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# Variation in absorption and excretion of calcium in grazing sheep in a semi-arid grazing ranch, Punjab, Pakistan

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## Summary

The calcium status of lactating, non-lactating, and male sheep on farm in southwestern Punjab, Pakistan was studied. Pasture and animal were sampled at the end of the first month of summer and winter. All samplings were carried out fortnightly. A mineral supplement was available to all animals throughout the year. Plasma calcium in all animals was not affected by the physiologic state and gender as it was severely deficient in all classes of animals. The calcium concentrations of forage and plasma in all classes of sheep, and milk in lactating sheep were affected by the season ($P<0.001$). In the lactating sheep, the marginal deficient levels of calcium for requirement (1200-2600 mg/kg) in summer and moderate deficiency (1200-2600 mg/kg) for sheep requirement during winter were found. In non-lactating sheep, plasma calcium was moderately deficient during winter and marginally deficient in summer. In male sheep, plasma calcium level in summer was marginally deficient while plasma calcium during winter was moderately deficient. Soil and forage calcium concentrations were found within the same range during both seasons and showed a positive association between them ($P<0.001$). Based on these analyses, it was concluded that the calcium status of grazing sheep in this specific region needs supplementation with specifically tailored mixture to achieve the maximum potential of grazing sheep in this ranch.

**Key words:** Calcium, Seasonal variation, Soil, Forage plant, Grazing sheep

## Introduction

The health and productivity of livestock depend on balanced and adequate quantities of all the necessary nutrients to meet their requirements for a given physiologic conditions. For grazing ruminant livestock which obtain all or most of their required nutrients from forages, knowledge of the nutrient composition of such forage is, therefore, essential. The mineral status of grazing goats and sheep in Pakistan has 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 (in particular in imported breeds), high mortality, and wool production with inferior quality are typical for sheep production in certain parts of the world (Proyecto Ovino Colombo Britanico, 1979).

Mineral imbalances have been reported to inhibit ruminant production systems (McDowell, 1985). It has been shown that none of the macro-minerals may have adequate concentrations in grazing animals (Khan, 2003). Forage and soil mineral imbalances are common and forages are frequently low in essential minerals. Many naturally occurring deficiencies in grazing livestock can be attributed to soil characteristics (McDowell, 1992; McDowell and Valle, 2000). The pastures are commonly considered to be adequate in

nutritive value (i.e., energy and protein) for sustaining mature animals. The quality of grasses is however often inadequate for growing animals whose physiologic demands are high (McDowell, 1985; Tejada *et al*., 1987; Pastrana *et al*., 1991; McDowell *et al*., 1993; Tudsri and Kaewkunya *et al*., 2002; Manyayu *et al*., 2003; Evitayani *et al*., 2004).

No, doubt, forage analysis is a much better indicator of mineral status for ruminants than is soil analysis. Likewise, animal tissue or fluid mineral concentrations are better indicators of the availability of most minerals than are forage mineral analyses. Grazing livestock obtain part of their mineral needs by consumption of water, soil, leaves, tree bark, etc, rather than entirely from forages. Livestock tissue or fluid-mineral concentrations are therefore more accurately portray the contribution of the total environment in meeting the mineral requirements of the grazing animals (McDowell, 1985; McDowell *et al*., 1993, McDowell and Valle, 2000). Research in the area of calcium status of soil, food-stuffs and grazing ruminants is limited in spite of its importance in animal nutrition. Therefore, there is a need to further investigate the status of calcium in soil, forages and ruminants. Mineral supplementation is the least cost input for improvement of livestock production. Nevertheless, mineral supplements should be used only when the mineral requirements of animal cannot be met within the available feed, and only as local conditions dictate. The provision of additional minerals beyond these needs is economically wasteful and unnecessary, and confers no benefits on the animal (Underwood, 1981). It is important to determine mineral concentrations of soil, forage, other feedstuffs, and animals to estimate the mineral needs of grazing ruminants, as well as the season of the year when they are most required. The district Leiah of the province of Punjab, Pakistan is well-known for livestock production. The Livestock Experimental Station Rakh Khaire Wala in this district is a specific place where sheep and goats herds are being reared. This experimental station is sparsely treed covered with native grasses and some varieties of sown forages, the great majority of soils are sandy which have low natural fertility. The climate is characterized by two weather pattern; the first extending from December to April, is characterized by little rain, or the dry season, and the second has medium rainfall from June to August. Under these conditions, forages or grasses produce a high dry matter yield and moderate nutritional content. In this region, the pasture is only feed for grazing animals during the year. The mineral supplements mostly are not provided, especially for sheep and goats, consequently, mineral deficiencies occur in animals. Potassium, copper, Na, Mg, and cobalt deficiencies occur in this region (Khan, 2003). In this specific region of Punjab state, low to deficient selenium concentrations in soil, forages, and blood plasma have been reported (Khan, 2003). In most species Ca is maintained close to 10 mg/dl in blood 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 entirely devoid of Ca, but the plasma, in health, contains from 9 to 12 mg/dl 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 levels of Ca are fed. Some Ca is lost during profuse sweating (Cheeke, 1987; McDowell, 2003).

