

## **TRANSFER OF MAGNESIUM FROM SOIL AND FORAGE TO GOATS GRAZING IN A SEMIARID REGION OF PAKISTAN: INFLUENCE OF SEASONS AND SAMPLING PERIODS**

**ZAFAR IQBAL KHAN<sup>1\*</sup>, MUHAMMAD ASHRAF<sup>2</sup>, KAFEEL AHMAD<sup>1</sup>,  
L.R. MCDOWELL<sup>3</sup> AND EHSAN ELAHI VALEEM<sup>4</sup>**

<sup>1</sup>*Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan.*

<sup>2</sup>*Department of Botany, University of Agriculture, Faisalabad-38040, Pakistan.*

<sup>3</sup>*Animal Science Department University of Florida P.O. Box 110910, USA.*

<sup>4</sup>*Public Private Partnership Unit (PPPU), Planning and Development Department, Government of Sindh, Karachi-74200, Pakistan.*

\*Corresponding Authors: drzafar10@hotmail.com; valeem@hotmail.com

### **Abstract**

The investigations were conducted in a goat farm at "Livestock Experimental Station" located in the South-western Punjab. The purpose of the investigations was to determine the influence of seasons and sampling periods, the transfer of magnesium from soil and forages to grazing goats in this semiarid region as to evaluate Mg requirement of the grazing livestock. Soil, forage and blood samples from lactating/ non-lactating and male grazing animals were taken fortnightly, four times during summer and winter. Soil samples taken from the pasture grazed by goats had adequate levels of Mg during winter and summer, while forage contained marginal deficient Mg. The marginal deficient levels of Mg in lactating goats were observed during winter and summer, while marginal deficient levels of Mg in the plasma of non-lactating goats were observed during both seasons. Plasma of male goats contained marginal deficient levels of Mg during summer. On the whole, the plasma Mg concentration may be considered inadequate mainly due to unavailability of this mineral from the dietary sources. It is concluded that high bio-available Mg supplementation is needed for increased animal productivity in this region.

### **Introduction**

Hypo-magnesemia tetany in animals is a serious disease of the first eight weeks of lactation. The incidence is highest during one to four weeks after lambing. In Australia, however, a high incidence of hypo-magnesemia in breeding ewes has been correlated with periods of rapid winter growth of pastures (Underwood and Suttle, 1999). The signs of Mg deficiency in adult ewes are similar to those of younger animals but death may occur more rapidly after convulsions (McDowell, 2003).

Subnormal serum magnesium (Mg) is an indicator of a serious metabolic disorder in cattle known as lactation tetany, grass tetany, and hypo-magnesemia (Sjollem, 1930). Ruminants grazing immature forages, can have decreased Mg absorption from the reticulorumen due to high dietary potassium (K) thereafter resulting in hypo-magnesemia (Ram *et al.*, 1998; Schonewille *et al.*, 1999). Adult ruminants are more vulnerable to this disease because of a low capacity of mobilization of skeletal Mg (Chicco *et al.*, 1972). Lactation exacerbates the condition and lush, fast growing early spring forage as a primary source of nutrition makes cows more likely to develop tetany (McDowell, 2003). Clinical signs of grass tetany include stiffness in walking and a staggered gait. As the condition aggravates, the animal shows visible muscular tremors, as well as the blinking of the third eyelid. At a severe stage the animal will collapse with continual tetanic muscular spasms.

**Table 1. Mean Mg concentrations of soil, forage (mg kg<sup>-1</sup> dry matter) and blood plasma (mg L<sup>-1</sup>) of different animal classes as a function of the seasons and sampling times.**

Variable	Season	Sampling period				Mean
		I	II	III	IV	
Soil	Winter	445.87 ±9.80	398.20 ± 7.85	365.25 ± 13.45	345.30 ± 6.25	388.66 ± 9.34
	Summer	410.90 ±6.30	422.25 ± 5.80	290.20 ± 8.12	267.20 ± 9.45	347.64 ± 7.42
Forage	Winter	2830.00 ± 142.83	2725.0 ± 124.5	2475.0 ± 135.3	2580.0 ± 115.10	2650.50 ± 126.00
	Summer	1430.00 ± 90.85	1390.50 ± 110.80	1365.00 ± 125.70	1245.00 ±130.20	1357.65 ± 116.15
Plasma (Lactating)	Winter	24.68 ± 2.30	26.33 ± 2.35	25.76 ± 1.20	25.95 ± 2.40	25.68 ± 0.71
	Summer	24.30 ± 1.70	20.35 ± 1.12	18.30 ± 1.25	17.25 ± 2.66	20.05 ± 3.11
Plasma (Non-lactating)	Winter	22.95 ± 1.23	20.94 ± 0.95	23.14 ± 1.15	25.60 ± 0.85	23.16 ± 1.91
	Summer	21.64 ± 0.62	22.60 ± 0.85	20.76 ± 0.54	18.69 ± 1.10	20.92 ± 1.67
Plasma (male)	Winter	18.50 ± 0.95	17.25 ± 0.85	17.56 ± 1.20	16.16 ± 1.30	17.37 ± 0.96
	Summer	22.66 ± 0.75	21.74 ± 0.65	19.50 ± 1.35	18.45 ± 1.75	20.59 ± 1.95

