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SEASONAL VARIATION OF CALCIUM IN SOIL – PLANT – ANIMAL SYSTEM AT SHEEP RANCH, PUNJAB, PAKISTAN

Z. I. Khan, A. Hussain, E. E. Valeem[2]*, M. Ashraf[1] and L.R. McDowell[3]

[1]*Department of Botany, University of Agriculture, Faisalabad, Pakistan.*
[2]*Department of Botany, Govt. Degree Sci. & Com. College Gulshan-e-Iqbal, Karachi, Pakistan.*
[3]*Animal Science Department University of Florida P.O. Box 110910 Gainesville, F.L. 32611-0691*

ABSTARCT

A study was conducted to determine the calcium nutrition status of different classes of grazing sheep during two different seasons on a farm located in southern Punjab (Pakistan). A complete free-choice supplement was available to all animals throughout the year. Soil, forage, feed, water, and animal samples like plasma, milk, faeces, and urine from lactating, plasma, faeces, and urine from non-lactating, and that of plasma and faeces from male animals were taken eight times during the year (four times in each season). The calcium concentrations of forage, feed and plasma in all classes of sheep, and milk in lactating sheep were affected by the seasonal fluctuations. While the marginal deficient levels of calcium in lactating sheep in summer and moderately deficient plasma calcium level in winter was found. While in non-lactating sheep plasma calcium was in moderate deficient level in winter and marginally deficient level in summer. In male sheep, plasma calcium level in summer was marginally deficient while plasma calcium during winter was moderately deficient. Plasma calcium in all animals was not affected by the physiological state and gender, as it was overwhelmingly deficient in all classes of sheep.

Soil and forage calcium concentrations were above the critical level during both seasons and showed a positive association between them. The feed and forage calcium collectively was sufficiently above the required range for ruminants, but was found ineffective in elevating the plasma calcium levels in all classes of animals showing the low bioavailability in spite of high concentration in the diet. From these analyses, it would suggest that the calcium status of grazing sheep in this specific region needs supplementation with specifically tailored mixture containing calcium with high bioavailability rather than high calcium contents showing antagonism.

Keywords: *Soil, Forage, Minerals, Sheep Ranch, Seasonal Variation*

INTRODUCTION

Poor animal performance and reproductive problems in grazing livestock are associated with mineral deficiencies (Underwood, 1977; Velasquez Pereira *et al*., 1997). The health and degree of productivity of livestock are dependent on balanced and adequate quantities of all the necessary nutrients to meet their requirements for a given physiological condition. For grazing ruminant livestock, which obtains all or most of the nutrients required from forages, knowledge of the nutrient composition of such forage is, therefore, essential. The mineral status of grazing goats and sheep in Pakistan had shown deficiencies in Ca, Mg, Na, K, and in some instances Cu (Khan, 2003). Field observations indicated sub-fertility and retained placenta in animals and slow growth in sheep and goats. Poor growth rate of lambs, low fertility of, in particular imported breeds, high mortality, and wool production with inferior quality are typical for sheep production in certain parts of the world (Proyecto Ovine Colombo Britanico, 1979).

Mineral imbalances have been reported to inhibit ruminant production systems (McDowell, 1985). It has been shown that none of the micro- minerals may have adequate concentrations in grazing animals (Khan, 2003). Calcium is the most abundant mineral element in animal body (1 to 2%), with 99% of it occurring in bone and teeth and the remainder, constituting the physiologically active pool of free Ca, is found in extra-cellular parts and within cells. Variable amounts of Ca are present in almost all feedstuffs. Calcium is generally deficient in grains and abundant in forage. Its content in natural feeds varies widely, depending on the species of plant and plant parts. The non-legume roughages such as grass hay and mature range forages are intermediate in Ca content (0.31 to 0.36%), and legume forage such as alfalfa and clover contain 1.2 to 1.7% Ca (NRC, 1980; McDowell, 2003).