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 in severe deficiencies, tetany (McDowell, 2003). Analysis of soils, forages, feed, water, and animal fluids plasma/serum Ca composition is important for obtaining mineral status of an area with 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 southwestern Punjab in order to formulate mineral supplements to have rapid and economic improvement in livestock production.

## Materials and Methods

The study was conducted using a flock of Thalli breed of lactating, non-lactating and male sheep grazing similar pastures on a farm "Livestock Experimental Station Rakh Khaire Wala" located in southern Punjab, Pakistan. Samples were collected during the summer (wet season) and winter (dry season) of 2005. Pastures were predominately grasses, legumes, tree leaves and crop wastes. In addition, all animals on the farm had access at all times to a free-choice complete mineral mixture. This sheep ranch comprises 400 hectares and receives annual precipitation of 250–750 mm mainly restricted to July and August. The soils are sandy and vertisols. The ranch has about 7,000 animals of which 2,000 are breeding sheep. This ranch is in a low-lying semi-arid region of south-western Punjab between latitudes of 23° and 36° N and longitudes 60° to 75° E. The average temperature during the experimental year was 38 ± 5°C during summer and 15 ± 7°C during winter with a relative humidity of 48 ± 5% during summer and 80 ± 8% during winter. The livestock farm was characterized by two pastures, denoted as feeding sites; one was intensively managed with fertilized soils, irrigated with canal water and with grazing reserves characterized by the availability of sown forage species including *Panicum*, *Andropogon*, *Penisetum*, *Setaria*, *Medicago sativa*, *Trifolium alexandrium*, *Hordeum vulgare*, *Cichorium intybus*, *Cynodon genera* vernal grass, imported velvet grass, *Tall fescue*, *Orchard grass*, *Molasses grass*, *Elephant grass*, *Pangola grass*, and *Jaragua grass*. The other pasture with unfertilized soils was a barren and uncultivated area with natural weed like vegetation and low intensity cropping largely accessible to grazing animals. This pasture was over-grazed with extensive replacement of perennial grasses by annual grasses, and forbs and bush encroachment by *Accacia spp*., *Ziziphus mucronata*, *Trachipogon spp*., *Cyperus rotandus*, *Tribulus terrestris*, *Chenopodium morale*, *Lathyrus odoratus*, *Alhagi spp*., *Salavadora spp. Calotropis spp.* and some wild spp. of plants. Each pasture maintained three animals/ha/year under a rotational grazing system.

### Animals

Ten clinically healthy lactating, non-lactating and male Thalli sheep were used for this study. The mean body weights were 40–45 kg. These animals were with variable degrees of cross breeding. The lactating sheep were in their second lactation. All experimental animals were the same throughout the study period. All animals were grazing similar pastures with the same botanical composition and stocking rate throughout the investigation.

### Housing and management

The lactating, non-lactating and male sheep were housed in a well-ventilated building which was divided by solid wood walls into pens, provided with straw bedding, 30 cm thickness and supplied with fresh clean water containing buckets, feed troughs and salt blocks. The animals were provided a good opportunity to consume their mineral mixture. All hygienic measures were good in the farm.

### Feeding and nutrition of animals

This work was carried out under two different feeding systems which included stable-diet (indoors) and grazing (outdoors) conditions according to the season of the year. Diets for animals on stable diets were formulated to cover their nutrient requirements according to the NRC (1984). However, all the animal classes were

gradually transferred to the grass pasture for grazing. The time of grazing was 2.5–5.0 hrs. The animals were fed on the grass pasture *ad libitum* and supplemented with free-choice mineral mixture having the various proportions of different minerals (Tables 1A and B).

**Table 1A: Ingredients of the feed supplement**

| Sr. No. | Ingredient | Percent |
|---|---|---|
| 1 | Salt | 47 |
| 2 | Dicalcium phosphate | 47 |
| 3 | Copper sulphate | 0.1170 |
| 4 | Iron sulphate | 0.3000 |
| 5 | Zinc oxide | 0.0744 |
| 6 | Manganese sulphate | 0.1872 |
| 7 | Cobalt sulphate | 0.0120 |
| 8 | Potassium iodide | 0.0042 |
| 9 | Wheat bran | 2.61 |
| 10 | Corn bran | 2.61 |

**Table 1B: Chemical analysis of salt in the feed supplement**

| Minerals | % |
|---|---|
| P | 6.3% |
| Ca | 15.7% |
| Mg | 2.0% |
| K | 0.3% |
| Na | 11% |
| Mn | 2400 ppm |
| Fe | 6000 ppm |
| Zn | 2630 ppm |
| Cu | 448 ppm |
| Co | 16.4 ppm |
| Mo | 10.8 ppm |
| Se | 0.8 ppm |

Each composite soil sample which was derived from five sub-samples taken at a depth of 20 cm as described by Sanchez (1976). As with soil samples, 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 were then 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 cloth bags for air-drying.

