

## SEASONAL VARIATION IN CALCIUM AND MAGNESIUM STATUS OF SOIL, DIETARY SOURCES AND SMALL GRAZING RUMINANTS IN THE SEMI-ARID REGION OF PAKISTAN

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### ABSTRACT

Investigations were conducted in the southern part of Punjab, Pakistan to evaluate the mineral status of goats, to determine the classes of animals which are most susceptible to specific mineral deficiencies or toxicities, as reflected by low tissue fluids mineral concentrations in animals, and to study the effect of season of the year on the mineral availability to animals. During both the winter and summer season, soil, forage, feed, water from pasture and blood, milk, faeces, and urine samples from lactating, non-lactating, and male goats were collected fortnightly and analyzed for macro- and micro mineral concentrations. Soil samples taken from the pasture grazed by goats had adequate levels of both calcium and magnesium during winter and summer. While forage contained marginal deficient  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ . Feed  $\text{Ca}^{2+}$  concentrations were moderately deficient during both seasons. The effects of feed supplement at goat ranch in raising the plasma mineral level was different in different groups of animals in different seasons. The moderate deficient level of plasma  $\text{Ca}^{2+}$  and marginal deficient level of  $\text{Mg}^{2+}$  during winter and summer in lactating goats, while in non-lactating goats in plasma, moderate levels of  $\text{Ca}^{2+}$  and marginal deficient levels of  $\text{Mg}^{2+}$  during both seasons were found. Plasma of male goats contained marginal deficient levels of  $\text{Ca}^{2+}$  during winter, and  $\text{Mg}^{2+}$  during summer, while moderate deficient levels of  $\text{Ca}^{2+}$  were found in summer. The fecal  $\text{Ca}^{2+}$  in non-lactating animals were high in summer, and  $\text{Mg}^{2+}$  high in winter. Urine mineral concentrations of lactating animals were above optimum level during winter, and in non-lactating animals both the urine minerals were high during the winter. Concentrations of milk minerals such as  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$  were lower in winter than those in summer.  $\text{Ca}^{2+}$  in lactating goats, and  $\text{Mg}^{2+}$  in male goats were excreted more during winter when comparison was made among different classes of goats because animal had low absorption for these minerals or for their body requirements. Urine mineral concentrations such as  $\text{Ca}^{2+}$  in lactating goats,  $\text{Mg}^{2+}$  in non-lactating goats were higher in winter than that in summer. The minerals which had more absorptive capacity through intestinal tract had been excreted more in urine and translocated to plasma of the animals.  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$  contents of milk in lactating goats was found to be lower during winter showing less availability and absorption of these minerals in this season. The findings of this work showed that the distribution of nutrients in different parts of the animal body was dependent on the rate of absorption through gastrointestinal tract of the animals. Despite the adequate levels of certain minerals found in forage and feed in particular season, the animals showed deficiency of these elements. So further study to eliminate unnecessary minerals in the supplement showing antagonism and thus responsible for reducing the bioavailability of certain essential minerals in a particular season, is needed. On the basis of these results both minerals in soil were adequate for the normal growth of plant. But were deficient in forage on goat ranch particularly during summer season. However, the supplementation of feed containing minerals seemed to have contributed much to the well being of the animals.

**Keywords:** Pasture allowance, season, physiological state, mineral content, availability, grazing livestock

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### INTRODUCTION

Agriculture, including livestock is the dominant sector of Pakistan's economy. Agriculture sector contributes about 26 percent of gross domestic product (GDP). It employs about 52 percent (30 millions) of the country's labour force and provides 59 percent of export earnings. Crop and livestock activities are closely integrated in the country. Dairy farming is an integral part of agriculture and there is hardly any farmer in the country who does not maintain livestock. Livestock production accounts for about 30 percent of agricultural GDP and about 8 percent of total GDP. It is primarily a subsistence activity characterized by wide ownership and the predominance of small units. Milk is the main product in case of buffaloes while cattle are kept mainly for draft power. Sheep and goats are kept for meat, milk, and wool as secondary purpose. Generally speaking, livestock serve first to meet dietary and farm work requirements and second as a source of cash income. Almost every rural family owns some animals and adult as well as children are involved in their husbandry. It is estimated that livestock plays an important role in the lives of 30-35 million people (Gillani, 1993).

Plants absorb most of the nutrients from the soil and in turn they are assimilated by animals. In order to meet the continued demand of crops, these nutrients have to be replenished through the addition of synthetic fertilizers. In Pakistan, N, P, and small quantity of  $\text{K}^+$  are being applied through commercial fertilizers, but no application of micro-nutrients is normally made. The crops are continuously mining the macro- and micro-nutrients from the soil reserves. Increased removal of micro-nutrients leads to their deficiencies. To diagnose their deficiencies in animals, soil, plant, and tissue and fluid analyses are necessary. In Pakistan, the research on micro-nutrients availability and their assimilation in fodder crops and animals was initiated in late fifties on a very limited scale. The literature pertaining to the present study is reported in this manuscript.

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At least 15 minerals are essential to animals. They are the major elements calcium (Ca), chlorine (Cl), phosphorous (P), magnesium (Mg), potassium (K) sodium (Na) and sulfur (S), and the trace elements cobalt (Co), copper (Cu), iron (Fe), iodine (I), manganese (Mn), selenium (Se), zinc (Zn) and molybdenum (Mo). There is some evidence that a further 15 elements may be required in ultratrace amounts in the diet [ $<50\mu\text{g}/\text{kg}$  dry matter (DM)], particularly for arsenic, boron, chromium, nickel, silicon and vanadium, although no specific biochemical functions have been identified for these elements (Mertz, 1993; Nielsen, 1996).

