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# Periodic Transfer of Calcium Concentration from Forage Plants and Soil to Small Ruminants

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**Abstract:-** An experiment was conducted to determine the calcium status of male and female (lactating and non-lactating) grazing goats (Thalli breed) during summer and winter seasons at a private goat farm in Punjab, Pakistan. The samples of forage plants, soil, water, milk, blood, urine and fecal matter of animals were gathered fortnightly for two months of each season for the estimation of calcium. The highest amount of calcium in urine, blood plasma, milk and feces were 102±4.56, 135±4.98, 117±5.0 mg $L^{-1}$ and 280± 4.56 mg $kg^{-1}$, respectively. The maximum amounts of calcium 530±0.21 and 124±2.65 mg $kg^{-1}$ was found in soil and forage samples while minimum amount 54±0.54 mg $L^{-1}$ in canal water. It was concluded that the available concentrations of calcium to animals through forage plants were in-sufficient to meet the dietary requirements of animals. It is therefore, an emergent need of calcium supplementation either through soil or in the feed of animals that will ultimately improve the animal health by minimizing metabolic disorders.

**Key words**: Calcium deficiency, ruminants, forage calcium.

## INTRODUCTION

Pakistan is facing a serious shortage of meat and milk. Most of the lands are comprised of arid or semiarid which is deficient of macro and micronutrients necessary for plant and animal's growth. Due to urbanization, livestock are limited only to small number of villages, therefore, causing the shortage of milk and other products involving the ruminants (Ashraf *et al.*, 2006; Khan *et al.*, 2010a). Minerals are the essential nutrients bearing significant role in the animal nutrition because their excess or deficiency produces "detrimental effect" in the performance of livestock. Forage calcium requirements for ruminants are, commonly, influenced by the age, weight of animals and levels of production. The goats have wide distribution from temperate zone to semi-arid and super-humid environments. They are very inquiring/selective animals for food than other ruminants.

Deficiencies of minerals are the most limiting adversaries to ruminants all over the world and these are difficult to detect in early stages or in milder forms (Khan *et al.*, 2010a). Variables amounts of calcium are present in almost all feedstuffs but it is generally deficient in grains and abundant in most forage. Absorption of calcium through the membranous lining of gut required the binding proteins which is ultimately regulated by the vitamin-D, necessary sunlight, calcitonin and parathyroid hormones (Soetan *et al.*, 2010). Productivity and health of livestock depends upon balanced quantities of nutrients to their requirements at a given physiological stage of animal (Youssef *et al.*, 1999). Milk is the natural source of high-quality calcium and phosphorus for growing mammals and their concentrations in milk are relatively constant and little affected by diet, but they differ between regular milk and colostrums. Calcium may cause weakened bones, slow growth and tetany (convulsions). Keeping in view the importance of macro-nutrient especially calcium for ruminants the studies were conducted to assess its translocation from soil through forage plants to goats and its status in their milk, blood, urine and feces.

## MATERIALS AND METHODS

Studies were carried out by selecting lactating, non-lactating females and male, three each, clinically healthy goats (Thalli breed) Four samples were collected after interval of each fortnight from all classes of animals. The goats were allowed to free grazing for four hours daily. All

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experimental goats remained same throughout study period. Soil samples up-to 15-20 cm depth were collected from six different sites of fodder fields and canal banks. Soil samples were air dried ground, thoroughly mixed and sieved for chemical analysis. One gram air dried soil was taken in 100 ml conical flask and added 20 ml ammonium bicarbonate diethylene-triamine-pentaacetic acid (AB-DTPA) solution. After shaking (GFL-Shaker, Germany) samples were filtered through Whatman filter paper No. 42 for half an hour. Samples were centrifuged (Hermle, Z-233 M-2, Abnet, Germany) for 20 minutes at 3500 rpm and stored at -20°C. Water samples from tube well, canal and tap were collected from where goats drank. Samples were collected in plastic bottles (100 ml) and stored at -20°C for further analysis. Forage plant samples were gathered from same site where the soil samples were collected. Washed with 1% HCl followed by four washings with distilled water in order to remove dust and unwanted particles. Samples were oven dried (Thelco, model 6, Germany) at 70±2°C and ground to powder stored in brown paper bags. Fecal matter samples were collected twice a daily at every fortnight from all types of animals under study. Samples were mixed to form a uniform sample. Samples were collected in polyethylene bags and stored at -20°C (Sanyo, biochemical freezer, Model MDF-U333, Japan). Ground and oven dried samples were processed according to method of Wolf (1982).