For sampling purpose animals were divided into three 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. All samplings were carried out fortnightly.

Blood samples were anaerobically collected by jugular vein puncture with a syringe and needle. The sample was then drawn by vacuum into evacuated tubes containing lithium heparin. The plasma was separated by centrifuging, harvested into polyethylene tubes and frozen at -20°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. A 10 ml aliquot of urine was transferred to polyethylene tubes, acidified with 0.3 ml concentrated HCl, and frozen for subsequent analysis (Tucker *et al*., 1991). The faecal samples were kept in open bags and allowed to dry in sun to constant atmospheric moisture (<30%) (Holdo *et al*., 2002). Milk samples were collected in 125 ml Nalgene 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).

Mineral mixture samples consumed by the animals were collected in five replicates for estimation of calcium in cloth bags and were air-dried. The samples of forages, feed, and faeces were dried in an oven at 60°C for 48 hrs.

Air- and oven-dried soil samples were pulverized in a ceramic mortar to pass through a 2-mm sieve and were analysed for Ca concentrations using a Mehlich-1 (Hesse, 1972; Rhue and 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 the

final volume was analysed.

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°C until the solution changed to colorless 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 and Judson, 1986; AOAC, 1990; Neathery *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 quantity of each plasma or milk sample was taken into crucible after thawing. To pre-digest, the samples were pretreated with 50% $HNO_3$ over an electric heater until smoking ceased to char the majority of organic matter. These samples were then ashed for 6 hrs at 550°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 labeled plastic tubes suitable to fit the auto sampler of atomic absorption spectrophotometer. The samples were diluted to determine Ca (Fick *et al*., 1979; AOAC, 1990; Nockels *et al*., 1993; Mpofu *et al*., 1999). 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 analyzed for concentration of Ca by atomic absorption spectrophotometry (Perkin-Elmer Model 5000).

## Statistical analysis

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 Ca concentrations were compared with established critical values to determine the various categories of deficient levels. The critical level for soils indicates the Ca 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 this nutrient.

# Results

Tables 2, 3A, 3B and 4 illustrate the analyses of variance and fluctuation of mean values of various pasture and animal samples during the experimental period. From the analysis of variance of data for different pasture samples it is evident that seasons had significant effect on forage ($P<0.001$) and feed ($P<0.05$) $Ca^{2+}$ concentrations and had non-significant effect on soil and water $Ca^{2+}$ levels ($P>0.05$) while $Ca^{2+}$ in all the samples but water was affected significantly ($P<0.001$) by sampling periods. Variations in $Ca^{2+}$ levels were found due to the interaction of season by fortnight for soil and forage $Ca^{2+}$ concentrations ($P<0.01$) during this investigation. During winter, there was a gradual decrease in $Ca^{2+}$ concentration from the 1st to 3rd fortnights, while soil $Ca^{2+}$ level at the 4th fortnight was almost the same as that at the 3rd fortnight. In contrast, during summer, there was a consistent decrease in soil $Ca^{2+}$ level from

**Table 2: Analysis of variance of data in soil (mg/kg), forage (mg/kg) at different fortnights during winter and summer seasons at sheep ranch**

| Source of variation | Degree of freedom | Mean squares | |
|---|---|---|---|
| | | Soil | Forage plants |
| Season (S) | 1 | $5227.20^{Ns}$ | $541450083.33^{***}$ |
| Error | 28 | 29984.46 | 44991547.98 |
| Fortnight (FN) | 3 | $6787.28^{***}$ | $161179635.80^{***}$ |
| S × FN | 3 | $785.05^{***}$ | $28084988.24^{***}$ |
| Error | 84 | 78.25 | 4876525.47 |