Under nutrition is commonly accepted to be the most important limitation to livestock production in different countries (Ogebe and McDowell, 1998; McDowell and Valle, 2000). Lack of sufficient energy and protein is often responsible for suboptimum production. Numerous investigators, however, have observed that ruminants sometimes deteriorate in spite of an abundant feed supply. Mineral deficiencies or imbalances in soils and plants have long been held responsible for low production and reproduction problems among tropical livestock (McDowell, 1985; McDowell *et al.*, 1993; Vargas and McDowell, 1997; Tiffany *et al.*, 2001).

Mineral deficiencies and imbalances for herbivores are reported from almost all tropical regions of the world. Phosphorus (P) deficiency has been reported in 25 Latin American countries and deficiencies of calcium (Ca) in 11, sodium (Na) in 15, magnesium (Mg) in 14, cobalt (Co) in 13, copper (Cu) in 21, selenium (Se) in 17, and zinc (Zn) in 16 countries (McDowell *et al.*, 1993). Iodine (I) deficiency is reported worldwide.

Mineral supplementation is required to correct mineral deficiencies in livestock and poultry diets. Prior to the turn of the 20<sup>th</sup> Century, the only supplemental minerals that were generally recognized as of value and provided to livestock and (or) humans, were common salt (NaCl), iron (Fe), or I, but administered on a very infrequent basis. In the early part of this century, pasture, other forages, distiller's soluble or grains, brewer's grains, fermentation products, meat and bone scraps, bone meal, and fish by-products were used in large quantities in livestock diets. In addition to providing energy and proteins, these feeds likewise provided sources of minerals and vitamins. As animal feeding became more sophisticated, fewer feed sources were used, faster growing and higher-producing animals were developed and the industry tended towards more intensified operations, and it became necessary to add an increasing number of minerals to properly fortify animal diets (McDowell, 1997).

For many classes of livestock including pigs, poultry, feedlot cattle, and dairy animals, mineral supplements are incorporated into concentrate diets, which generally ensure that animals are receiving required minerals. However, for grazing livestock to which concentrate feeds cannot be economically fed. It is necessary to rely on both indirect and direct methods of providing minerals. Self-feeding of 'free-choice' mineral supplements is widely used for grazing livestock, which confers no added benefits on the animal (Rojas *et al.*, 1993).

Although highly variable, livestock obtain some minerals from drinking water but at concentrations that are generally quite inadequate for meeting daily dietary requirements. Average concentrations of minerals in surface waters in the United States have been calculated to provide beef cattle with approximately the following percentages of their daily requirements: NaCl, 34%;  $\text{Ca}^{2+}$ , 10-16%;  $\text{Mg}^{2+}$ , 4-11%; S, 28%  $\text{Co}^{2+}$ , 12%, Mn, 6% and for P,  $\text{K}^+$ ,  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cu}^{2+}$ , 1-2% or less (NRC, 1974; Shirley, 1985). Due to the extreme variability of minerals in water and the generally low concentrations, water mineral analysis for evaluation ruminant mineral status would frequently be of little value. Likewise, Mo determination of water has been reported to be useful as water naturally high in Mo can induce  $\text{Cu}^{2+}$  deficiencies in grazing animals (Viek and Lindsay, 1977; McDowell, 1987). Water analyses could also be useful to establish if S in water is resulting in a conditioned  $\text{Cu}^{2+}$  deficiency or if  $\text{Cu}^{2+}$  piping is contributing to  $\text{Cu}^{2+}$  intake (Cseh *et al.*, 1995). Further more, the contribution of water to the mineral intake of animals has largely been ignored and underestimated.

The soil and its parent materials are the primary sources of mineral elements on which soil-plant-animal relationships are built. An excellent comprehensive study on soil-plant relationships and deficiency diseases has been prepared by Reid and Horvath (1980). When few data are available about the minerals to be investigated, general introductory information can be obtained by examining the nature of the geological formations. According to White *et al.* (1999) soils vary widely in their micronutrient content and in their ability to their supply micronutrient in quantities sufficient for optimal crop growth. It was concluded that soils deficient in their ability to supply micronutrients to crops are alarmingly wide spread across the globe. Certain naturally occurring mineral deficiencies in herbivores are associated with specific regions and are directly related to soil characteristics. A general observation by a number of researchers is that the level of a mineral in the soil does not necessarily indicate its availability to plants growing on the soil (Underwood, 1977; Reid and Hovath, 1980).

Lamand (1979) and Davies (1997) reviewed the subject of nutrient balance in the soil and limitations of soil trace element analysis to predict available nutrients to plants and soil tests help identify nutrients needed to enhance plant nutrition (Hanlon *et al.*, 1990; Tiffany *et al.*, 2000). It was concluded that forage analysis was the

only reliable method of estimating nutrient availability to grazing animals, although this is often modified by interference and interactions between trace elements (McDowell, 1987).

In some instances, a soil survey can provide clues to potential livestock deficiencies. However, considerable evidence indicates that there is often little relationship between soil chemistry and mineral composition of farm crops and vegetation growing on that soil. A more satisfactory soil analysis to relate mineral concentrations for livestock is the use of soil extractants (*i.e.*, 0.05N HCl + 0.025N H<sub>2</sub>SO<sub>4</sub>) which are the more available forms of soil minerals (Rhue and Kidder, 1983). Analyses to determine the available forms of soil minerals can sometimes provide evidence of livestock mineral deficiencies but, unfortunately, more often they are unreliable and difficult to interpret.

