefore be used to manipulate NDF and ADF contents in alfalfa fibre feed. Early mowing can secure lower fibre fractions in the feed and thus improve digestibility of nutrients (*Grubić et al.*, 1997).

Conclusion

Contents of crude proteins decline in different plant organs from the first to third stage of development. NDF, ADF, hemicellulose and lignin contents increase with the progress of alfalfa development both in the leaf and stem. Changes in the amounts of cell wall constituents are most prominent in the stem. Leaves are more abundantly supplied with nutrients than the stem.

These findings clearly suggest that harvesting of fibrous feeds in the optimal stage of vegetation secures high contents of nutrients provided that appropriate methods of harvesting and storage are applied to preserve maximum leaf biomass.

UTICAJ FAZE RAZVITKA NA HEMIJSKI SASTAV LISTA I STABLA LUCERKE

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Rezime

Ispitivan je sadržaj sirovih proteina i konstituenata ćelijskog zida u listu i stablu lucerke (*Medicago sativa* L.) cv K – 22 u zavisnosti od fenofaze razvitka. Uzorkovanje je obavljeno u sedmodnevnim intervalima u drugom otkosu. Sa porastom i razvitkom, sadržaj sirovih proteina se smanjuje, u listu od 332,6 do 328,8gkg^{-1}SM, a u stablu od 138,6 do 122,0gkg^{-1}SM. Od prve do treće faze razvitka sadržaj NDF se povećao, u listu od 352,0 do 476,7gkg^{-1}SM, a u stablu od 592,8 do 867,1gkg^{-1}SM. Najveći sadržaj ADF i hemiceluloze je takođe u trećoj fazi razvitka, u listu 187,4 i 289,3gkg^{-1}SM, a u stablu 499,4 i 367,7gkg^{-1}SM. Promene u sadržaju lignina su najveće u stablu, dok se u listu sadržaj lignina povećao od 42,0 do 49,0gkg^{-1}SM.

Ključne reči: lucerka, faza razvitka, sirovi proteini, NDF, ADF, lignin

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