$^{***}$ = Significant at 0.001 level. NS = non-significant

**Table 3A: Analysis of variance of data for $Ca^{2+}$ concentration in blood plasma, urine, milk, and (mg/L) faeces (mg/kg) of lactating sheep at different fortnights during winter and summer seasons**

| Source of variation | Degree of freedom | Mean squares | | | |
|---|---|---|---|---|---|
| | | Plasma | Faeces | Urine | Milk |
| Season (S) | 1 | 6281.80*** | 127174.9Ns | 4899.9Ns | 2839566.3*** |
| Error | 18 | 3872.88 | 24769530.2 | 3264.3 | 22429.6 |
| Fortnight (FN) | 3 | 169.88*** | 25425.9Ns | 1329.2** | 27196.8*** |
| S × FN | 3 | 75.87 | 1435881.9*** | 185.7Ns | 479.9Ns |
| Error | 54 | 16.09 | 43967.8 | 175.6 | 889.2 |

**, *** = significant at 0.01 and 0.001 levels, respectively. NS = non-significant

**Table 3B: Analysis of variance of data for $Ca^{2+}$ concentration in blood plasma, urine (mg/L) and faeces (mg/kg) of non-lactating sheep and that of plasma and faeces of male sheep at different fortnights during winter and summer seasons**

| Source of variation | Degree of freedom | Mean squares | | | | |
|---|---|---|---|---|---|---|
| | | Non-lactating sheep | | | Male sheep | |
| | | Plasma | Faeces | Urine | Plasma | Faeces |
| Season (S) | 1 | 17850.00*** | 9613475.6Ns | 591.9Ns | 17008.00*** | 144284.8Ns |
| Error | 18 | 1599.38 | 14087237.8 | 457.7 | 165.7 | 3771975.5 |
| Fortnight (FN) | 3 | 9.67Ns | 174167.07Ns | 414.9*** | 0.275Ns | 1202873.05Ns |
| S × FN | 3 | 6.68Ns | 678446.5*** | 2179.5*** | 83.25*** | 162295.7Ns |
| Error | 54 | 18.07 | 64679.7 | 18.8 | 5.8 | 671895.5 |

*** = Significant at 0.001 level. Ns = non-significant

the 1st to 4th fortnights. Overall, soil $Ca^{2+}$ concentration during winter was higher as compared to that during summer.

Forage $Ca^{2+}$ concentration in winter were 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 the 1st, 2nd, 3rd and 4th fortnights was almost the same. $Ca^{2+}$ concentrations in plasma of all the three groups of sheep were affected by the seasonal fluctuations ($P<0.001$) and by fortnight only in lactating sheep ($P<0.001$) and variations due to the interaction of season and fortnight were found in lactating ($P<0.05$) and male sheep ($P<0.001$). Plasma $Ca^{2+}$ concentration of lactating sheep were 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 1st fortnight; afterwards, there was a sudden increase at the 2nd fortnight which remained uniform statistically up to the 3rd fortnight. However, at the last fortnight, $Ca^{2+}$ concentration again decreased markedly. $Ca^{2+}$ concentration of plasma of non-lactating sheep during summer was higher than that during winter. $Ca^{2+}$ level was very low in winter and there was almost no statistical difference at all fortnights. During summer, plasma $Ca^{2+}$ level remained unchanged with time of sampling. In male sheep, a maximum plasma $Ca^{2+}$ concentration was observed in summer and a very low amount during winter. There was a slight decrease in summer in $Ca^{2+}$ concentration with time; in winter a slight increase was recorded with periods of sampling in contrast to summer season. Faecal $Ca^{2+}$ levels were neither affected by season, nor fortnight ($P>0.05$) in all the three groups of animals and only variations were found in faecal $Ca^{2+}$ levels due to the interaction of season and fortnight in lactating and non-lactating sheep ($P<0.001$). Nevertheless, during winter, a consistent decrease was found at different fortnights of sampling. However, in summer, there was a gradual increase in faecal $Ca^{2+}$ concentration, though there was no statistical difference between $Ca^{2+}$ level at the 1st and 2nd fortnights and that of the 3rd and 4th fortnights in lactating sheep. In non-lactating sheep, the $Ca^{2+}$ concentration in winter was almost the same at all fortnights with tendency of a gradual decrease with time. In contrast, during summer, the $Ca^{2+}$