Number of samples during each season: soil 60, forage 60, plasma 120.

Use of small grain forages in cool weather, such as ryegrass and oats, increases the risk of the disease. Incidence of Mg deficiency can be influenced by management considerations as well as geographical location (McDowell, 2003). Grass tetany is a common problem in many regions of the world (McDowell, 2003). The purpose of this study was to evaluate Mg status of grazing goats on natural and improved forages in pasture including arid region of Punjab, Pakistan. A major emphasis of the investigation was on the assessment of magnesium status and the risk of grass tetany in this region of Pakistan.

### Materials and Methods

The investigations were carried out using a herd of Daira Din Pannah breed of goats, grazing pasture at the Livestock Experimental Station Rakh Khairwala, District Layyah in southern Punjab (Pakistan). The farm was established about 40 years ago which consists of 14,000 hectares land. It allows almost 7000 animals to graze. The climate is sub-tropical, semi-arid continental characterized by two distinct seasons, winter and summer. Samples from those animals were collected which had been in the pastures for not less than 1-2 years prior to sample collection. All the animals at the farm had access to graze the improved varieties of forages throughout the year.

For sampling purpose, 30 animals were grouped into three classes, according to age, physiological status and gender, with 10 animals per class as follows: Class one contained 10 lactating goats, class two comprised 10 non-lactating goats, and class three consisted of 10 male goats. These animals were ear tagged at this ranch.

Samples of soil, forage and animal blood plasma, were collected from the goat ranch of the farm fortnightly in each season. Sampling periods were January, February and June, July, corresponding to the winter and summer seasons, respectively. The mean temperature of the year ranged from 25-28°C and the average relative humidity was 25-45 %.

Samples of forage were collected from those species that were most frequently grazed by goats at this ranch. The forage species collected were: *Medicago sativa*, *Avena sativa*, *Trifolium alexandrinum*, *Hordeum vulgare*, *Cichorium intybus*, *Lathyrus odoratus*, *Chenopodium morale* during winter and *Cyperus rotundus*, *Tribulus terrestris*, *Pennisetum glaucum*, *Cynodon dactylon*, *Digitaria decumbens*, *Cynodon plectostachyum*, *Panicum milliacum*, *Sorghum bicolor*, *Setaria italica* during summer. As mineral status of the soil differed from place to place, therefore, soil and corresponding forage samples were collected at three different places with five replications from each place. All the samples were analyzed for magnesium. Soil, forage, and plasma minerals concentrations were compared to established critical values to determine the various categories of deficient levels. The critical level for soils indicates the element concentration below which normal growth and/ or mineral composition of forage may be adversely affected. For forage samples, it indicates the lowest requirement of the element or organic constituent to avoid deficiency symptoms in animals. Plasma critical levels indicate the concentration below which specific signs of deficiency may occur. Interpretation of these critical values was done with caution taking into consideration the management, nutritional, environmental and individual factors that affect the availability, supply and utilization of each nutrient.

#### Collection of samples

**Soil:** Soil samples were taken from different surfaces up to 15-20 cm depth at three different points from each pasture using a stainless steel sampling auger. The samples were air-dried and ground using a Wiley mill with a 2 mm sieve and mixed. These samples were stored in plastic bags.

**Forage:** Using the hand plucked method, forage samples were collected from three different points in the pasture ranch on the same spots from where soil samples were collected, twice a day, in the morning and the late afternoon, after following the grazing animals closely and hand plucked materials comparable to those grass species and plant parts eaten. The plucking of forage samples was done at 15 cm from the ground to simulate the grazing behavior of animals. The samples were washed with 1% HCl followed by 3-4 washings with distilled water to remove foreign material. Then they were air-dried. The air-dried samples were oven dried at 65 °C. These were ground to powder and stored in clean and dry plastic bags for chemical analysis.