Serum/plasma Ca concentration varies little in spite of changes in dietary Ca because of endocrine regulation. The homeostatic or physiological mechanisms regulating serum/plasma Ca are more effective than those for other minerals. In most species Ca is maintained closely about 10 mg/100 ml by regulatory action of PTH calcitonin, and the active metabolite of vitamin-D (1, 25, $(OH)_2$-D) (McDowell, 2003). The blood cells are almost or entirely devoid of Ca, but the plasma, in healthy animal, contains from 9 to 12 mg/100 ml in most species. In all species, the faeces are a primary path for Ca excretion. Faecal Ca is a combination of unabsorbed dietary and unabsorbed endogenous factors that affect Ca absorption. It also affects the amount found in the faeces. Urinary loss is minimal owing to efficient re-absorption by the kidneys. The most animals may, however, excrete considerable amounts of Ca in urine when high level of Ca are fed. Some Ca is lost during profuse sweating (Cheeke, 1987; McDowell, 2003; Hafez & Dyer, 1969).

*Corresponding author: *Ehsan Elahi Valeem, Govt. Degree Sci. & Com. College Gulshan-e-Iqbal, Karach-75300, Pakistan. (valeem@go.com).*

Like other minerals, calcium concentration in dietary component in comparison with requirements of various species, is useful in detecting the status of this nutrient. Inadequate intake of Ca will cause weakened bones, slow growth, low milk production and tetany (convulsions) in severe deficiencies (McDowell, 2003). A number of response criteria have been used to evaluate calcium status of grazing livestock including growth rate, feed intake, and feed efficiency; serum/plasma Ca levels; and numerous dimensional, compositional, and mechanical criteria for several bones.

Analysis of soils, forages, feed, water, and animal fluids plasma/serum Ca composition is important for obtaining mineral status of an area with a view to providing supplements to grazing animals. Assessment of mineral status of grazing animals has been considered an important strategy to increase animal productivity, especially, in those areas where mineral deficiencies or imbalances are commonly found. McDowell (1987) indicated that tissue mineral concentrations or their functional forms must be maintained within narrow limits if growth, health, and productivity of animals are to be maintained. The adequate information on soil characteristics and mineral composition in Pakistan is lacking despite the importance of this state to livestock production.

The objective of this study was to evaluate the Ca status of grazing sheep in central Punjab in order to formulate mineral supplements to have rapid and economic improvement in livestock production. These results would have applications to the remaining areas of Pakistan as well as other countries with regions of similar soils and climates.

MATERIALS AND METHODS

Soil, forage, feed, water, and animal samples were taken from the farm "Livestock Experimental Station" located in southern Punjab, owned by the Government of Punjab, Pakistan. These collections were made eight times fortnightly during the year (four times each during the summer and winter seasons). Composite soil and forage samples were collected at three sites from the pasture. The five sub-samples of soil and forages were taken from the beginning, middle, and end of the pasture.

Each composite soil sample was derived from five sub-samples taken at a depth of 20 cm as described by Sanchez (1976). Similarly, each of the composite forage sample came from five sub-samples of the same predominating forage species that was most frequently grazed by sheep on the farm. Forages were collected after careful observation of sheep grazing pattern. The forage samples were clipped to a height of 3-6 cm, from the ground to simulate the grazing behaviour of animals. Individual forage samples were collected at the same spots from where soil samples were collected. Representative samples of the forages then were placed in polyethylene bags at the laboratory where they were given a rapid wash with tap water followed by a glass-distilled water to remove any soil, which was present. Soil and forage samples were placed in clean clothe bags for air-drying.

For sampling purpose animals were divided into 3 classes, lactating/non-lactating and male animals respectively, with 10 animals per class. Blood plasma, milk, faeces and urine samples from lactating, plasma, faeces, and urine from non-lactating and plasma, and faeces from male sheep were taken at the farm concurrently with the soil and forage samplings.