Undoubtedly, forage analysis is a much better indicator of mineral status of ruminants than is soil analysis. Likewise, animal tissue-mineral concentrations are better indicators of the availability of most minerals than are forage mineral analyses. Grazing livestock obtain a part of their mineral supply from the consumption of water, soil, leaves tree bark, etc. rather than entirely from forages. Livestock tissue-mineral concentrations, therefore, more accurately portray the contribution of the total environment in meeting the mineral requirements of grazing animals. As in illustration, neither the available Cu<sup>2+</sup> content of the soil nor the Cu<sup>2+</sup> content in the herbage show any positive relationship with the Cu<sup>2+</sup> status of animals (Sutherland, 1980; Minson, 1990).

Animal tissue and fluid levels of minerals, in addition to concentrations of particular enzymes, metabolites, or organic compounds with which the mineral in question is associated functionally, are important indicators of mineral status. The diagnostic significance of tissue and fluid analysis is based upon evidence that mineral element deficiencies result in subnormal concentrations of the element, in altered concentrations of related metabolites or in changed activities of affected enzymes. The concentrations of minerals in tissues, or of their functional forms, such as thyroxine (I) and vitamin B<sub>12</sub> (Co), must be maintained within narrow limits, if growth, health, and productivity of the animal are to be sustained. Deviation from these normal limits, which is now well defined for most elements, therefore, constitutes useful diagnostic indicators (Cagle *et al.*, 1985; McDowell, 1985; Wildeus *et al.*, 1992). A further valuable aspect of such tissue composition changes is that they frequently arise prior to the appearance of adverse clinical signs (Underwood, 1979).

Ideally, animal scientists would like to determine the mineral status of an animal by measuring the mineral content of one tissue that is readily available from a live animal. Unfortunately, no one tissue or fluid will portray the status of all minerals. Blood, urine, saliva, milk, feces, and hair may be easily sampled, and even liver and bone may be routinely biopsied with a minimum of time and danger to the animal. Liver take neither by biopsy from sacrificed animals is an excellent indicator of the status of certain trace elements as an example, Cu<sup>2+</sup> liver concentrations of less than 25 ppm coincide with Cu<sup>2+</sup> deficiency signs in cattle (Judson and McFarlane, 1998). However, bone is the preferred tissue for evaluating bone-forming minerals, particularly Ca<sup>2+</sup> and P (Little 1984). Other non-chemical techniques for evaluating the mineral status of bone minerals include bone breaking strengths, ultrasound procedures and photon absorption (Williams *et al.*, 1985).

The organ, tissue, or fluid chosen for analysis varies with the element, but estimations of whole blood, plasma or serum trace element, or enzyme concentrations have wide applicability and do not, of course, require sacrifice of the animal. The levels of certain mineral elements in hair or wool, urine, and even milk are also of value in the detection of deficiency or toxicity states, although individual variability can be very high and external contamination provides problems for trace element status evaluation (Szabo *et al.*, 1999).

Whole blood or blood serum or plasma is widely used for studies in mineral nutrition. Blood is widely employed for studies in mineral nutrition because it invariably reflects in some aspects of its composition, and the mineral status of the animal. Blood can be obtained easily and frequently without harm to or death of the animal. Values significantly and consistently above or below "normal" concentrations or ranges provide suggestive but no conclusive evidence of a dietary excess or deficiency of particular minerals (McDowell, 1985; Wildeus, 1992). Due to homeostasis mechanisms, some minerals will often remain at normal concentrations (*i.e.*, Ca<sup>2+</sup>) even though the animal is deficient. Low Cu<sup>2+</sup> status in cattle can be established by both liver and blood concentrations in cattle (Binnerts, 1986). However, with a normal blood Cu<sup>2+</sup> level, the serum does not indicate the size of the Cu<sup>2+</sup> reserve, while liver not only allows evaluation of the actual situation but also show reserve and overall likelihood of future deficiency. Most mineral blood concentrations are very tightly controlled by regulatory process resulting in a mineral concentration changes over a wide dietary input range. This minimize their usefulness in diagnostic evaluations. Blood analyses are not only part of the diagnostic process and should not be the sole bases for nutritional management decisions.

Due to the many factors that cause variation in mineral content of hair, hair analyses are not likely to be precise indicators of the mineral status of animals. While concentrations of Ca<sup>2+</sup>, P and Cu<sup>2+</sup> in hair are not affected by dietary intake, Zn<sup>2+</sup> and Se<sup>2+</sup> contents of hair may reflect dietary intake as well as toxic consumptions of Pb, As, and possibly Cd (Combs *et al.*, 1982). Urinary and fecal levels for some minerals provide a means of status

assessment as higher excretion rates reflect dietary adequacy (Ternouth, 1990). Urine and fecal P excretion was significantly higher in P supplement than in control cattle (Call *et al.*, 1978). Determination of urine Mg is one of the better methods of evaluation of this mineral (NCMN 1973). Cohen (1974) and Caple and Halpin (1985) reported that fecal P was linearly related to dietary P levels and thus can be used to predict dietary P intake. A disadvantage of fecal P is that for mature tropical forages, a large portion of P in the faeces represents the undigested mineral and excretion is not reflected by metabolic need. Random urine samples are strongly affected by current dietary intake and also suffer from dilution effects. For grazing ruminants it would be impractical to collect 24-h urine samples.

Because of its rapid reaction to deficiency long before clinical signs appear, the best criterion for assessment of Na<sup>+</sup> status is the concentration of Na<sup>+</sup> and K<sup>+</sup> in saliva (Morris *et al.*, 1980). Deficiency causes a fall in Na<sup>+</sup> and a rise in K<sup>+</sup>. It has been suggested the normal Na:K ratio in saliva to be from 17:1 to 25:1 and suggested that if it is between 10:1 and 15:1, Na deficiency can be suspected (Ortolani, 1997).