Blood samples were collected from jugular vein by disposable syringes. A pinch of anticoagulant (EDTA) was added in it in order to avoid clotting of blood sample. Samples were centrifuged (Hermle, Z-233 M-2, abnet, Germany) at 3000 rpm for 20 minutes to separate blood plasma. Samples were stored in freezer at -20°C and further processed following the methods of Mpofu *et al.* (1998). Morning and evening samples of milk were collected from lactating goats mix to make them uniform sample. One ml of $K_2Cr_2O_7$ (30%) was added for preservation. Samples were stored at -20°C in freezer (Model MDF-U333, Japan). Ten ml of milk was digested with mixture of perchloric acid and nitric acid (2:1). Final volume was made up to 25 ml with distilled water. Samples were analyzed for calcium following the procedure of Stelwagen *et al.* (1999). Data was analyzed statistically (Duncan, 1980) for determining the significance of treatment means at 5% level of probability (Steel and Torrie, 1980).

## RESULTS

For blood samples inconsistent pattern was recorded for both seasons at different sampling intervals. The highest value 144±3.47 mg $L^{-1}$ of calcium was found at first fortnight sampling of lactating (winter) and male (summer and winter) goats while lowest (36±2.58 mg $^{-1}$L) at first fortnight during summer and winter seasons in non-lactating and male goats respectively. Calcium concentration in blood plasma was significantly at par in lactating goats during winter season while non-lactating and male goats statistically showed no significant variations. The seasonal means varied from 66±1.65 to 135±4.98 mg $L^{-1}$ for winter and summer season (Table I). In lactating goats, a highly significant positive correlation (r=0.971) was observed between plasma and soil in summer season. The calcium secreted through milk was higher (117±5.0 mg $L^{-1}$) during summer than winter (86±2.14 mg $L^{-1}$) (Table I). The highest fortnight value of calcium at second and third fortnight was 144 mg/L in summer and the lowest 72±2.11 mg $L^{-1}$ in the months of winter in lactating goats. Significant ($p<0.01$) differences were recorded between the summer and winter levels among all the animal types except non-lactating ($p>0.01$) animals.

Calcium levels in male animals excreted through urine were higher in the months of winter (102±4.56 mg $L^{-1}$) as compared to summer season while lowest (38±3.21 mg $L^{-1}$) in winter season for lactating goats (Table I). However, a significant decrease was found in lactating goats in winter season. In lactating goats during winter season the high significant positive correlation (r=0.971) was recorded between urine and plasma whereas a strong negative correlation (r=-0.982). Significant ($p<0.01$) variations were observed between urine and forage samples for non-lactating goats in summer season was observed. No significant difference was observed in all goat types in calcium concentrations excreted through the fecal matter were maximum 280±4.56 mg $kg^{-1}$ in male during

**Table I.- Calcium concentrations (mean±SE) in feces, urine, plasma and milk samples of lactating, non-lactating and male goats during summer and winter seasons.**

