ДИНАМИКА НА СЪДЪРЖАНИЕТО НА КАЛЦИЙ В ЛИСТА И СТЕБЛА ОТ ЛЮЦЕРНА (*Medicago sativa*) В ЗАВИСИМОСТ ОТ СТАДИЯ НА ВЕГЕТАЦИЯТА
DYNAMICS OF CALCIUM CHANGES OF ALFALFA (*Medicago sativa*) LEAVES AND STEMS AS AFFECTED BY MATURITY STAGE

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Резюме**

Въпреки че бобовите треви, ползвани като фуражи, са добър източник на калций, високомлечните дойни крави демонстрират признаци на остър недостиг. Проведено е изследване за съдържанието на калций в стебла и листа от люцерна, ползвайки свободен блоков опит на три местоположения и в три стадия на вегетация. Стадиите са: преди бутонизация (без видими пъпки), бутонизация (без видими цветове) и начало на цъфтеж (10-15% цветове). След прибиране на цялата биомаса от 1 m^2 бяха взети представителни проби и след разделянето листата и стеблата бяха изсушени и смлени по стандартна процедура. Резултатите сочат доказан ефект на стадия на зрелостта, като най-високо е съдържанието на Ca в листата ($p<0.05$) в сравнение със стеблата и цялото растение. Най-високо съдържание на Ca в листата е установено в стадия начало на цъфтеж (2.43±0.0797), докато във фаза бутонизация най-високите съдържания са съответно 1,57 и 0,88%. Поради съществените разлики, отчетени между различните вегетационни фази на всички видове проби, може да се заключи, че стадият на вегетацията на люцерната и/или действителното определяне на калциевото съдържание трябва сериозно да се вземат предвид при формулирането на дажбите.

Abstract

Even though legumes used as forage are good sources of Ca, high lactating dairy cows still express signs of acute deficiencies. A study using random block design of three localities, three consecutive mowing and three maturity stages of alfalfa on Calcium content of leaves and stems of alfalfa was done. Maturity stages were: vegetative (no visible buds), bud stage (no visible flowers) and bloom stage (10-15% flowers). After collecting whole biomass from one square meter, a subsample was taken and after separation of leaves and stems, standard protocol of drying and grinding was performed. Results show significant effect of maturity stage with the highest Ca content in leaves ($p<0.05$) as compared with whole plant and alfalfa stems. The highest Ca content of leaves was observed at the beginning of flowering (2.43±0.0797), while bud stage resulted in higher Ca concentration (1.57 and 0.88% respectively). Due to significant differences observed between maturity stages for all types of samples, it may be concluded that stage of harvesting of alfalfa and/or proper determination of Ca content of alfalfa should be seriously considered in ration formulations of animals.

Ключови думи: люцерна, листа, стебла, калций, вегетация.
Key words: alfalfa, leaves, stems, calcium, maturity.

INTRODUCTION

Feedstuffs quality is a very complex notion, but mainly reveals on feeds aptitude to fulfill animals nutritional requirements (Pop et al., 2010).

Minerals represent one of the most important groups of nutrients necessary for the body of every living organism. Calcium (Ca) is surely the most important mineral, both quantitatively (being the most abundant mineral present) and qualitatively playing many physiological functions in the animal body directly or through interactions with other minerals.

Although forages are generally satisfactory sources of calcium for grazing livestock, particularly when they contain leguminous species (Suttle, 2010), still high

production dairy cows are commonly faced with acute shortage of Ca (Underwood and Suttle, 1999). Acute or chronic nutritional deficiencies of both macro and microminerals, plays crucial role in production performances of animals (Pinchak et al., 1989). Kastrati (1997), in a study on chemical composition and nutritional value of feedstuffs, reported high variations in Ca content of fresh alfalfa and alfalfa hay. even though, minerals of alfalfa can be replaced in dairy rations from inorganic sources easily and inexpensively they still have nutritional and some economic value under most circumstances in meeting the nutritional requirements of dairy cows (Robinson, 1998).