Evaluation of mineral status on the basis of tissue analysis would seem to be the ultimate solution. However, as with all techniques, limitations exist. Some disadvantages of tissue and fluid analysis include: (1) the wide variability or tissue mineral concentrations among animals consuming the same diets, (2) trace mineral contamination or other factors (*i.e.*, blood hemolysis) encountered during field collections, (3) the need for selecting the appropriate animal class that is most suitable to indicate status, (4) uncertainty of correct critical level and proper interpretation of results, (5) more complication in method of collection, preparation, analysis and storage of samples, and (6) often the need for animal case histories to substantiate analytical mineral levels.

Tissue and fluid mineral concentrations are excellent indicators of mineral status for certain minerals but less valuable for others. For some trace minerals such as Se<sup>2+</sup> and Cu<sup>2+</sup>, a wide range of tissues and/or fluids (*i.e.*, blood, liver, kidney, enzymes, etc.) accurately reflect the animal status, as a deficiency significantly reduces concentrations of these elements. However, for Zn<sup>2+</sup>, and Mn<sup>2+</sup>, tissues are less sensitive to dietary change and thus status evaluation is more difficult.

Mineral deficiencies likely to affect production of grazing livestock at pasture include those of the major elements Ca, P, Mg, Na and S and the trace elements Co, Cu, I, Mn<sup>2+</sup>, Se<sup>2+</sup> and Zn<sup>2+</sup> (Little 1982; Judson *et al.*, 1987). Excessive intakes of minerals can also have an adverse effect on animal health, the more commonly encountered problems have been associated with excessive intakes of the minerals Cu<sup>2+</sup>, Mo, Fe<sup>2+</sup>, S, Na<sup>+</sup>, K<sup>+</sup> and fluorine (F).

The health and degree of productivity of livestock are dependent on balanced and adequate quantities of all necessary nutrients to meet their requirements for a given physiological stage. For grazing ruminant livestock which obtain all or most of the nutrients required from forages, feed, and water, a knowledge of nutrient composition of such dietary factors is, therefore, essential. The adequate supplemental feeding of minerals which are frequently deficient in forages can improve livestock production and result in a favourable cost-to-benefit ratio. Through a systematic mapping technique which involves collection of soil, forage, feed, water, and animal tissue fluids for analysis, it is possible to determine appropriate mineral supplement formulas for various grazing livestock regions.

This research work therefore was carried out for two years with the following objectives:

- To evaluate mineral status of soil, forage, along with water, and feed supplement consumption at the goat farm
- To appraise the mineral concentration of blood plasma, milk, urine, and faeces of goats grazing in the same pasture
- To relate, soil, forage, water, and feed sources of minerals to the requirements of animals, and recommend the best supplementation program for each zone of the farm, to promote efficient and profitable goat production
- To assess the incidence of deficiencies and toxicities of minerals in animals and to compare the mineral status of different classes of goats grazing in the pasture
- To investigate the effect of seasons and sampling periods (fortnights) on the bioavailability of minerals to animals
- To have the knowledge for the provision of essential mineral fortification for particular class of animal species, level of production, and for the correct season of the year to complement the available minerals in forage and concentrates

## MATERIALS AND METHODS

This study was conducted, during 2000-2002, using a herd of Darah Din Pannah breed of goats, grazing pastures on two different ranches at the Livestock Experimental Station Rakh Khairwala, District Layyah, in southern Punjab, Pakistan. The farm was established about 40 years ago and consisted of 14,000 hectares land and close to 7000 animals. The climate is sub-tropical, semi-arid continental characterized by two distinct seasons, winter and summer. Samples from those animals were collected which had been in the pastures for not less than 1-2 years prior to sample collection. All the animals at the farm had access to feed containing mixture of different minerals, in addition to grazing the improved varieties of forages throughout the year.

For sampling purpose, 30 animals were grouped into 3 classes, according to age, physiological status and gender, with 10 animals per class as follows: Class 1 contained 10 lactating goats, class 2 comprised 10 non-lactating goats, and class 3 consisted of 10 male goats. These animals were ear tagged at each ranch.

Samples of soil, forage, feeds, water, and animal blood plasma, milk, faeces, and urine (urine only from female animals) were collected at two different ranches of the farm fortnightly in each season. Sampling periods were January, February and June, July, corresponding to the winter and summer seasons. The mean temperature of the year ranged from 25-28°C and the average relative humidity was 25-45%.

Samples of forage were collected from those species that were most frequently grazed by goats at this ranch. The forage species collected were: *Medicago sativa*, *Avena sativa*, *Trifolium alexandrium*, *Hordeum vulgare*, *Cichorium intybus*, *Lathyrus odoratus*, *Chenopodium morale* during winter, and *Cyperus rotundus*, *Tribulus terrestris*, *Pennisetum glaucum*, *Cynodon dactylon*, *Digitaria decumbens*, *Cynodon plectostachyum*, *Panicum milliaceum*, *Sorghum bicolor*, *Setaria italica* during summer. As mineral status of soil differed from place to place, therefore soil and corresponding forage samples were collected at three different places with 5 replications from each place. All the samples were analyzed for calcium and magnesium. Soil, forage, and plasma minerals concentrations were compared to established critical values to determine the various categories of deficient levels. The critical level for soils indicates the element concentration below which normal growth and / or mineral composition of forage may be adversely affected. For forage samples, it indicates the lowest requirement of the element or organic constituent to avoid deficiency symptoms in animals. Plasma critical levels indicate the concentration below which specific signs of deficiency may occur. Interpretation of these critical values was done with caution taking into consideration the management, nutritional, environmental and individual factors that affect the availability, supply and utilization of each nutrient.