| Sample | Animal types | Season | Sampling periods (fortnights) I | II | III | IV | Seasonal means |
|---|---|---|---|---|---|---|---|
| Feces (mg kg$^{-1}$) | Lactating | Summer | 300±10.20 | 172±2.54 | 240±1.36 | 194±1.55 | 226±7.11$^{a}$ |
| | | Winter | 190±5.21 | 250±2.54 | 260±3.69 | 280±4.11 | 245±8.22$^{a}$ |
| | Non-lactating | Summer | 220±5.47 | 190±3.22 | 300±5.82 | 180±5.66 | 222±9.55$^{a}$ |
| | | Winter | 200±6.24 | 300±3.11 | 240±4.71 | 324±1.55 | 266±2.33$^{a}$ |
| | Male | Summer | 224±4.87 | 424±3.22 | 180±2.11 | 260±2.54 | 272±1.65$^{a}$ |
| | | Winter | 330±3.54 | 320±1.25 | 290±3.55 | 180±2.31 | 280±4.56$^{a}$ |
| Urine (mg L$^{-1}$) | Lactating | Summer | 90±1.25 | 100±1.00 | 108±2.66 | 46±1.46 | 86±7.89$^{a}$ |
| | | Winter | 40±2.54 | 38±3.00 | 38±1.25 | 37±5.79 | 38±3.21$^{c}$ |
| | Non-lactating | Summer | 46±6.47 | 48±2.54 | 47±1.47 | 61±2.65 | 50±5.64$^{bc}$ |
| | | Winter | 36±1.58 | 90±3.65 | 67±5.82 | 108±1.00 | 75±8.97$^{ab}$ |
| | Male | Summer | 56±0.21 | 110±1.47 | 71±2.66 | 36±5.00 | 68±1.23$^{abc}$ |
| | | Winter | 107±2.65 | 107±2.58 | 100±2.44 | 96±6.44 | 102±4.56$^{a}$ |
| Plasma (mgL$^{-1}$) | Lactating | Summer | 72±3.21 | 67±6.39 | 77±1.36 | 82±2.33 | 74±7.89$^{bc}$ |
| | | Winter | 144±2.69 | 96±1.47 | 108±8.14 | 132±1.54 | 120±3.21$^{a}$ |
| | Non-lactating | Summer | 86±1.58 | 36±2.58 | 108±0.22 | 92±2.66 | 80±2.65b$^{c}$ |
| | | Winter | 66±1.58 | 66±6.39 | 67±0.36 | 68±4.21 | 66±1.65$^{c}$ |
| | Male | Summer | 144±3.47 | 136±7.11 | 126±0.25 | 126±4.22 | 135±4.98$^{a}$ |
| | | Winter | 144±2.65 | 78±8.55 | 108±0.41 | 88±6.11 | 104±2.00$^{bc}$ |
| Milk (mg L$^{-1}$) | Lactating | Summer | 108±1.23 | 144±9.00 | 144±0.74 | 75±4.10 | 117±5.00$^{a}$ |
| | | Winter | 88±4.56 | 72±2.11 | 96±0.85 | 88±1.30 | 86±2.14$^{a}$ |

For respective animal class similar alphabets followed by letters in rows did not differ significantly (*P* >0.01)
P values are given in table

**Table II.- Calcium concentrations (Mean±SE) in forage plants, soil, canal and tube well waters during summer and winter seasons.**

| Sample types | Season | Sampling periods (fortnights) I | II | II | IV | Seasonal means |
|---|---|---|---|---|---|---|
| Forage (mg kg$^{-1}$) | Summer | 144±2.56 | 108±3.22 | 120±0.96 | 124±4.11 | 124±2.65$^{a}$ |
| | Winter | 130±3.78 | 140±2.11 | 100±0.25 | 100±2.36 | 117±0.01$^{a}$ |
| Soil (mg kg$^{-1}$) | Summer | 540±6.78 | 440±3.66 | 580±0.14 | 560±5.36 | 530±0.21$^{a}$ |
| | Winter | 234±2.69 | 250±2.54 | 260±0.69 | 280±8.96 | 256±0.54$^{b}$ |
| Canal water (mg L$^{-1}$) | Summer | 40±1.36 | 36±0.25 | 90±0.85 | 50±1.00 | 54±0.54$^{a}$ |
| | Winter | 90±1.58 | 88±0.36 | 78±0.74 | 60±5.00 | 79±4.32$^{a}$ |
| Tube well water (mg L$^{-1}$) | Summer | 72±1.69 | 90±0.14 | 72±0.85 | 80±2.44 | 78.5±2.45$^{a}$ |
| | Winter | 90±3.58 | 78±0.41 | 88±0.96 | 90±3.25 | 86.5±1.00$^{a}$ |

For respective animal class similar alphabets followed by letters in rows did not differ significantly (*P* >0.01)

winter season. Lowest seasonal mean 222±9.55 mg kg$^{-1}$ in non-lactating during summer season was observed (Table I).