**Table 4: Calcium concentrations of soil, forage and different animal sample types as related to seasons**

| Variable | Season | Sampling periods (Mean ± SE) | | | | |
|---|---|---|---|---|---|---|
| | | I | II | III | IV | Seasonal means |
| Soil (mg/kg) | Winter | 821.07 ±21.26 | 810.20 ±20.19 | 797.07 ±21.51 | 750.93 ±22.17 | 806.82 ±10.45 |
| | Summer | 809.20 ±22.18 | 799.20 ±22.18 | 792.07 ±21.06 | 773.47 ±20.29 | 793.00 ±10.47 |
| Forage (mg/kg) | Winter | 11593.33 ±1640.11 | 9633.33 ±1282.17 | 6266.67 ±777.47 | 5466.67 ±775.01 | 8240.00 ±646.17 |
| | Summer | 5080.00 ±659.30 | 4893.33 ±679.73 | 3220.00 ±135.65 | 2773.33 ±154.76 | 3991.67 ±270.15 |
| Plasma (L) (mg/L) | Winter | 59.18 ±2.62 | 65.73 ±3.05 | 61.73 ±3.31 | 55.25 ±1.88 | 60.47 ±1.46 |
| | Summer | 78.86 ±3.51 | 77.53 ±2.88 | 76.14 ±2.76 | 74.33 ±2.12 | 76.72 ±1.40 |
| Plasma (NL) (mg/L) | Winter | 64.04 ±4.24 | 62.40 ±1.93 | 63.44 ±1.92 | 64.67 ±1.79 | 63.64 ±1.29 |
| | Summer | 92.75 ±1.22 | 91.63 ±1.18 | 91.68 ±1.33 | 91.06 ±1.30 | 91.78 ±0.61 |
| Plasma (M) (mg/L) | Winter | 62.75 ±1.89 | 63.55 ±2.00 | 65.04 ±2.00 | 66.47 ±2.04 | 64.46 ±0.98 |
| | Summer | 91.82 ±1.09 | 90.5 ±1.37 | 89.5 ±1.52 | 87.97 ±1.57 | 89.96 ±0.71 |
| Faeces (L) (mg/kg) | Winter | 11114.6 ±668.26 | 10867 ±678.13 | 10637.8 ±644.93 | 10495.00 ±630.38 | 10778.6 ±317.463 |
| | Summer | 10448.5 ±514.80 | 10635.2 ±541.27 | 10834.5 ±590.11 | 10903.4 ±592.48 | 10705.4 ±270.83 |
| Faeces (NL) (mg/kg) | Winter | 10371.7 ±617.98 | 10306.8 ±613.73 | 10229.0 ±602.24 | 10075.2 ±569.23 | 10245.6 ±289.30 |
| | Summer | 9815.00 ±394.23 | 9858.30 ±395.15 | 10170.2 ±473.87 | 10383.7 ±517.74 | 1005.8 ±218.61 |
| Faeces (M) (mg/kg) | Winter | 8411.90 ±331.47 | 8375.30 ±329.49 | 8092.20 ±258.24 | 8320.50 ±330.75 | 8299.97 ±152.17 |
| | Summer | 8390.50 ±288.45 | 8374.50 ±292.96 | 7759.10 ±647.87 | 8348.10 ±303.68 | 8218.05 ±202.71 |
| Milk (L) (mg/L) | Winter | 593.00 ±25.65 | 556.00 ±23.10 | 543.00 ±25.52 | 511.00 ±25.32 | 550.75 ±12.86 |
| | Summer | 953.00 ±16.80 | 908.00 ±24.94 | 888.00 ±24.03 | 851.00 ±25.19 | 900.00 ±22.52 |
| Urine (L) (mg/L) | Winter | 104.30 ±8.77 | 116.60 ±8.40 | 120.30 ±8.23 | 126.50 ±8.25 | 116.96 ±4.25 |
| | Summer | 124.30 ±10.78 | 134.20 ±10.62 | 136.30 ±9.58 | 134.20 ±10.83 | 132.25 ±5.08 |
| Urine (NL) (mg/L) | Winter | 99.40 ±7.88 | 90.80 ±7.24 | 84.70 ±7.26 | 75.52 ±6.50 | 87.60 ±3.75 |
| | Summer | 86.60 ±7.12 | 91.00 ±7.27 | 94.80 ±6.96 | 99.00 ±7.39 | 92.85 ±3.53 |

L: lactating, NL: non-lactating and M: male sheep. Least square means of the following number of samples: soil (60), forage (60) and plasma, faeces, and urine (40) samples from each class of animals during each season

remained almost unchanged up to the 2nd fortnight. Thereafter, there was a gradual increase in $Ca^{2+}$ concentration up to the last fortnight. The $Ca^{2+}$ level at all the fortnights in male sheep during both seasons remained almost uniform except at the 3rd fortnight when there was a very low $Ca^{2+}$ concentration in summer as compared to that at the same fortnight in winter. Urine $Ca^{2+}$ levels were not affected ($P>0.05$) by the season in animals but affected only by the fortnight ($P<0.001$) in both lactating and

non-lactating sheep and variations due to interaction of season; the effect of fortnight was only significant in non-lactating sheep ($P<0.001$). In lactating sheep during both seasons, there was a gradual increase in $Ca^{2+}$ concentration in urine, with time, except at 4th fortnight of summer, where the $Ca^{2+}$ concentration was almost the same as that at the 3rd fortnight. In non-lactating sheep, 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. While milk $Ca^{2+}$ level was affected both by the season and fortnight ($P<0.001$), no significant effect ($P>0.05$) due the interaction of season and fortnight was found in this study. 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.