Blood samples were anaerobically collected by jugular vein puncture with a syringe and needle, then drawn by vacuum into evacuated tubes containing lithium heparine as an anticoagulant, and plasma was separated by centrifugation and harvested into polyethylene tubes and frozen at -20^{o}C for subsequent analysis for calcium. Faecal samples were collected from the rectum of the animals manually and urine samples collected via manual stimulation of the vulva of female animals and a 10 ml aliquot was transferred to polyethylene tubes, acidified with 0.3 ml concentrated HCl, and frozen for subsequent analysis (Tucker *et al*., 1990). The faecal samples were kept in open bags and allowed to dry in sun to constant atmospheric moisture (<30%). Milk samples were collected in 125ml bottles using the first drawn milk. All lactating animals were sampled shortly after administration of 1.0 ml oxytocin injection to stimulate milk let down. Milk samples were taken in plastic vials and stored frozen until analysis (Fick *et al*., 1979).

Feed samples consumed by the animals were collected in five replicates for assay of calcium at each sampling period in cloth bags and were air-dried. Water samples were taken in borosilicate vials from pans fortnightly during both sampling seasons along with other samples in five replicates. The samples of forages, feed, and faeces were dried in an oven at 60 ^{o}C for 48 h.

Air- and oven-dried soil samples were pulverized in a ceramic mortar to pass through a 2 mm sieve and were analyzed for Ca concentrations using a Mehlich-1 (Hesse, 1972; Rhue & Kidder, 1983) extraction procedure: 5 g of soil were added to 20 ml of 0.05 *M* HCl in 0.025 *M* H_2SO_4 and final volume was analyzed.

Water and urine samples were filtered into sterilized plastic beakers, and 1 ml aliquots were used to prepare serial dilutions for analysis. Air and oven dried samples of forage, feed and faeces were ground with a Wiley mill to pass through a 1-mm mesh. To prepare samples for estimation of calcium, dried and ground samples of about 2 g each of forages, feed, and faeces were digested by nitric acid and perchloric acid (3:1) at 250 ^{o}C until the solution changed to colourless and thick white fumes appeared in the flask. The contents of the flask were washed with pure water and diluted to constant volume. The supernatant obtained from centrifugation was used for analysis (Koh & Judson, 1986, Anonymous, 1990; Neathary *et al*., 1990). Direct dry or wet ashing of plasma and milk was not possible because of high fat, protein and moisture as spattering and swelling might result in loss of sample. Therefore, appropriate quantities of each plasma and milk sample were taken into crucible after thawing. To pre-digest, the samples were pre-treated with 50% HNO_3 over an electric heater until smoking ceased to char the majority of organic matter. These samples then were ashed for 6 hours at 550 ^{o}C in a muffle furnace.

The residues were dissolved in 1 % HCL and transferred into a volumetric flask to make up a constant volume of 50 ml. Samples were poured into labelled plastic tubes suitable to fit the auto-sampler of Atomic absorption spectrophotometer. The samples were diluted to determine individual elements (Mpofu *et al*, 1999; Nockels *et al*., 1993; Anonymous, 1990; Fick *et al*., 1979).

All the samples were filtered through Whatman filter paper No. 42 and brought to appropriate volume with double distilled water and stored in polyethylene tubes. Samples were analysed for concentration of Ca by atomic absorption spectrophotometry (Perkin-Elmer Model 5000).

The data were analyzed using a split-plot design (Steel and Torrie, 1980). Differences among means were ranked using Duncan's New Multiple Range Test (Duncan, 1955).

Soil, forage, and plasma calcium concentrations were compared with established critical values to determine the various categories of deficient levels. The critical level for soils indicates the calcium 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.

RESULTS AND DISCUSSION

Pasture Samples

Soil:

From the analysis of variance of data for soil Ca^{2+} it is evident that seasons had no significant effect whereas the effects of sampling intervals was significant on soil Ca^{2+} level (Table 1). During winter, there was a gradual decrease in Ca^{2+} concentration from 1st to 3rd fortnights, while soil Ca^{2+} level at the fortnight 4 was almost same as that at fortnight 3. In contrast, during summer, there was a consistent decrease in soil Ca^{2+} level from 1st to 4th fortnights (Fig. 1a). Overall, soil Ca^{2+} concentration during winter was higher as compared to that during summer.