**Blood:** Blood samples were taken from male and female goats that were offered feed using the same ingredients being raised at the farm. Blood disposable needles for goats were used to collect the blood from the jugular vein. Blood from each animal was taken in a standing position by holding the animal in between knees. Hair or wool over the site of jugular vein was sheared. The jugular veins were raised, while pressing the posterior side of the neck with thumb and afterward, needles were inserted into the vein. Twenty ml of the blood were drawn

into a clean sterile test tube having anticoagulant (EDTA). The blood samples were centrifuged at 3000 rpm for 20 minutes to separate the plasma. The plasma samples were stored at  $-20^{\circ}\text{C}$  till further analysis.

### **Sample preparation**

**Soil:** Minerals were extracted from soil using the Mehlich-1 extracting solution method (0.05 N HCl + 0.025 N H<sub>2</sub>SO<sub>4</sub>) following Rhue and Kidder (1983). Ten grams of air-dried soil were taken in 125 ml conical flask and 40 ml Mehlich-1 extracting solution was added to it and shaken for 15 minutes on a reciprocating shaker, filtered through a medium porosity filter paper (Whatman filter paper No. 2). Clear supernatant was obtained by centrifugation for 5 minutes at 180 rpm. The supernatant was stored in plastic bottles for macro-and micro-nutrients determination.

**Forage:** One gram of the dried forage sample was taken in a 50 ml conical flask, and kept overnight after adding 5 ml concentrated HNO<sub>3</sub> and 5 ml perchloric acid (HClO<sub>4</sub>). Next day, again 5 ml HNO<sub>3</sub> was added to each sample. All the samples were digested on hot plate at 250<sup>o</sup> C in fuming hood till the material was clear. After digestion the material was cooled down and the volume was made up to 50 ml with double distilled water and stored in clean airtight bottles for analysis of metal ions (Anon., 1990).

**Plasma:** A quantity of 5 ml of blood plasma was digested with a 4 ml mixture of perchloric acid and nitric acid (1:1). After digestion, the volume was made to 25 ml with distilled de-ionized water. Further dilution was prepared for macro mineral determination following Kamada *et al.*, (2000).

### **Analytical procedure for determination of minerals**

The above samples were diluted as required, and analyzed for Mg concentrations. An aliquot of above samples was used for determination of Mg using an atomic absorption spectrophotometer (Py Unicam Ltd. York street, Cambridge UK). To ensure the quality of the analysis, a certified standard was analyzed after every six samples. The samples were diluted as required and concentrations of elements were measured. The final quantities were computed by comparison of sample reading with standard curves.

### **Statistical analysis**

The data were analyzed using a split-plot completely randomized design with the effects of seasons as the whole plots and effects of sampling periods as the sub-plots (Steel and Torrie, 1980). Differences among means were ranked using Duncan's New Multiple Range Test (Duncan, 1955).

## **Results**

### **Pasture samples**

**Soil:** Soil Mg concentration was affected significantly ( $p < 0.01$ ) both by seasons or sampling periods of sampling. The amount of Mg in soil was higher in winter as compared to that in summer except at the 2<sup>nd</sup> sampling period. During both seasons there was a consistent decrease in soil Mg from 2<sup>nd</sup> sampling period to onwards (Table 1).

**Forage plants:** Significant seasonal or sampling period effects ( $p < 0.001$ ) were observed on forage Mg level and concentration of forage Mg during winter was markedly higher than that during summer. During winter a decreasing trend in Mg level was found with time but during summer no significant variation in forage Mg was found at different time intervals (Table 1).

#### **Animal samples**

##### **Lactating goats**

**Plasma:** The sampling period had a significant effect ( $p < 0.001$ ) on plasma Mg but there was no significant seasonal effect ( $p < 0.05$ ) on it. Mg concentration in plasma remained almost uniform at all sampling intervals during winter. On the other hand, during summer a consistent decrease in plasma Mg level was found with time (Table 1).

##### **Non-lactating goats**

**Plasma:** Plasma Mg level did not vary with seasonal change ( $p < 0.05$ ), but sampling periods had a significant effect ( $p < 0.01$ ) on plasma Mg. Plasma Mg contents were found almost uniform at all sampling periods during winter (Table 1), whereas during summer the plasma Mg levels were uniform at the last three sampling periods.