## Discussion

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 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 contents. With increasing acidity of soils there is impaired absorption of $Ca^{2+}$ (Reid and Horvath, 1980; McDowell and Valle, 2000; Islam *et al*., 2003). In this study, the high soil $Ca^{2+}$ levels may perhaps be related to high pH of soil.

The 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 a $Ca^{2+}$ 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 a Ca:P ratio of almost 1:1 to 2:1 (Underwood, 1981; Kumagai *et al*., 1990). Livestock will tolerate dietary Ca:P ratios of more than 10:1 without any serious effect provided the P intakes are adequate (Ternouth, 1990; Aregheore, 2002). 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.

Free-choice mineral mixture $Ca^{2+}$ contents were same during both seasons to complement the 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 $Ca^{2+}$ since it may contribute to a P deficiency because of imbalance of these two elements.

The feed or supplemental 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 were not seemed to have been affected by the physiologic status and gender; 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 (Serra *et*

*al*., 1996, 1997; Hayashida *et al*., 2003, 2004). 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 than in late lactation which showed less absorption through gastrointestinal tract in winter like the plasma $Ca^{2+}$ during this season.

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 during this season. Urine $Ca^{2+}$ excretion was higher during summer than that during 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, the dietary intake was low in $Ca^{2+}$ as compared to that during winter, the absorption was the 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 physiologic 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) and Fujihara *et al*. (1992) while assessing the mineral status of sheep in the Paramo region of Colombia. High incidence of plasma $Ca^{2+}$ below the critical level as reported here indicates that sheep had a very severe $Ca^{2+}$ deficiency, particularly, during winter.

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 $Ca^{2+}$ absorption; when dietary $Ca^{2+}$ is inadequate, $Ca^{2+}$ is reabsorbed from body reserves (Rowlands, 1980; Nasrullah *et al*., 2003). 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 that 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; Hayashida *et al*., 2004). 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 Ca salts of phytic acid present in diet are completely hydrolyzed only in the presence of vitamin D (NRC, 1984; McDowell, 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 to maintain the plasma $Ca^{2+}$ at a constant level. It exists as protein-bound $Ca^{2+}$ and a soluble $Ca^{2+}$ which is physiologically active. Almost half of the plasma Ca is bound to protein, and only a small portion is freely diffusible and non-ionized. The regulation of plasma $Ca^{2+}$ is highly dependent on parathyroid hormones; thyrocalcitonin or calcitropic hormones (McDowell, 1997) are responsive to plasma $Ca^{2+}$ concentration. Therefore, the low plasma $Ca^{2+}$ levels observed in some animals in this study, might be due to certain malfunctions and different levels of these hormones.

## References

Adams, RS (1975). Variability in mineral and trace element content of dairy cattle feeds. J. Dairy Sci., 58: 1538-1548.

A.O.A.C (1990). *Official methods of analysis of the association of analytical chemists*. 14th. Edn., Virginia, USA, Arlington.

Aregheore, EM (2002). Voluntary intake and digestibility of fresh, wilted and dry Leucaena (*Leucaena leucocephala*) at four levels to a basal diet of guinea grass (*panicum maximum*). Asian-Aust. J. Anim. Sci., 15: 1139-1146.

Black, HE; Capen, CC; Yarrington, JT and Rowland, GN (1973). Effect of a high calcium prepartal diet on calcium homeostatic mechanisms in thyroid glands, bone, and intestine of cows. Lab. Invest., 29: 437-448.

Cheeke, RR (1987). *Rabbit feeding and nutrition*. 1st. Edn., Academic Press. NY. P: 376.

Cuesta, PA; McDowell, LR; Kunkle, WE; Bullock, F; Drew, A; Wilkinson, NS and Martin, FG (1993). Seasonal variation of soil and forage mineral concentrations in north Florida. Commun. Soil Sci. Plant Anal., 24: 335-347.

Duncan, DB (1955). Multiple range and multiple F-test. Biometrics. 11: 1-42.

Espinoza, JE; McDowell, LR; Wilkinson, NS; Conrad, JH and Martin, FG (1991). Monthly variation of forage and soil minerals in Central Florida. I. Macro-minerals. Commun. Soil Sci. Plant Anal., 22: 2023-2056.

Evitayani, L; Warly, L; Fariani, A; Ichinohe, T and Fujihara, T (2004). *In vitro* rumen degradability and gas production during dry and rainy seasons in North Sumatra, Indonesia. In: Wong, HK; Liang, JB; Jelan, ZA; HO, YW; Goh, YM; Panandam, JM and Mohamed, WZ (Eds.), *Proceedings of the 11th. Animal Science Congress*. Volume 3, 5-9 September (2004), Kuala Lumpur, Malaysia. The Asian-Australasian Association of Animal Production Societies, Kuala Lumpur. PP: 382-384.