### Collection of samples

#### Soil

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

#### Forage

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

#### Water

Water samples were collected from the particular channel or site from where it is being supplied to the animals at farm. These samples were stored in plastic bottles till further analysis. One drop of 0.1% of sodium hexametaphosphate ( $\text{Na PO}_3$ )<sub>6</sub> per 25 ml was added in each bottle to check the precipitation of salt during storage.

#### Feed

Feed samples were picked up from the feed that is being offered to animals. Feed samples were dried at 60°C for six hours, while mixing the samples regularly. The samples were preserved in polyethylene bags for the analyses of macro-and micro-nutrients.

#### Blood

Blood samples were taken from male and female goats that were offered feed using the same ingredients being raised at the farm. Blood disposable needles for goats were used to collect the blood from the jugular vein. Blood from each animal was taken in a standing position by holding the animal in between knees. Hair or wool over the site of jugular vein was sheared. The jugular veins were raised, while pressing the posterior side of the neck with thumb and afterward, needles were inserted into the vein. Twenty ml of the blood were drawn into a clean sterile test tube having anticoagulant (EDTA). The blood samples were centrifuged at 3000 rpm for 20 minutes to separate the plasma. The plasma samples were stored at -20°C till further analysis.

**Milk**

The milk samples were collected from goats by hand milking. First 100 ml milk were cleared and discarded. Then 20 ml of milk samples were collected in test tube containing 100 mg of sodium azide as preservative. Samples were frozen at  $-20^{\circ}\text{C}$  until analyzed.

**Urine**

After cleaning and thorough washing the external genitalia of the animals with toilet soap and luke-warm water, catheters were fixed in urethra of female animal only, the urine was collected into clean glass beakers or bags. The urine samples thus collected, were kept at  $-20^{\circ}\text{C}$  for further investigation.

**Faeces**

Fecal samples from each animal were collected manually from the rectum of the animals and put in small plastic bags, oven-dried at  $55^{\circ}\text{C}$  for 96 h and were stored in polyethylene bags for further analysis.

**Sample preparation****Soil**

Minerals were extracted from soil using the Mehlich-1 extracting solution method ( $0.05\text{ N HCl} + 0.025\text{ N H}_2\text{SO}_4$ ) following Rhue and Kidder (1983). Ten grams of air-dried soil were taken in 125 ml conical flask and 40 ml Mehlich-1 extracting solution was added to it and shaken for 15 minutes on a reciprocating shaker, filtered through a medium porosity filter paper (Whatman filter paper No.2). Clear supernatant was obtained by centrifugating for 5 minutes at 180 rpm. The supernatant was stored in plastic bottles for macro-and micro-nutrients determination.

**Forage**

One gram of the dried forage sample was taken in a 50 ml conical flask, and kept overnight after adding 5 ml concentrated  $\text{HNO}_3$  and 5 ml perchloric acid ( $\text{HClO}_4$ ). Next day, again 5 ml  $\text{HNO}_3$  was added to each sample. All the samples were digested on hot plate at  $250^{\circ}\text{C}$  in fuming hood till the material was clear. After digestion the material was cooled down and the volume was made up to 50 ml with double distilled water and stored in clean airtight bottles for analysis of metal ions (AOAC, 1990).

**Plasma**

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

**Urine and water**

After thawing the urine and water samples at room temperature, they were filtered through Whatman filter paper No. 42 into sterilized plastic bottles. For the analyses of macro-elements such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , 1-ml aliquots were used to prepare serial dilutions. While for micro-elements determination water and urine samples were processed according to methods described by Fick *et al.* (1979). For this purpose 100 ml of each urine and water samples were measured in a graduated cylinder, poured into 100ml crucibles, evaporated to dryness on a hot plate in a fuming hood. The final volume of water samples was made up to 10 ml by rinsing and washing the crucibles. Urine samples were completely ashed in a muffle furnace followed by acid  $\text{HNO}_3$  digestion to prepare ash solution. These samples were filtered through Whatman filter paper No. 42 and made the volume up to 10ml in a volumetric flask making it 10 times more concentrated than the original samples and stored for further analysis.

**Milk**

Fat free milk was obtained by centrifugation of whole milk at 3000 rpm in a refrigerated centrifuge at  $4^{\circ}\text{C}$  for 10 minutes. Ten (10) ml defatted milk samples were treated with 4 ml mixture of perchloric acid and nitric acid (1:1) and final volume was made up to 25 ml with distilled water and stored in capped plastic vials at  $4^{\circ}\text{C}$  for analysis (Knowles *et al.*, 1999; Stelwagen *et al.*, 1999).

**Feed and faeces**

One gram of air-and oven-dried samples were put into crucibles and these were placed in muffle furnace. Temperature was raised slowly until maximum temperature of  $550^{\circ}\text{C}$  reached. This temperature was maintained for 8 hours. After removing from the furnace, crucibles were cooled in a desiccator upto  $200^{\circ}\text{C}$  by placing for 2 hours.