Extractable soil Ca^{2+} concentrations were sufficiently higher during both seasons and found equally important during both seasons for the normal growth of plants. However, these concentrations were substantially higher than those observed by Cuesta *et al*. (1993) in North Florida and Rojas *et al*. (1993) in Venezuela. Similar levels of soil Ca^{2+} had already been reported in North Florida (Tiffany *et al*., 2000, 2001). The soil content of an element is the one of the most important limitations for plant growth. However, availability factors, including soil pH, texture, moisture content and organic matter are probably more often the limiting factors rather than mineral content. With increasing acidity of soils there is impaired absorption of Ca^{2+} (Reid and Horvath, 1980; McDowell and Valle, 2000). In this study the high soil Ca^{2+} levels perhaps may be related to high pH of soil.

Forage plants:

Analysis of variance of data for forage Ca^{2+} showed that both seasons and fortnights had significant effects on its concentration (Table 1). Data for Ca^{2+} concentration of different forage species were pooled within each season and were analysed and presented in Fig. 1b. Ca^{2+} concentration in winter forages was markedly higher as compared to that in summer. During winter, there was a gradual decrease in forage Ca^{2+} concentration with time, whereas in contrast during summer the Ca^{2+} concentration of forage species at 1st and 2nd and at 3rd and 4th fortnights was almost same.

Mean forage Ca^{2+} concentrations were adequate and sufficiently higher than the requirements of ruminants. Very high concentration was found in winter as compared to that in summer. Forage Ca^{2+} requirements of grazing

ruminants is a subject of considerable debate as the requirement is influenced by animal type and level of production, age and weight. Reuter and Robinson (1997) suggested Ca^{2+} requirement for maintenance, growing and lactating sheep to be 1200-2600-mg/kg. Thus the forage Ca^{2+} values found in this study was considered adequate for the optimum performance of ruminants. Similar forage Ca^{2+} values as found in summer were reported by Pastrana *et al*., (1991) in Colombia, Tiffany *et al*., (2000, 2001) in North Florida, Espinoza *et al*., (1991) in Central Florida and Cuesta *et al*., (1993) in North Florida. It is generally recommended that diets of livestock should have Ca:P ratio of about 1:1 to 2:1 (Underwood, 1981). Livestock will tolerate dietary Ca:P ratios of more than 10:1 without any serious effect provided the P intakes are adequate (Ternouth, 1990). Temperate forages generally contain more Ca^{2+} than those grown in the tropics. However, hay from Ireland had a mean Ca^{2+} concentration almost similar to that found in this study during winter from the forage which was also similar to that reported by the Pennsylvania State Forage Test Service (Adams, 1975). The forage mean level of Ca^{2+} was found higher than that the requirements of grazing ruminants.

Water:

Water Ca^{2+} concentration was not affected by the seasons and fortnights (Table1). Non-consistent fluctuations in water Ca^{2+} at different fortnights during both seasons were observed (Fig. 1c). However, water Ca^{2+} was higher in winter than that in summer at all fortnights except fortnight 3.

Feed:

Ca^{2+} concentration in feed was significantly affected by different seasons and sampling intervals (Table1). Generally, high concentration was observed during winter as compared to that during summer. During summer and winter, a gradual decrease in Ca^{2+} concentration was found with time (Fig. 1d).

Feed Ca^{2+} contents were slightly higher than the requirements of ruminants but within the tolerable range during both seasons. Water Ca^{2+} contents were same during both seasons to complement the feed and forage Ca^{2+} concentrations for the requirements of animals. The availability of different minerals from feed or supplement differs widely. An unbalanced mixture that is high in Ca^{2+} will not be considered adequate source of Ca^{2+}, it may contribute to a P deficiency because of imbalance of these two elements.