Fick, KR; McDowell, LR; Miles, PH; Wilkinson, NS; Funk, JD and Conrad, JH (1979). *Methods of mineral analysis for plant and animal tissues*. 2nd. Edn., Dept. Anim. Sci., Univ. Florida, Gainesville. PP: 1-74.

Fujihara, T; Matsui, T; Hayashi, S; Robles, AY; Serra, AB; Cruz, LC and Shimizu, H (1992). Mineral status of grazing Philippine goats. 2. The nutrition of selenium, copper, and zinc of goats in Luzon Island. Asian-Aust. J. Anim. Sci., 5: 389-395.

Hayashida, M; Orden, EA; Cruz, EM; Cruz, lC and Fujihara, T (2004). Effect of concentrate supplementation on blood mineral concentration of growing upgraded Philippine goats. Anim. Sci. J., 75: 139-145.

Hayashida, M; Orden, EA; Cruz, LC and Fujihara, T (2003). Effect of intraruminal soluble glass bolus on blood selenium and plasma mineral level of grazing does under backyard conditions in selected areas in Nueva Ecija, Philippines. Asian-Aust. J. Anim. Sci., 16: 189-197.

Hesse, PR (1972). *A textbook of soil analyses*. Chemical Publishing Co., NY. PP: 91-105.

Holdo, RM; Joseph, P; Dudley, J and McDowell, LR (2002).Geophagy in the African elephant in relation to availability of dietary sodium. J. Mammalogy. 83: 652-664.

Islam, MR; Saha, CK; Sharker, NR; Jahilil, M and Hasanuzzaman, M (2003). Effect of variety on proportion of botanical fraction and nutritive value of different Napier grass (*Pennisetum purpureum*) and relationship between botanical fraction and nutritive value. Asian-Aust. J. Anim. Sci., 16: 177-188.

Khan, ZI (2003). Effect of seasonal variation on the availability of macro- and micronutrients to animals (sheep and goats) through forage from soil. PhD Thesis, University of Agriculture, Faisalabad, Pakistan. P: 286.

Koh, TS and Judson, GT (1986). Trace elements in sheep grazing near a lead-zinc smelting complex at port pirie, South Australia. Bull. Environ. Contam Toxicol., 37: 87-95.

Kumagai, H; Ishida, N; Katsumata, M; Yano, H; Kawashima, R and Jachja, J (1990). A study on nutritional status of macro-minerals of cattle in Java in Indonesia. Asian-Aust. J. Anim. Sci., 2: 7-13.

Manyayu, JG; Chakoma, C; Sibanda, S; Mutisi, C and Chakoma, IC (2003). The intake and palatability of four different types of Napier grass (*Pennisetum purpureum*) silage fed to sheep. Asian-Aust. J. Anim. Sci., 16: 823-829.

McDowell, LR (1985). *Nutrition of grazing ruminants in warm climates*. 1st. Edn., New York, Academic Press. P: 443.

McDowell, LR (1987). Assessment of mineral status of grazing ruminants. World Rev. Anim. Prod., 33: 19-32.

McDowell, LR (1992). *Minerals in animal and human nutrition*. Academic Press, San Diego, Calif.,

McDowell, LR (1997). *Minerals for grazing ruminants in tropical regions*. Extension Bulletin, Anim. Sci. Dept. Centre for Trop. Agric.,Univ. Florida. P: 81.

McDowell, LR (2003). *Minerals in animal and human nutrition*. 2nd. Edn., University of Florida, Gainesville. PP: 235-276.

McDowell, LR; Conrad, JH and Hembry, FG (1993). *Mineral for grazing ruminants in tropical regions*. University of Florida, Gainesville.

McDowell, LR and Valle, G (2000). Major minerals in forages. In: Givens, DI; Owen, E; Oxford, RFE and Omed, HM (Eds.), *Forage evaluation in ruminant nutrition*. (1st. Edn.), CAB International, Wallingford, UK. PP: 373-393.

Mpofu, IDT; Ndlova, LR and Casey, NH (1999). The copper, cobalt, iron, selenium and zinc status of cattle in the Sanyati and Chinamhora small holder grazing area of Zimbabwe. Asian-Aust. J.

Anim. Sci., 12: 579-584.

Nasrullah Niimi, M; Akashi, R and Kawamura, O (2003). Nutritive evaluation of forage plants in South Sulaweshi, Indonesia. Asian-Aust. J. Anim. Sci., 16: 693-701.

