Biotechnology in Animal Husbandry 25 (5-6), p 1225-1231, 2009 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 633.31

EFFECTS OF GROWTH STAGE ON THE MINERAL CONCENTRATIONS IN ALFALFA (*Medicago sativa* L.) LEAF, STEM AND THE WHOLE PLANT

J. Marković, R. Štrbanović, M. Cvetković, B. Anđelković, B. Živković

Institute for Forage Crops, 37000, Kruševac, Republic of Serbia
Corresponding author: jordan.markovic@ikbks.com
Original scientific paper

Abstract: The proper balance of protein, energy, vitamins and all nutritionally-important minerals is needed to make a nutrition program successful. Nutrient balance is the key to any effective nutrition program. Mineral concentrations in forages vary much more than do protein and energy concentration. In general, alfalfa leaves were higher in nitrogen, phosphorus, calcium, magnesium, copper, zinc, iron and manganese than alfalfa stem and whole plant. In contrast, stem of alfalfa tended to be higher in potassium than leaf of alfalfa. Forage mineral concentration was affected by maturity. Generally, there was a rapid uptake of mineral during early growth and gradual dilution as the plant matured. Nitrogen, potassium, magnesium, iron, copper, zinc and manganese are the most common minerals affected by plant maturity. Phosphorus and calcium concentrations increased with growth and development in both anatomical fractions as well as in whole plant. This experiment was conducted to determine the mineral composition of alfalfa (*cv* K-28) leaf, stem and whole plant during growth and development.

Key words: alfalfa, leaf, stem, stage of growth, minerals

Introduction

Mineral contents of forages are very important for animal feeding. A number of inorganic elements are essential for normal growth and reproduction of animals *(NRC, 2001)*. Grazing livestock have to depend largely upon forage to fulfill their mineral requirements *(Espinoza et al., 1991)*. The concentration of individual minerals in forages varies greatly depending on soil, plant and management factors.

But acute and chronic dietary deficiencies in macro and micro minerals have significant impact on production efficiency of rangelands throughout the world *(Pinchak et al., 1989)*. Mineral elements most likely to be lacking under grazing conditions for ruminants are Ca, P, Cu and Zn *(McDowell, 1996)*.

Therefore, it is important that rich forages in terms of mineral content are used in animal feeding. Among the forage plants, alfalfa is one of the best forage plants to meet the mineral demands of livestock.

The object of this investigation was to examine the difference between stages of growth of alfalfa leaf, stem and whole plant in contents of nitrogen, phosphorus, potassium, calcium, magnesium, iron, zinc, copper and manganese.

Material and Methods

The experiment was designed as randomized block system in three replicates. Three stages of growth of alfalfa (*Medicago sativa* L.) *cv* K-28 were examined in the second cut, in the first year of production. 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 65ºC and grinding and sieving them to 1 mm particle size.

Forage was cut and a sample of the whole plants was collected. A separate sample, collected at the same time was separated into the leaves and stems. The concentrations of K, Ca, Mg, Fe, Zn, Cu and Mn were measured by atomic absorbance spectrophotometry. Samples for Ca and Mg analysis were prepared with $1gL^{-1}$ lanthanum. Phosphorus was measured colorimetrically, according to the ISO 6491 method. The amount of total nitrogen was measured by the Kjeldahl method modified by *Bremner (1960)*.

Data were processed by the analysis of variance in a randomized block design. The significance of differences between arithmethic means was tested by LSD test. Soil type was with an organic matter content of approximately 3,5 % and a pH of 6,5.

Results and Discussion

The macro- and micro-minerals of different botanical fraction of alfalfa, as well as the whole plant harvested at three different development stage are presented in tables 1 and 2. The relative changes of mineral composition discussed in this paper are largely associated with between plant anatomical fraction and stage of plant development.

For the leaf tissue of alfalfa, nitrogen contents at all stages of maturity were higher than 48.0 gkg^{-1} DM (Table 1). In contrast, the stem fraction of alfalfa generally had lower content of nitrogen, and with growth and development decreased from 22.88 to 20.70 gkg^{-1} DM. Profile of whole plant tissue reflected the relative proportions of each tissue and were consistent with other findings for

alfalfa *(Frame, 2005)*. It suggests an importance of harvesting the plants in their early stage of growth and shows the great nutrient value of leaves.

In the second development stage phosphorus concentration in leaves, stems and whole plant were significantly higher, but in the third stage of growth content of phosphorus declined and were 2.70, 2.06 and 2.39 gkg^{-1} DM in leaf, stem and whole plant, respectively.

Leaves had higher concentrations of minerals than stems, except for potassium (Table 1). This agrees with *Halgerson et al. (2004)*, who reported that concentrations of most minerals were greater in leaves than in stems, but who also found potassium concentration to be greater in stems than in leaves.