The feed and forage Ca^{2+} contents during both seasons were sufficient for the requirements of ruminants. The sources Ca^{2+} level were found to be higher in winter than those in summer. Plasma Ca^{2+} levels in animals did not seem to have been affected by the physiological status and gender, and in all classes it was higher in summer than that in winter. It was slightly high in male sheep during winter and in non-lactating sheep during summer. Plasma Ca^{2+} concentrations in winter and summer were below the normal limits in all groups of animals and these were considered deficient levels. Deficiency in plasma Ca^{2+} during both seasons in lactating and in non-lactating and male sheep was found. Lactation period affected the milk Ca^{2+} contents, being lower in early lactation than in late lactation which showed less absorption through gastrointestinal tract in winter like the plasma Ca^{2+} during this season.

Plasma Samples

Lactating Sheep:

Prominent effects of seasons and fortnights were observed on plasma Ca^{2+} as is evident from analysis of variance (Table-2a). Plasma Ca^{2+} concentration of lactating sheep was higher in summer as compared to that during winter. There was a gradual decrease in Ca^{2+} level in summer. In contrast during winter, a low concentration of plasma Ca^{2+} was found at the 1^{st} fortnight, afterwards there was a sudden increase at the 2^{nd} fortnight which remained uniform statistically up to the 3^{rd} fortnight but at the last fortnight Ca^{2+} concentration again decreased markedly (Fig. 2a).

Non-Lactating Sheep:

Ca^{2+} concentration of plasma during summer was higher than that during winter. There was no marked effect of different sampling periods on plasma $Ca^{2+,}$ but in contrast, a significant effect of seasons was observed. (Table 2b). Ca^{2+} level was very low in winter and there were almost no statistical differences at all fortnights. During the summer plasma Ca^{2+} level remained unchanged with time of sampling. (Fig. 2e).

Male Sheep:

Fortnights did not show any significant effect on Ca^{2+} of plasma unlike the seasons as is evident from the analysis of variance (Table 2b). Maximal plasma Ca^{2+} concentration was observed in summer and very low amount

during winter. There was a slight decrease in summer in Ca^{2+} concentration with time and in winter a slight increase was recorded with periods of sampling in contrast to summer season (Fig. 2h).

It has been reported that in sheep and cattle a mechanism exists for controlling the blood Ca^{2+} concentrations within narrow limits by adjustment of dietary Ca^{2+}, absorbed and when dietary Ca^{2+} is inadequate, by reabsorbing Ca^{2+} from body reserves (Rowlands, 1980). It is reported that plasma Ca^{2+} is directly affected by dietary intake provided there is small amount of P and Mg in the diet, and dietary Ca^{2+} and P, are good indicators in assessing the status of Ca^{2+} in animals (Black *et al*., 1973; NCMN, 1973; Pastrana *et al*., 1991). Low availability of Ca^{2+} to animals as found in this study during both seasons irrespective of the higher concentration in the diets may be attributed to its low availability through digestive canal and its absorption rate. Vitamin D is involved in the absorption of Ca^{2+} only when calcium salts in the intestine are insoluble. It has been suggested that calcium salts of phytic acid present in diet are completely hydrolysed only in the presence of vitamin D (NRC, 1984; McDowell *et al*., 1987).

A normal animal on good balanced diet will absorb about one-third of the Ca^{2+} level ingested. Since more than 99% of the Ca^{2+} in the animal body is located in the skeleton system (McDowell, 1997), this store of Ca^{2+} must serve as a source of Ca2+ to maintain plasma Ca2+ at a constant level. It exists as protein bound Ca2+ and a soluble ionized Ca2+, and only the last one is physiologically active. About half the plasma Ca2+ is bound to protein, and only a small portion is freely diffusible and non-ionized. The regulation of plasma Ca2+ is heavily dependent on parathyroid hormones and thyrocalcitonin or calcitropic hormones (McDowell, 1997) are responsive to plasma Ca2+ concentration. Therefore, the low plasma Ca2+ levels may possibly be considered due to certain malfunctions and different levels of these hormones in the animals involve in this study.