Of many factors that influence the chemical composition and nutritional value of alfalfa, maturity stage is certainly a crucial one contributing in the total amount and the balance of nutrients accumulated.

Based on the importance of alfalfa as forage for ruminants and Ca as a nutrient, the primary objective of this study is to ascertain the effect of maturity stage at harvesting of alfalfa on the level of accumulation of Ca in leafs and stems.

MATERIALS AND METHODS

A random block design of experiment involving three localities, three consecutive mowing and three maturity stages of alfalfa was used. Maturity stages were: vegetative (no visible buds), bud stage (no visible flowers) and bloom stage (10-15% flowers). Three replicate samples were taken from each locality by collecting whole biomass from one square meter cut at 5 cm height. 30 plants were taken to separate leafs from stems. After 24 hours drying at 60^{0}C, samples were ashed in muffle furnace at 550^{0}C and Ca was determined using titrimetric procedure as described by AACCI Method 40-21.01. Results were statistically processed using JMP IN software (a business unit of SAS).

RESULTS AND DISCUSSION

The results of statistical processing of the data obtained in this study show significant effects of sample type on Ca content in alfalfa (Prob>.0001). While there is no significant effect of either locality, mowing or maturity stage (P>0.05)

When looking into Ca content in different parts of the plant, presented in table 2, the highest Ca content is found in leafs as compared with whoole plant, and stems. Results show that irespective of locality, maturity stage and mowing, leafs accuulated significantly higher amounts of Ca. Higher Ca of leafs was also reported by Markoviq (2009). These authors have studied the dinamics of Ca accumulation of alfalfa during bud stage, 40% flowering and full bloom, and found 2.87, 3.03 and 3.2% Ca in leafs for respective maturity stage. These results are higher than results obtained in our study. In another study, done by Halgerson et al. (2004), higher Ca in leafs was reported. These authors found 2.5% Ca, which is a bit higher than in our study, while Ca content of stems was very similar. It is worth to be mentioned that the method of sample preparation employed by Halgerson et al. (2004) was the same, but they used NIRS as analytical procedure.

Clark et al. (1987), reported mean values of 1.4% with the variations 0.58-1.9% Ca in samples of alfalfa hay

Таблица 1. Ефекти на изследваните фактори върху Ca съдържание
Table 1. The effects of investigated factors on Ca content

Source/Източник	DF	Sum of squares Сума от квадратите	F Ratio Съотношение	Prob > F
Sample type / Тип проба	3	80,103407	136,0960	<,0001
Locality / Нахождение	2	0,983062	2,5053	0,0837
Mowing / Откос	2	0,391122	0,9968	0,3706
Maturity stage Вегетационен стадий	2	0,603350	1,5376	0,2170

Таблица 2. Ефект на типа на пробата върху Ca съдържание
Table 2. The effect of sample type on Ca content

Sample type/Тип проба	LSM	SE, ±
Full plant / Цяло растение	1,4954482 [b]	0,04921525
Leaf / Листо	2,1934824 [a]	0,05023239
Stem / Стебло	0,7946816 [c]	0,05023239
Prob> F	<0001	

Levels accompanied with different letters in superscript differ significantly/ Нивата, отразени с различни букви като суперскрипт, определят статистическата достоверност

Таблица 3. Ефект на мястото на пробовземане върху Ca съдържание
Table 3. The effect of locality on Ca content

LOCALITY / Локализация		LSM	SE, ±
I.	BARILEVË	1,2840446 [ab]	0,05727444
II.	MAKOVC	1,3722391 [ab]	0,05053073
III.	PRISHTINË	1,4408445 [a]	0,05228235
	Prob> F	0,0837	

Levels with different letters in superscript are significantly different./Нивата с различни букви определят статистическата достоверност

Таблица 4. Ефект на откоса върху Ca съдържание
Table 4. The effect of mowing on Ca content

Mowing	LSM	SE, ±
FIRST	1,3153046 [a]	0,04548946
SECOND	1,3661098 [a]	0,05727444
THIRD	1,4157138 [a]	0,05899543
Prob> F	0,3706	