For soil sampling, inconsistent trend was recorded for both seasons during all fortnights. Seasonal means of calcium for summer and winter seasons were 530±0.21 and 256±0.54 mg kg$^{-1}$ respectively (Table II). The highest fortnight value

580±0.14 mg $kg^{-1}$ was recorded at third fortnight. A strong positive correlation (r=0.971) was observed between soil and blood plasma of lactating animals in summer season. In summer, more forage calcium (124±2.65 mg $L^{-1}$) was found as compared to winter season (Table II). The highest calcium level was (144±2.56 mg $kg^{-1}$) at first fortnight while lowest (100±0.25 mg $kg^{-1}$) at third fortnight of winter season. While in winter, maximum calcium was present after second fortnight (140 mg $kg^{-1}$). The comparison of calcium in tube well and canal waters showed inconsistent variations in tube well water during both seasons of summer and winter. Seasonal means during summer and winter seasons were 54±0.54-79±0.32 mg $L^{-1}$ for canal water and 78.85±2.45-86.5±1.0 mg $L^{-1}$ for tube well water. Highest levels for both waters were 90±0.85 at third fortnight in summer and 90±3.58 mg $L^{-1}$ in first fortnight of the winter season (Table II). A strong positive correlation was observed for water with soil (r=0.995) and forage plants (r=0.922). During both seasons statistically non significant ($p>0.01$) results were recorded in canal water, tube well water and forage except soil samples ($p<0.01$).

## DISCUSSION

Among different macro-mineral, calcium play a very important role in various metabolic processes in both animals and forage plants. Imbalances of minerals in forage plants and soils in arid and semi-arid regions are the main hurdle in achieving the maximum production from livestock (Khan *et al*., 2010a). Calcium concentrations not only varied from plant to plant but also in different parts of the forage plants. It can have a very hazardous effect on the health and performance of ruminants if included in the diet at very high levels. The tolerance levels of each macromineral varied from animal-to-animal and from time-to-time in the same animal (Khan *et al*., 2004). However, it is necessary to determine precise amount of mineral supplements and tolerance levels for specific type of animals (Bribiesca, 2001; Knowlton and Herbein, 2002). The concentration of calcium in blood is important for bones formation. The highest calcium level in plasma was found at first fortnight of lactating and male goats during both seasons. Its levels ranged from 66±1.65 to 135±4.98 mg $L^{-1}$ during both seasons. The possible reason for this high value of calcium in lactating goats is more production of milk in winter as compared to summer. The maximum calcium estimation in soils is related to its more translocation through forage in ruminants. In the process of blood coagulation calcium converts the pro-thrombin to thrombin and helps in blood clotting. Its reduced levels increase the irritability of nerve cells and very low level increase the spontaneous discharges of nerve cells which leads tetany and convolution. Excessive calcium depresses the heart functioning, respiratory and heart failure (Soetan *et al*., 2010). Its lowered amount in forage plants than requirements results a lowered levels in milk samples (Ashraf *et al*., 2006). Variations in calcium status in milk may be due to the various chemical combinations with other elements, production levels, intake from forages and animal adoption. Its lowered amount found in lactating and male but the higher in non-lactating animals in the winter season. Recent values of calcium in lactating and non lactating during the months of summer were in close association with the finding of Khan *et al*. (2009) and Pasha *et al*. (2009).