Calcium is necessary for the proper growth and functioning of root tips and meristems. Calcium pectate is a component of the cell walls, increasing mechanical strength of the plant. Therefore, calcium tends to be stored in the leaf. Much higher content of calcium (from 28.66 to 31.98 gkg^{-1} DM) were found in the leaf than in the stem (the highest value 13.36 gkg^{-1} DM in the third, and the lowest 10.95 gkg^{-1} DM in the first stage of development). The calcium concentration in whole plant increased with growth and development, as well as in leaves and stems. This data are not in agreement with *Ignjatović et al. (1998)*. Although forages are generally high in calcium, the availability of calcium in some forages may be low because of the presence of calcium oxalate. In alfalfa, 20 to 33 % of the calcium was present as insoluble calcium oxalate and apparently unavailable to the animal *(Martz et al., 1990)*.

Table 1. Content of macro-minerals in leaf, stem and whole plant of alfalfa, cv K-28 at different stage of maturity (g kg^{-1} DM)

Macro-minerals	Plant anatomical fraction	I stage	II stage	III stage	LSD 0.05	LSD 0.01
N	Leaf	54.76	51.93	48.96	0.597	0.991
	Stem	22.88	21.96	20.70	0.737	1.222
	Whole plant	42.55	38.81	35.17	0.416	0.689
P	Leaf	2.93	3.42	2.70	0.153	0.254
	Stem	1.91	2.35	2.06	0.119	0.198
	Whole plant	2.54	2.96	2.39	0.098	0.162
K	Leaf	21.42	19.38	18.26	0.443	0.735
	Stem	27.61	22.88	22.30	0.902	1.495
	Whole plant	23.79	20.91	20.22	0.502	0.833
Ca	Leaf	28.66	30.28	31.98	3.865	6.409
	Stem	10.95	9.76	13.36	0.625	1.037
	Whole plant	21.88	21.29	22.91	1.973	3.271
Mg	Leaf	9.50	9.12	8.83	0.378	0.628
	Stem	4.51	3.85	4.25	0.484	0.803
	Whole plant	7.59	6.81	6.59	0.401	0.666

Magnesium is closely associated with calcium and phosphorus, and the magnesium content of forage crops varies widely. The concentration of magnesium declined with growth and development in leaf, stem and whole plant. The highest magnesium content was in the first stage of development (9.50, 4.51 and 7.59 gkg^{-1} DM in leaf, stem and whole plant, respectively).

The present investigation has revealed that concentration of microelements change in both plant anatomical fractions, as well as in whole plant with plant maturation. The considerable decline in the iron concentration of alfalfa stem with increasing crop maturity and the consistently high iron concentration of leaf are in agreement with earlier experiments *(Kidambi, 1990)*. The whole plant tissue reflected the relative proportions of each tissue type and the highest concentration was in the first stage, and the lowest in the third stage of development (Table 2). At all harvest times, leaf of alfalfa contained higher content of zinc than alfalfa stem (Table 2). The zinc concentration in whole plant of alfalfa declined from 33.26 gkg^{-1}DM to 23.37 gkg^{-1} DM in the full flowering stage. The data in Table 2 show little difference in copper and manganese concentrations between three different stages of growth in leaf, stem and whole plant of alfalfa. The leaf contained higher concentration of Cu and Mn than stem, during development. In the both investigated plant anatomical fraction, and in whole plant of alfalfa concentrations of these microelements decreased in the second stage, and increased in the third development stage. The concentrations of copper and manganese were higher in full boot stage than in full flowering stage.

Table 2. Content of micro-minerals in leaf, stem and whole plant of alfalfa, cv. K-28 at different stage of maturity (mg kg^{-1} DM)

Micro-minerals	Plant anatomical fraction	I stage	II stage	III stage	LSD 0.05	LSD 0.01
Fe	Leaf	309.46	222.56	208.15	41.69	69.14
	Stem	219.66	158.29	161.40	24.39	40.45
	Whole plant	275.06	194.40	185.38	31.93	52.95
Zn	Leaf	40.62	31.15	28.91	3.891	6.453
	Stem	21.41	14.79	17.55	1.323	2.194
	Whole plant	33.26	23.98	23.37	3.215	5.332
Cu	Leaf	18.12	17.27	17.85	2.888	4.788
	Stem	13.02	10.96	11.62	1.387	2.301
	Whole plant	16.16	14.50	14.81	2.203	3.653
Mn	Leaf	70.30	61.55	74.76	7.544	12.510
	Stem	28.46	20.46	26.76	1.926	3.194
	Whole plant	54.27	43.55	51.38	3.897	6.462

The mineral content of forages is greatly influenced by the quantity and the availability of minerals in the soil. Availability is very important because, for many

minerals, only a small portion of the total mineral is available for uptake by the plant. Low concentrations of these minerals in forage may impair the ability of microorganisms to digest fiber and synthesize protein *(Whitehead et al., 1985).*

Large decreases in forage manganese concentration have been observed due to reletively small increases in soil pH. Zinc and copper may decrease slightly with increasing soil pH. Macrominerals are less affected than microminerals by soil pH *(Masters et al., 1985).* In addition to maturity, climatic and seasonal changes can influence forage minerals *(Emanuele et al., 1990).*

Conclusion

Stage of forage maturity affects the content of a number of minerals in forages. General observation that a decrease in contents of nitrogen coincides with plant aging was confirmed in this study. The highest content of nitrogen was in leaf of alfalfa, cv K-28 at the first stage of development.