Levels with different letters in superscript are significantly different/Нивата с различни букви определят статистическата достоверност

Таблица 5. Ефект на стадия на зрелост върху Ca съдържание на люцерна
Table 5. The effect of maturity stage on Ca content of alfafa

Stage/Стадий зрелост	LSM	SE, ±
BLOOM / Цъфтеж	1,4338556 [a]	0,05258332
BUD / Бутонизация	1,3388759 [a]	0,05258332
VEGETATIVE / Преди бутонизация	1,3243967 [a]	0,05428289
Prob> F	0,2170	

Levels with different letters in superscript are significantly different/Нивата с различни букви определят статистическата достоверност

Table 6. The effect of sample type x maturity stage of alfalfa on Ca content
Таблица 6. Ефект на вид проба х стадий на зрелост на люцерната върху Ca съдържание

Sample type/Maturity stage/ Тип проба/стадий зрелост	LSM	SE ±
Leaf, Bloom / Листа, цъфтеж	2,4338594 [a]	0,07968874
Leaf, Bud / Листа, бутонизация	2,0752581 [b]	0,07968874
Leaf, Vegetative / Листа, преди бутонизация	2,0455824 [b]	0,08408481
Whoole plant, Bud / Цяло растение, бутонизация	1,5760508 [c]	0,07968874
Whoole plant, Bud / Цяло растение, цъфтеж	1,5102178 [cd]	0,07968874
Whoole plant, Bud / Цяло растение, преди бутонизация	1,400076 [cd]	0,07975206
Steam, Bud / Стебло, бутонизация	0,8866828 [e]	0,07968874
Steam, Vegetative / Стебло, преди бутонизация	0,7533933 [e]	0,08408481
Steam, Bloom / Стебло, цъфтеж	0,730067 [e]	0,07968874

Levels with different letters in superscript are significantly different/Нивата с различни букви определят статистическата достоверност

collected from different maturity stages, mowing and localities. These results are very close to those obtained for full plant in our study.

According to Kastrati (1997), fresh alfalfa and alfalfa hay taken from different localities of Kosova and harvested at different marurity stages showed varations between 0.58-0,93% and 0,79-3,07% espectively. According to Dale and Batal (2002), Ca content in dried alfalfa ranged from 1.2-1.5% while NRC (2001) reports average values of 1.47-1.52%.

There are very few studies on the Ca content of alfalfa in Kosovo, while comparisions ammong localitiies are missing.

As shown in table 5, there are no significant differences in Ca concentration of alfalfa harvested at different maturity stages. This is due to differences between

localities, mowwings and parts of the plants analyzed. When looking in table 2 and table 6, it may be cleraly seen that maturity stage is important in the accumulation of this mineral since leafs contains far more Ca than stems. By ineracting the effects of type of the sample and the maturity stage, only stems show no difference, while leafs and whoole plant are dependent on the maturity stage.

Results of the effect of sample type (table 5) and interaction of the type of sample with the maturity stage (table 6), on the concentration of Ca are not in full compliance with those reported by NRC (1996) and NRC (2001). According to these sources, the amount of Ca in alfalfa hay decreases as alfalfa becommes more mature. Results of our research shows no difference between vegetative and bloom stage, while bud stage resulted in significantly higher Ca content of whoole plant. Higher Ca content in leafs can be linked with the increase of cell vall constituents Kamberi (2011), and the statement of Hepler (2005) who says that lower Ca content makes cell walls softer and more permeable while increased Ca levels hardens it making less plastic. Freeden (1989), reports a high availability of Ca 56% for alfalfa harvested in the vegetative state and given as hay to non-lactating goats while alfalfa harvested at the full-bloom stage had a lower calcium absorption of 47%.

CONCLUSIONS

This study confirms the effect of the stage of development of alfalfa on the level of accumulation of Ca in leafs. Since different amounts of Ca may be supplied to animals fed alfalfa harvested at different maturity stages and different mowing due to differences in Ca concentration in leafs and stems, it is advised to take care on the time of harvesting and proper postharvest management to protect leafs.

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