The concentrations of calcium secreted through milk were higher during summer than in winter season which is related to more intake of calcium through forage as the summer forage showed more calcium than winter. In-significant amount of calcium in milk might be due to its excretion through feces in winter season. Recorded levels of milk calcium in both seasons were not in close association with values found by Ashraf *et al.* (2006) who recorded 1.40 mg/g in lactating buffaloes. Deficiency of mineral nutrients causes the under nourishment, low productivity, parasitism, epidemics and developmental problems in animals (Inam-ur-Rehman *et al*., 2008). Homeostasis mechanism tends to protect the animals against excessive calcium absorption, thus animals are excreting calcium excreted through the feces (Ashraf *et al.*, 2006). Calcium levels in male animals excreted through urine were higher in the months of winter amongst all other variables. Decreasing trend was found for winter season in lactating goats. It was found that lesser amount of calcium was excreted through urine as compared to feces because feces are the major path for calcium excretion. Urinary loss of calcium is negligible in ruminants because it can either be re-absorbed by kidney or lost through the sweating. Lowered levels in urine for lactating and non-lactating animals during summer season reflect its efficient absorption by kidney. Possible causes of differences in urine calcium in animals and seasons were physiological processes, re-absorption by kidney, a-biotic factors and genetic variations. In lactating goats during winter season strong positive correlation (r=0.971) was found between urine and plasma.

In all ruminants, the excretion of calcium through feces is very common. Fecal calcium is the combination of un-absorbed dietary calcium and un-absorbed endogenous calcium from intestinal mucosal secretions. Calcium concentrations excreted through the fecal matter were maximum in male during winter season. In lactating animals during the summer season more calcium was acquired by animals but less excreted through feces as compared to winter because it might be transferred to blood or milk. Differences in feces calcium levels among animals and seasons may be due to preferences for forages, efficiency of absorption, genetic variations and animal adaptation (Khan *et al*., 2010a). Major portion of calcium and phosphorus in feces includes the un-absorbed dietary portion (Soetan *et al*., 2010). The strong positive correlation (r=0.975) evaluated between feces and forage in lactating goats. Assessment of mineral status of animals is greatly influenced by forage plants and soil status upon which plants are growing (Mtmuni *et al.*, 1990). It is used as major nutrient for plant growth and also reduces the acidity of soil (Ashraf *et al*., 2006). Estimated soil calcium in winter was in close agreement with the levels as reported by Tiffany *et al.* (1999). Mtimuni *et al*. (1990) suggested that useful informations about the inter relationships among mineral nutrients could give a precise mineral profile of blood plasma. The comparison of calcium in tube well and canal waters showed inconsistent variations of calcium concentration in tube well water during both seasons. The higher calcium in waters may be due to mixing of various chemical wastes in canal water as industry effluents by cotton and ginning factories. Strong positive correlation was observed for waters with forage plant (r=0.922). Forage calcium requirement of grazing ruminant is a subject of considerable debate as the requirement is influenced by animal types and level of production, age and weight (Khan *et al.*, 2004). In summer, more calcium concentration (124 mg/L) was found as compared to forage of the winter. Calcium and magnesium are one of those necessary elements which are necessary for normal growth of ruminants. Stressful conditions like temperature fluctuations, low annual rainfall and dry winds causes the considerably variations in essential minerals in forage plants than plants growing under normal environmental conditions (Ahmad *et al*., 2008; Bruce *et al*., 2000). Many researchers in their earlier studies have reported that many macro-minerals decrease with the increase in maturity of forage plants (Khan *et al*., 2010a, b). Similarly Khan *et al*. (2009) evaluated 0.18% and 0.14% of forages calcium in summer and winter seasons.

## CONCLUSIONS

The levels of minerals varied from time-to-time and space-to-space in this region of Punjab. Soil forage and milk calcium were higher in summer season than in winter. However, the amount of calcium was higher in feces, urine and plasma in male animals than in lactating and non-lactating goats. The overall conclusion drawn by this study was that there is a definite additional requirement of feed supplement for ruminants (goats) to overcome calcium deficiency for better growth, development, milk production and resistance against diseases.

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