The leaf tissue contained higher concentrations of macroelements, except potassium. In both investigated plant anatomical fraction and in whole plant, the highest content.of macroelements were in the first stage of growth, except for phosphorus and calcium. Phosphorus and potassium decrease markedly with increasing maturoty, while calcium is not greatly altered by stage of maturity.

The highest content of iron, zinc and copper recorded in leaves in the first stage of plant development. The manganese concentration was the highest in leaves, in the third development stage.

Uticaj faze razvića na koncentraciju mineralnih elemenata u listu, stablu i celoj biljci lucerke (*Medicago sativa* L.)

J. Marković, R. Štrbanović, M. Cvetković, B. Anđelković, B. Živković

Rezime

Pravilna izbalansiranost proteina, energije, vitamina i za ishranu važnih minerala neophodan su uslov za sprovođenje adekvatne ishrane. Izbalansiranost hranljivih materija jeste osnova pravilne ishrane. Koncentracije mineralnih elemenata u kabastim hranivima variraju više od proteina i energijom bogatih supstanci. Generalno, list lucerke sadrži veću koncentraciju azota, fosfora, kalcijuma, magnezijuma, bakra, cinka, gvožđa i mangana, nego stablo i cela biljka. Suprotno ovome, stablo sadrži veću količinu kalijuma nego list lucerke. Koncentracija mineralnih elemenata u lucerki zavisi od faze razvića. Usvajanje

mineralnih elemenata tokom ranih faza razvića je intenzivno, i smanjuje se sa napredovanjem faze razvića. Koncentracija fosfora i kalcijuma se povećava sa rastom i razvićem u listu, stablu i celoj biljci lucerke, dok je koncentracija azota, kalijuma, magnezijuma, gvožđa, bakra cinka i mangana usko povezana sa napredovanjem faze razvića. Ova istraživanja su sprovedena da bi se odredile koncentracije mineralnih elemenata u listu, stablu i celoj biljci lucerke, *cv* K-28, tokom rasta i razvića.

Acknowledgment

Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project 20048.

References

ESPINOZA J.E., MCDOWELL L.R., WILKINSON N.S., CONRAD J.H., MARTIN F. G. (1991): Forage and soil mineral concentrations over a 3 years period in a warm climate region of central Florida. II. Trace minerals. Livest. Res. Rural Dev., 1, 3-10.

EMANUELE S.M., STAPLES C.R. (1990): Ruminal release of minerals from six forage species. J. Anim. Sci., 68, 2052-2060.

FRAME J. (2005): Forage legumes for temperate grasslands. Food and agriculture organization of the United nations, Rome, 2005.

HALGERSON J.L., SHEAFFER, C. C., MARTIN, N. P., PETERSON, P. R., WESTON S.J. (2004): Near-infrared reflectance spectroscopy prediction of leaf and mineral concentrations in alfalfa. Agronom. J., 96, 344-351.

IGNJATOVIĆ S., DINIĆ B., KOLARSKI D., UROŠEVIĆ B. (1998): Chemical composition of first and second cut of lucerne (*Medicago sativa* L.) at different stages of maturity. Proc. 17th General Meeting EGF, may 18-21, Debrecen, Hungary.

KIDAMBI S.P. (1990): Mineral concentration in alfalfa and sainfoin as influenced by soil moisture level. Agron. J., 82, 229-236.

MARTZ F.A., BELO A.T., WEISS M.F., BELZEA R.L., GOFF, J.P. (1990): True absorption of calcium and phosphorus from alfalfa and corn silage when fed to lactating cows. J. Dairy Sci., 73, 1288-1295.

MASTERS D.G., FELS H.E. (1985): Zinc supplements and reproduction in grazing ewes. Biol. Trace Element Res., 7, 89-93.

MCDOWELL L.R. (1996): Feeding minerals to cattle on pasture. Anim. Feed Sci. Technol., 60, 247-271.

NATIONAL RESEARCH COUNCIL (2001): Nutrient requirements of dairy cattle, Seventh Revised Edition, National Academy Press, washington, DC., p. 304.

PINCHAK W.E., GREENE L.W., HEITSCHMIDT R.K. (1989): Mineral dynamics in beef cattle diets from a southern mixed-grass prairie. J. Range Manage, 42, 5, 431-433.
WHITEHEAD D.C., GOULDEN K.M., HORTHEY R.D. (1985): The distribution of nutrient elements in cell wall and other fractions of the herbage of some grasses and legumes. J. Sci. Agric., 36, 311-318.

Received 31 May 2009; accepted for publication 15 August 2009