Faecal Samples

Lactating Sheep:

Analysis of variance of data for faecal Ca2+ concentration of samples collected during the winter and summer is presented in Table 2a. The data showed that there was no seasonal or fortnight effect on faecal Ca2+. Although winter a consistent decrease was found at different fortnights of sampling (Fig. 2b). However in the summer season, there was a gradual increase in faecal Ca2+ concentration, but there was no statistical difference between Ca2+ level at the 1st and 2nd fortnights and that of 3rd and 4th fortnights.

Non-Lactating Sheep:

Analysis of variance of data for faecal Ca2+ showed no seasonal and fortnight effect on Ca2+ level (Table-2b). The Ca2+ concentration in winter was almost same at all fortnights with tendency of a gradual decrease with time. In contrast, during summer, the Ca2+ remained almost unchanged up to the 2nd fortnight, then there was a gradual increase in Ca2+ concentration up to last fortnight (Fig. 2f).

Male Sheep:

Analysis of variance of data for faecal Ca2+ showed no seasonal and sampling interval effect in relation to Ca2+ level (Table 2b). The Ca2+ level at all the fortnights during both seasons remained almost uniform except at the 3rd fortnight where there was a very low Ca2+ concentration in summer as compared to that at the same fortnight in winter (Fig. 2i).

Urine Samples

Lactating Sheep:

It is clear from the analysis of variance of data that Ca^{2+} concentration excreted via urine did not vary during summer and winter seasons, but sampling intervals affected it significantly (Table 2a). In both seasons, there was a gradual increase in Ca^{2+} concentration in urine, with time, except at 4^{th} fortnight of summer, where the Ca^{2+} concentration was almost same as that at the 3^{rd} fortnight (Fig. 2c).

Non-Lactating Sheep:

The seasonal effect was non-significant on urine $Ca^{2+,}$ whereas, sampling interval affected it significantly (Table-2b). A gradual increase in urine Ca^{2+} was observed in summer with time, while in winter, a sharp decrease in Ca^{2+} concentration was found with time of sampling (Fig. 2g).

Table 1. Analysis of variance of data for Ca^{2+} concentration in soil, forage plants, water, and feed at different fortnights during winter and summer seasons at sheep ranch.

S. O. V.	df	Mean squares		df	Mean squares	
		Soil	Forage plants		Water	Feed
S	1	5227.20^{ns}	541450083.33***	1	90.00^{ns}	47196.90*
Error	28	26904.47	34331511.90	8	77.33	8766.28
FN	3	4790.23***	121159638.89***	3	22.97ns	14050.50***
S x FN	3	565.05***	23084972.22***	3	95.67^{ns}	233.70^{ns}
Error	84	62.26	4076115.08	24	103.78	539.31

***, * * *** = Significant at 0.05 and 0.001 levels, respectively, **df** = Degree of freedom,
FN = Fortnight, **ns** = non-significant, **S** = Season, **S. O. V.** = Source of variation

Table 2a. Analysis of variance of data for Ca^{2+} concentration in blood plasma, faeces, urine, and milk of, lactating sheep at different fortnights during winter and summer seasons.

S. O. V.	df	Mean squares			
		Plasma	Faeces	Urine	Milk
S	1	5281.90***	107164.8^{ns}	4697.1^{ns}	2439511.3***
Error	18	262.85	14766530.5	3064.4	20427.9
FN	3	159.98***	23420.5^{ns}	1023.2**	29191.3***
S x FN	3	71.82	1135781.6***	142.7^{ns}	377.9^{ns}
Error	54	18.04	43767.9	180.9	856.8

***, * * *** = Significant at 0.05 and 0.001 levels, respectively, **df** = Degree of freedom,
FN = Fortnight, **ns** = non-significant, **S** = Season, **S. O. V.** = Source of variation

Table 2b. Analysis of variance of data for Ca^{2+} concentration in blood plasma, faeces and urine of non-lactating, sheep and that of plasma and faeces of male sheep at different fortnights during winter and summer seasons.