These crucibles were placed on hot plate in a fuming hood for acid digestion. The ash was wetted with a few drops of deionized water. 5ml 50% HNO<sub>3</sub> was added to the sample and the solution was evaporated to about half of the volume and then 10% HNO<sub>3</sub> was added to <sup>2</sup>/<sub>3</sub> volume of the crucible. Then the solution was evaporated to about 10 ml and deionized H<sub>2</sub>O was added to <sup>2</sup>/<sub>3</sub> the crucible volume. The solution was then heated to evaporate to about 5 ml. It was filtered through Whatman filter paper No. 42 and the final volume was made upto required level after rinsing the funnel, crucibles, and filter paper with deionized water. The samples so prepared were stored in plastic bottles for analysis (Fick *et al.*, 1979).

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

The above samples were diluted as required, and analyzed for Ca<sup>2+</sup> and Mg<sup>2+</sup> concentrations. An aliquot of above samples was used for determination of Ca<sup>2+</sup> and Mg<sup>2+</sup> using an atomic absorption spectrophotometer (Py Unicam Ltd. York street, Cambridge UK). To ensure the quality of the analysis, a certified standard was analyzed after every six samples. The samples were diluted as required and concentrations of elements were measured. The final quantities were computed by comparison of sample reading with standard curves.

#### **Statistical Analysis**

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

### **RESULTS AND DISCUSSION**

Samples of soil, forages, water, and feed were taken fortnightly in two different seasons during the year 2001. In addition, samples of blood plasma, faeces, urine, and milk were collected from lactating goats, those of plasma, faeces, and urine from non-lactating animals, and only plasma and faeces from male animals.

All the samples were analyzed to determine mineral composition by appropriate techniques.

#### **Calcium**

##### **Pasture samples**

##### **Soil**

Analysis of variance of data for soil Ca<sup>2+</sup> concentration showed non-significant effect of seasons but significant of fortnights (Table-1). In winter, the Ca<sup>2+</sup> level increased consistently from 1<sup>st</sup> to 3<sup>rd</sup> fortnight but at the 4<sup>th</sup> fortnight the Ca<sup>2+</sup> content was the same as that at the 1<sup>st</sup> fortnight. In contrast, in summer, the soil Ca<sup>2+</sup> was higher at the 1<sup>st</sup> and 3<sup>rd</sup> fortnights than that at the other fortnights. The overall seasonal difference with respect to soil Ca<sup>2+</sup> was not possible to discern from the data reported here (Fig.1a).

##### **Forage plants**

There was a significant effect of seasons and fortnights on forage Ca<sup>2+</sup> level (Table.1). In winter, the highest forage Ca<sup>2+</sup> was found at the first fortnight, whereas at the remaining three fortnights forage Ca<sup>2+</sup> remained almost uniform. Forage Ca<sup>2+</sup> concentration during summer did not vary significantly at different intervals (Fig.1b). Generally, high level of forage Ca<sup>2+</sup> was found during winter as compared to that in summer.

##### **Water**

No seasonal or fortnight influence on water Ca<sup>2+</sup> level was found (Table.1). However, the water Ca<sup>2+</sup> level was slightly higher in winter than that in summer during the first two fortnights whereas at the subsequent two fortnights no seasonal difference was found with respect to water Ca<sup>2+</sup>. During summer, there was a consistent increase in water Ca<sup>2+</sup> concentration with time from the 2<sup>nd</sup> fortnight (Fig.1c).

##### **Feed**

Non-significant seasonal and significant effects of sampling intervals were found on feed Ca<sup>2+</sup> concentration (Table.1). During both seasons the level of Ca<sup>2+</sup> decreased consistently with time (Fig-1d). Generally, feed Ca<sup>2+</sup> level was higher in summer than that in winter.

Table 1. Analysis of variance of data for Ca<sup>2+</sup> concentration in soil, forage plants, water and feed at different fortnights during winter and summer seasons at goat ranch.

Source of Variation S.O.V.	degree of freedom df	Soil	Mean squares Forage plants	Water	Feed
Season (S)	1	308.03ns	1312170250.00***	34.23ns	86211.23
Error	8	2961.14	1603125.00	94.11	58252.55
Fortnight (FN)	3	51998.49***	30781583.33***	27.03ns	35116.63***
S x FN	3	31057.69***	19828250.00***	11.16ns	1554.23ns
Error	24	2515.07	790125.00	30.45	2053.22

\*\*, \*\*\* = Significant at 0.01 and 0.001 levels, respectively.

Table 2(a). Analysis of variance of data for Ca<sup>2+</sup> concentration in blood plasma, faeces, urine and milk of lactating goats at different fortnights during winter and summer seasons at goat ranch.

Source of Variation S.O.V.	degree of freedom df	Plasma	Mean squares Faeces	Urine	Milk
Season (S)	1	29.76ns	4588820.00*	12276.01*	1346805.00***
Error	18	236.88	675240.28	1592.29	15817.50
Fortnight (FN)	3	429.51***	1161588.33***	6.95ns	5820.00ns
S x FN	3	5.76ns	1516743.33***	2249.35***	15578.33***
Error	54	17.00	161546.76	16.76	2259.35

Table 2(b). Analysis of variance of data for Ca<sup>2+</sup> concentration in blood plasma, faeces, and urine of non-lactating goats and that of plasms and faeces of male goats at different fortnights during winter and summer seasons.

Source of Variation S.O.V.	degree of freedom df	Non-lactating goats Plasma	Mean squares Faeces	Male goats Urine	Plasma	Faeces
Season (S)	1	18.51ns	122617.80ns	1540.01ns	107.18ns	98816796.80***
Error	18	460.08	5986197.91	1349	468.36	3331613.70
Fortnight (FN)	3	38.99***	20070.97ns	8.55ns	105.44***	3800381.35***
S x FN	3	42.50***	5780551.80***	108.55***	175.26***	193632.43ns
Error	54	5.06	164164.28	8.04	11.19	90056.76

\*, \*\* = Significant at 0.05 and 0.001 levels, respectively; ns = non-significant.