National Research Council (1984). *Nutrient requirements of domestic animals*. *Nutrient requirements of beef cattle*. (6th. Rev. Edn.), National Academic Sciences, Washington, DC., P: 190.

Neathery, MW; Crowe, NA; Miller, WJ; Crowe, CT; Varnado, JL and Blackmon, DM (1990). Influence of dietary aluminum and phosphorus on zinc metabolism in dairy calves. J. Amin. Sci., 68: 4326-4333.

Netherlands Committee on Mineral Nutrition (1973). *Tracing and treating mineral disorders in dairy cattle*. Netherlands Committee on Mineral Nutrition, Centre for Agriculture Publishing and Documentation-Wagenixgen, The Netherlands. PP: 11-16.

Nockels, CF; Deboniss, J and Torrent, J (1993). Stress induction affects copper and zinc balance in calves fed organic and inorganic copper and zinc sources. J. Anim. Sci., 71: 2539-2545.

Pastrana, R; McDowell, LR; Conrad, JH and Wilkinson, NS (1991). Macromineral status of sheep in the Paramo region of Colombia. Small Ruminant Res., 5: 9-21.

Proyecto Ovino Colombo Britanico (1979). Informe (Annual report) 1978-1979. ICA, Caja Agraria, Planeacion Nacional, Reino Unido, Bogota, Colombia. PP: 31-53.

Reid, RL and Horvath, DJ (1980). Soil chemistry and mineral problems in farm livestock: a review. Anim. Feed Sci. Tech., 5: 95-167.

Reuter, DJ and Robinson, JB (1997). *Plant analysis*. *An interpretation manual*. 2nd. Edn., CSIRO Publishing, Melbourne. P: 84.

Rhue, RD and Kidder, G (1983). *Analytical procedures used by the IFAS extension soil laboratory and the interpretation of results*. Soil Sci. Dept., Univ. Florida, Gainesville. PP: 38-60.

Rojas, LX; McDowell, LR; Wilkinson, NS and Martin, FG (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.

Rowlands, GJ (1980). A review of variations in concentrations of metabolites in the blood of beef and dairy cattle associated with physiology, nutrition and disease. World. Rev. Nutr. Diet., 35: 172-235.

Sanchez, PA (1976). *Properties and management of soils in tropics*. John Wiley and Sons, NY. PP: 97-101.

Serra, AB; Serra, SD; Orden, EA; Cruz, LC; Ichinohe, LC and Fujihara, T (1996). Monthly nutrient variation of paragrass (*Bracaria mutica*) and stargrass (*Cynodon plectostachyum*) collected from pastures grazed by goats. Asian-Aust. J. Anim. Sci., 9: 203-210.

Serra, AB; Serra, SD; Orden, EA; Cruz, LC; Nakamura, K and Fujihara, T (1997). Variability in ash, crude protein, detergent fiber and mineral content of some plant species collected from pastures grazed by goats. Asian-Aust. J. Anim. Sci., 10: 28-34.

Steel, RGD and Torrie, JH (1980). *Principles and procedures of statistics: a biometrical approach*. 2nd. Edn., New York, Mcgraw Hill Book Co. PP: 204-280.

Tejada, R; McDowell, LR; Martin, FG and Conrad, LH (1987). Evaluation of the macro-mineral and crude protein status of cattle in specific regions in Guatemala. Nutr. Rep. Int., 35: 989-998.

Ternouth, JH (1990). Phosphorus and beef production in northern Australia. 3. Phosphorus in cattle-a review. Trop. Grass., 24: 159-169.

Tiffany, ME; McDowell, LR; O'Connor, GA; Nguyen, H; Martin, FG; Wilkinson, NS and Cardoso, EC (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.

Tiffany, ME; McDowell, LR; O'Connor, GA; Nguyen, H; Martin, FG; Wilkinson, NS and Katzowitz, NA (2001). Effects of residual and reapplied biosolids on forage and soil concentrations over a grazing season in north Florida. II. Microminerals. Commun. Soil Sci. Plant Anal., 32: 2211-2226.

Tucker, WB; Hogue, JF; Waterman, DF; Swenson, TS; Xin, Z; Hemken, RW; Jackson, JA; Adams, GD and Spicer, LJ (1991). Role of sulfur and chloride in the dietary cation-anion balance equation for lactating cattle. J. Anim. Sci., 69: 1205-1213.

Tudsri, S and Kaewkunya, C (2002). Effect of Leucaena row spaceing and cutting intensity on growth of Leucaena and three associated grasses in Thailand. Asian-Aust. J. Anim. Sci., 15: 986-991.

Underwood, EJ (1981). *The mineral nutrition of livestock*. 2nd. Edn., Commonwealth Agricultural Bureaux, Slough, England, P: 180.