S. O. V.	df	Mean squares				
		Non-lactating sheep			Male sheep	
		Plasma	Faeces	Urine	Plasma	Faeces
S	1	15840.00***	713475.3^{ns}	551.3^{ns}	13005.00***	134234.1^{ns}
Error	18	138.30	11083237.2	2043.9	105.9	3871875.5
FN	3	6.62^{ns}	110167.08^{ns}	114.9***	0.283^{ns}	1002973.07^{ns}
S x FN	3	7.63^{ns}	778336.3***	1179.8***	53.20***	142215.2^{ns}
Error	54	12.06	54669.7	12.2	3.9	501893.3

***, * * *** = Significant at 0.05 and 0.001 levels, respectively, **df** = Degree of freedom,
FN = Fortnight, **ns** = non-significant, **S** = Season, **S. O. V.** = Source of variation

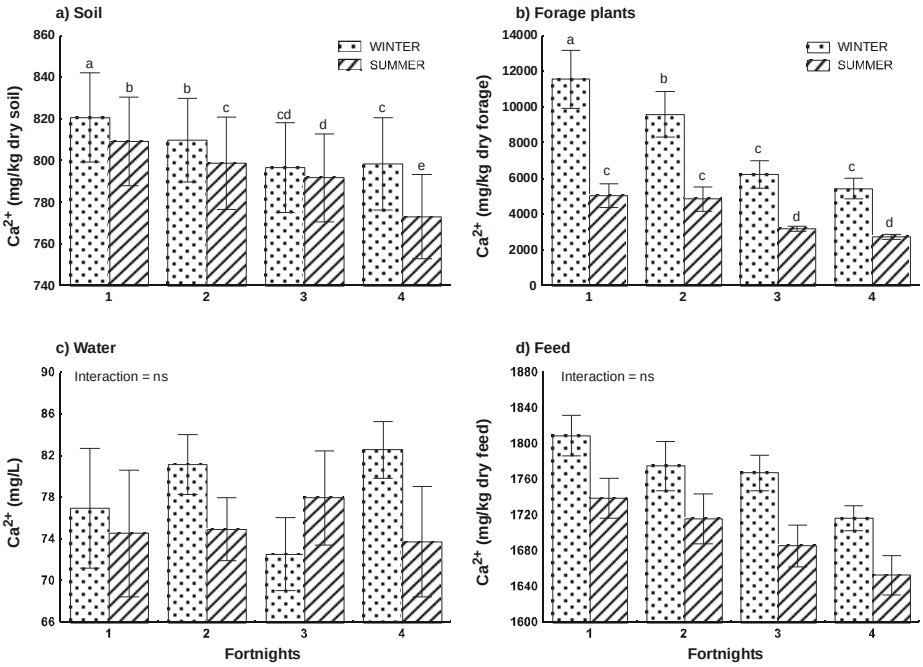

Fig.1: Ca^{2+} concentration in (a) soil, (b) forage plants, (c) water, and (d) feed at different fortnights during winter and summer seasons (sheep farm).

(Means with the same letters do not differ significantly at P<0.05)