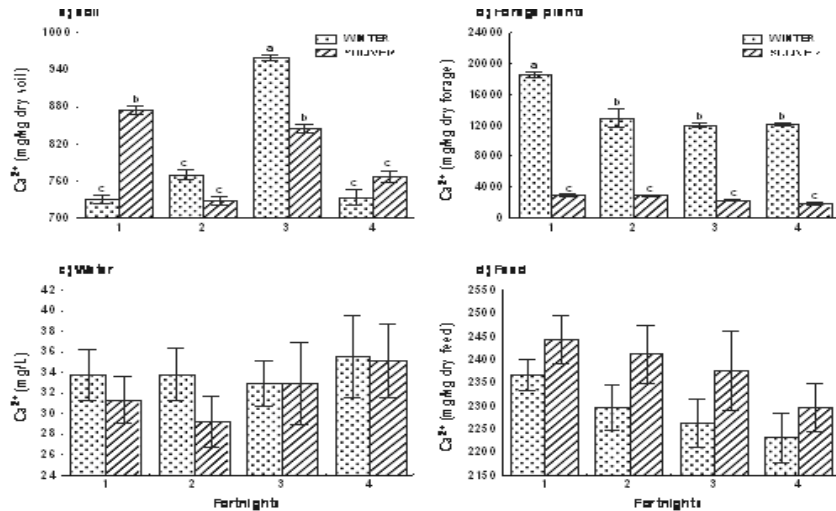


Fig. 1. Ca<sup>2+</sup> concentration in (a) soil, (b) forage plants, (c) water and (d) feed at different fortnights during winter and summer seasons at goat ranch. (Means with the same letters do not differ significantly at P≤0.05).

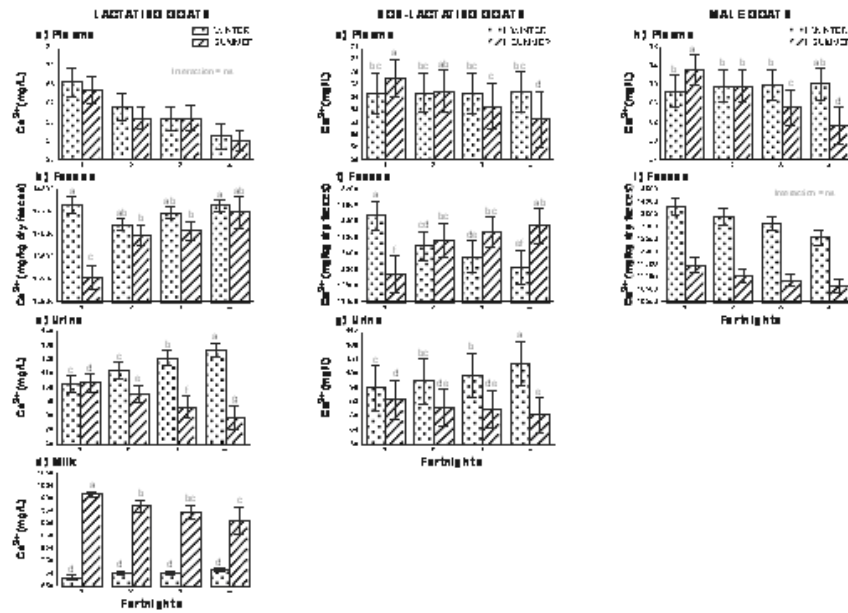


Fig. 2. Ca<sup>2+</sup> concentration in different sample types of lactating, non-lactating and male goats at different fortnights during winter and summer seasons. (Means with the same letters do not differ significantly at P≤0.05).

Table 3. Analysis of variance of data for Mg<sup>2+</sup> concentration in soil, forage plants, water and feed at different fortnights during winter and summer seasons at goat ranch.

Source of Variation	degree of freedom	Soil	Mean squares Forage plants	Water	Feed
S.O.V.	df				
Season (S)	1	20884.90**	34596000.00***	78.40ns	50410.00ns
Error	8	350.20	165625.00	84.59	17586.64
Fortnight (FN)	3	17092.57***	380333.33**	52.43ns	44072.47**
S x FN	3	3740.83***	312666.67*	66.07ns	9368.20ns
Error	24	458.95	74625.00	36.11	5868.94

\*\* , \*\*\* = Significant at 0.01 and 0.001 levels, respectively.

Table 4(a). Analysis of variance of data for  $Mg^{2+}$  concentration in blood plasma, faeces, urine and milk of lactating goats at different fortnights during winter and summer seasons at goat ranch.

Source of Variation	degree of freedom	Plasma	Faeces	Urine	Milk
Season (S)	1	3.50ns	3162908.1ns	32361.01ns	819.20ns
Error	18	34.49	8802791.78	16654.86	520.35
Fortnight (FN)	3	10.24**	582457.71***	1778.11**	202.22ns
S x FN	3	7.17*	26388.08ns	544.91ns	225.50ns
Error	54	1.80	37670.09	422.57	129.57

Table 4(b). Analysis of variance of data for  $Mg^{2+}$  concentration in blood plasma, faeces, and urine of non-lactating goats and that of plasma and faeces of male goats at different fortnights during winter and summer seasons.