##### **Male goats**

**Plasma:** Both seasonal and sampling interval effects were non-significant ( $p < 0.001$ ) on plasma Mg level. During winter, a slight increase in plasma Mg was observed up to the 3<sup>rd</sup> sampling period, whereas during summer, the plasma Mg decreased consistently with time (Table 1).

#### **Discussion**

Grass tetany is a complex ruminant metabolic disorder that is affected by a number of factors such as forage species and mineral composition, soil properties, fertilizer practices, season of the year, temperature, animal species, breed and age (McDowell and Valle, 2000).

In the present study there was a significant seasonal effect on mean soil Mg concentration so that Mg content in soil was higher in winter than that in summer. These concentrations were above the requirement of plant growth (Rhue and Kidder, 1983). The soil Mg concentration found in this study was higher than that reported earlier in north Florida (Cuesta *et al.*, 1993; Tiffany *et al.*, 2000). Forage Mg concentrations differed significantly during winter and summer. The high forage Mg level in winter was above the requirement of ruminants suggested by Anon. (1985), whereas in summer the forage Mg was below the critical level. Similar low values in summer and adequate values for winter forage Mg were reported by Salih *et al.*, (1983) in Florida.

Despite higher Mg level in forage, its biological availability is very low perhaps due to certain unknown interactions of various elements and conditions of rumen of the animals. According to Dua and Care (1995) the dietary Mg availability to stock is markedly affected by other dietary components, especially K. High dietary levels of K and N will inhibit Mg absorption from the rumen. Ca and soluble carbohydrates may respectively increase and decrease dietary Mg requirements of livestock, whereas raised dietary P levels appears to lower the requirements for both Ca and Mg (Judson and McFarlane, 1998). Mean plasma Mg levels in the lactating goats were not affected significantly during winter and summer. The levels were generally lower than the known

critical level (McDowell *et al.*, 1983). In the non-lactating goats, the plasma Mg levels were close to the critical level during both seasons with no seasonal effect. In contrast, in male goats, the plasma Mg levels were above the critical level and higher than that in the non-lactating and lactating goats. Seasonal influence in all cases was not prominent. The plasma Mg levels of the lactating goats were lower than those in the non-lactating and male goats. This may have been due to a large amount of Mg secreted through milk in the lactating goats. Mean plasma Mg values were lower than those reported in the previous studies conducted in Venezuela (Rojas *et al.*, 1993) and in Indonesia (Prabowo *et al.*, 1990). In view of Miller *et al.* (1972) plasma Mg concentration is controlled to some extent by homeostatic mechanism. For the lactating goats, Mg concentration was deficient during both seasons, although the source of Mg (forage, feed and water) had higher Mg level above the requirements of ruminants. The depression in plasma Mg may have been due to the fluctuations and other dietary interactions, or other factors affecting requirements (Baumgurtel and Judson, 1998).

In conclusion, forages were contained to be deficient marginally in Mg during summer. Forage Mg levels were ineffective in raising the plasma Mg levels in all classes of goats except in male animals during winter. The plasma Mg levels were likely to be deficient marginally in the lactating and non-lactating goats during winter and summer and in male animals only during winter. Therefore, supplementation is needed with a mixture containing Mg or by other safe and practical means of raising the Mg intake of animals sufficient to maintain normal plasma value during both seasons and to prevent losses from lactation tetany.

#### References

- Anonymous. 1985. Nutrient requirements of domestic animals, Nutrient Requirements of Sheep (5<sup>th</sup> ed.) Natl. Acad. Sci., N.R.C., Washington, D.C.
- Anonymous. 1990. Official Methods of Analysis of the Association of Analytical Chemists. (14<sup>th</sup> Ed.). Arlington, Virginia, U.S.A.
- Baumgurtel, K.L. and G.J. Judson. 1998. Evaluation of a sustained release bolus to supply trace elements and vitamins to beef cattle. *Anim. Prod. Aust.*, 22: 133-136.
- Chicco, C.F. C.B. Ammerman, W.G. Hillis and L.R. Arrington. 1972. Utilization of dietary magnesium by sheep. *Am. J. Phys.* 222:1469-1472.
- Cuesta, P.A., L.R. McDowell, W.E. Kunkle, F. Bullock, A. Drew, N.S. Wilkinson and F.G. Martin. 1993. Seasonal variation of soil and forage mineral concentrations in north Florida. *Commun. Soil Sci. Plant Anal.*, 24: 335-347.
- Cuesta, P.A., L.R. McDowell, W.E. Kunkle, F. Bullock, A. Drew, N.S. Wilkinson and F.G. Martin. 1993. Mineral concentration in liver and milk of grazing beef cattle as related to season and location. *Intl. J. Anim. Sci.*, 8:189-193.
- Dua, K and A.D. Care. 1995. Impaired absorption of magnesium in the etiology of grass tetany. *Brit. Vet. J.*, 151:413-26.
- Duncan, D.B. 1955. Multiple range and multiple F-test. *Biometrics*, 11:1-42.
- Judson, G.J and J.D. McFarlane. 1998. Mineral disorders in grazing livestock and the usefulness of soil and plant analysis in the assessment of these disorders. *Aust. J. Exp. Agric.*, 38:707-723.
- Kamada, H., H. K. Nishimura, P. Krongyuti, P. Sukkasame, N. Phoengpong and S. Intramane. 2000. Selenium status of soil, herbage and beef cattle in Southern Thailand. *Asian. Aus. J. Anim. Sci.*, 13:757-760
- McDowell, L.R and G. Valle, 2000. Major minerals in forages. In: Forage Evaluation in Ruminant Nutrition (Givens, D.I., Owen, E., Oxford, R.F.E. and Omed, H.M., eds). pp: 373. CAB International, Wallingford, UK.