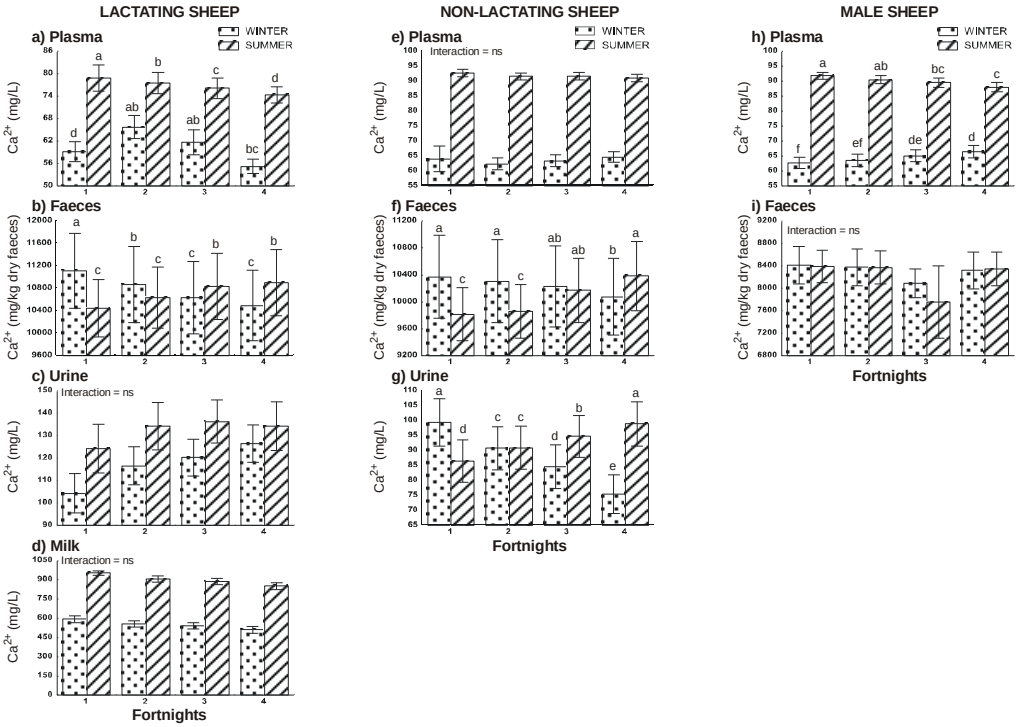

Fig.2: Ca^{2+} concentration in different sample types of lactating, non-lactating, and male sheep at different fortnights during winter and summer seasons.

(Means with the same letters do not differ significantly at P≤0.05)

Milk Samples of Lactating Sheep

Milk Ca^{2+} concentration was affected significantly by both the seasons and sampling intervals. (Table-2a). The milk Ca^{2+} concentration in summer was higher than that during winter and there was no statistical difference in Ca^{2+} level at different sampling intervals during both seasons (Fig. 2d).

Faecal Ca^{2+} contents during winter were higher in all classes of sheep than those in summer may be the possible explanation of low plasma Ca^{2+} in all these classes of sheep in this season. Urine Ca^{2+} excretion was higher in summer than that in winter in lactating and non-lactating sheep because of less absorption during winter through digestive tract of animals. In this study it was found that absorption of Ca^{2+} was lower in winter than that in summer, which was reflected by the low plasma Ca^{2+} levels in all classes of animals. While during summer although the dietary intake was low in Ca^{2+} as compared to that in diet during winter, but the absorption was maximum which was shown by the concentration of Ca^{2+} in milk and urine in this season. During winter faecal Ca^{2+} excretion was high reflecting the less availability or absorption during this particular period. Plasma Ca^{2+} in winter was not affected by physiological status of the animals and a slight depression in lactating sheep as compared to other groups was found. It was also found that the type of pasture did not affect plasma Ca^{2+} levels, since the Ca^{2+} contents in the pasture were higher in winter than that in summer. Thus a high pasture Ca^{2+} level was not believed to be responsible for a small rise in Ca^{2+} in plasma during summer. A positive association between Ca^{2+} content in the pasture and those in faeces was found in this study. Therefore, faecal Ca^{2+} values can be considered as a reflection of pasture type. Low plasma Ca^{2+} level as found in this study in all classes of sheep during both seasons, has earlier been reported by Pastrana *et al*. (1991) while assessing the macro, mineral status of sheep in the Paramo region of Colombia. High incidence of plasma Ca^{2+} below critical level as reported here indicates that sheep had a very severe Ca^{2+} deficiency, particularly, during winter.

Based on this study, it can be concluded that soil and forage Ca^{2+} was adequate for plants and animal requirements during both seasons. Blood plasma analyses showed moderate Ca^{2+} deficient level in all classes of sheep during winter and marginal deficient levels during summer indicating the very low availability of Ca^{2+} from the diet. Thus the status of sheep in relation to Ca^{2+} requirements needs further studies to determine requirement and economical benefit of mineral supplementation containing Ca^{2+} element and to clarify the cause of unavailability of Ca^{2+} in spite of high amounts of this element in the source used.

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