- McDowell, L.R. 1985. In: Nutrition of Grazing Ruminants in Warm Climates. Academic Press New York, pp-443.
- McDowell, L.R. 2003. Minerals in animals and human nutrition. 2<sup>nd</sup> ed. 144p. Elsevier Science BV. Amsterdam, The Netherlands.
- McDowell, L.R., 1999. Minerals para Ruminants sob Pastejoem Regioes Tropicais Enfatizando Brasil, Univ. Florida, Gainesville, FL.
- McDowell, L.R., J.H. Conrad, G.L. Ellis and L.K. Loosli. 1983. Minerals for Grazing Ruminants in Tropical Regions. Extension Bulletin Anim. Sci. Dept., Univ. of Florida.
- Miller, W.J and P.E. Stake. 1974. Uses and limitations of biochemical measurements in diagnosing mineral deficiencies. Proc. Georgia Nutr. Conf. Feed Ind., P.25. Univ. of Georgia, Athens.
- Prabowo, A., L.R. McDowell, N.S. Wilkinson, C. J. Wilcox and J. H. Cornad. 1990. Mineral status of grazing cattle in South Sulawesi, Indonesia; I. Macrominerals. *Am. J. Anim. Sci.*, 4:111-120.
- Ram, L., J.T. Schonewille, H. Martens, A.T. Van't Klooster, and A.C. Beynen 1998. Magnesium absorption by wethers fed potassium bicarbonate in combination with different dietary magnesium concentrations. *J. Dairy Sci.* 81: 2485-2492.
- Rhue, R.D. and G. Kidder. 1983. Analytical procedures used by the IFAS extension soil laboratory and the interpretation of results. Soil Sci. Dept., Univ. Florida, Gainesville.
- Rojas, L.X., L.R. McDowell, N.S. Wilkinson, and F.G. Martin. 1993. Mineral status of soils, forages, and beef cattle in South East Venezuela. I. Macro-minerals and forage organic constituents. *Int. J. Anim. Sci.*, 8:183-188.
- Schonewille, Schonewille, J.T., A.T. Van't Klooster, H. Wouterse, and A.C. Beyen. 1999. Effects of intrinsic potassium in artificially dried grass and supplemental potassium bicarbonate on apparent magnesium absorption in dry cows. *J. Dairy Sci.*, 82:1824-1830.
- Sjollem, B. 1930. On the nature and therapy of grass staggers. *Vet. Res.*, 10:425-430.
- Steel, R.G.D. and J.H. Torrie. 1980. *Principles and procedures of statistics*. A Biometrical approach (2<sup>nd</sup> Ed.). McGraw Hill Book Co. New York.
- Tiffany, M.E., L.R. McDowell, G.A. O'Connor, H. Nguyen, F.G. Martin, N.S. Wilkinson, and E. C. Cardoso. 2000. Effects of pasture-applied biosolids on forage and soil concentrations over a grazing season in North Florida. I. Macrominerals, crude protein, and in vitro digestibility. *Commun. Soil Sci. Plant Anal.*, 31:201-213.
- Underwood, E.J., Suttle, N.F., 1999. *The Mineral Nutrition of Livestock*. (3<sup>rd</sup> ed.). Midlothian, UK, pp. 283-392.

(Received for Publication 10 March 2007)