Source of Variation	degree of freedom	Non-lactating goats	Urine	Male goats	
S.O.V	df	Plasma	Faeces	Plasma	
Season (S)	1	18.38ns	1723725.61ns	13702.61ns	25.55ns
Error	18	35.04	1036013.67	51970.77	91.03
Fortnight (FN)	3	0.85*	19069.71**	11312.51**	1.86ns
S x FN	3	1.16**	35170.18***	1386.71	61983.77ns
Error	54	0.24	4036.71	2333.74	2.31

\*, \*\* = Significant at 0.05 and 0.001 levels, respectively; ns = non-significant.

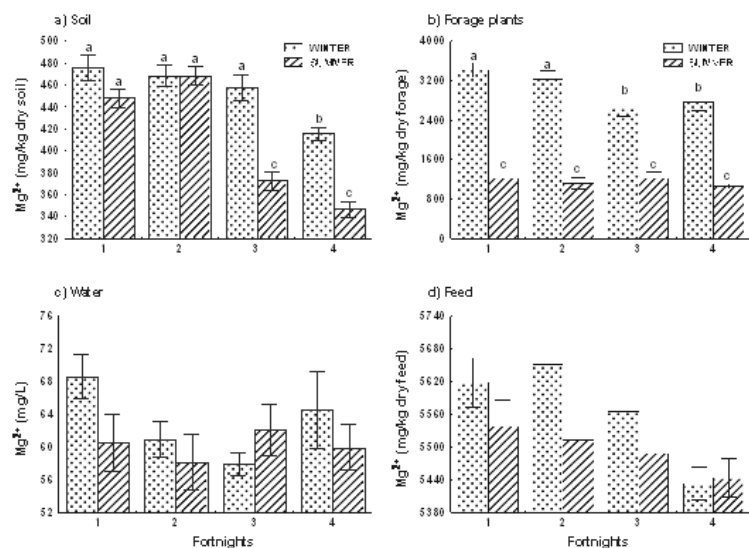


Fig. 3.  $Mg^{2+}$  concentration in (a) soil, (b) forage plants, (c) water and (d) feed at different fortnights during winter and summer seasons at goat ranch. (Means with the same letters do not differ significantly at  $P \leq 0.05$ ).

## Animal samples

### Lactating goats

#### Plasma

Analysis of variance of data showed non-significant effects of seasons but significant of fortnights on plasma  $Ca^{2+}$  concentration (Table-2a). During both seasons, there was a consistent decrease in  $Ca^{2+}$  level from 1<sup>st</sup> to last fortnight (Fig-2a).

#### Faeces

There was a significant effect of seasons and fortnights on fecal  $Ca^{2+}$  level (Table.2a). Fecal  $Ca^{2+}$  level was significantly higher in winter than that in summer. During winter, forage  $Ca^{2+}$  level remained almost unaffected with time, whereas during summer, it increased consistently with time (Fig.2b).

#### Urine

There was a significant seasonal effect on urine  $Ca^{2+}$ , but the sampling intervals had no significant effect (Table.2a). Urine  $Ca^{2+}$  level increased consistently with time during winter, whereas the reverse was true during summer. Generally urine  $Ca^{2+}$  level was higher in winter than that in summer (Fig.2c).

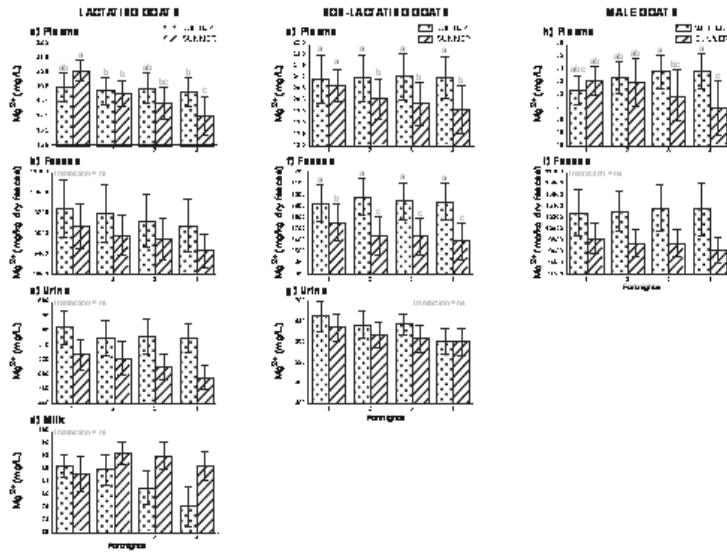


Fig.4.  $Mg^{2+}$  concentration in different sample types of lactating, non-lactating and male goats at different fortnights during winter and summer seasons.

(Means with the same letters do not differ significantly at  $P \leq 0.05$ ).

### Milk

Significant seasonal effect and non-significant of fortnights was observed on milk  $Ca^{2+}$  concentration (Table. 2a). During winter  $Ca^{2+}$  in milk remained unchanged with time, whereas a consistent decrease in milk  $Ca^{2+}$  was found with time during summer (Fig.2d). Overall, milk during summer contained higher  $Ca^{2+}$  than that in winter.

### Non-lactating goats

#### Plasma

A marked sampling period effect was observed on plasma  $Ca^{2+}$  level, but seasons had no effect in changing  $Ca^{2+}$  level in plasma (Table.2b). Plasma  $Ca^{2+}$  remained unchanged with time during winter. In contrast, during the summer, a consistent decrease in plasma  $Ca^{2+}$  was found with time (Fig.2e).

#### Faeces

Effect of seasons or fortnights on fecal  $Ca^{2+}$  was found to be non-significant (Table.2b). A consistent decrease in fecal  $Ca^{2+}$  with time was found during winter, Whereas, during summer it had an increasing trend with time (Fig.2f).

#### Urine

No seasonal or fortnight effect on urine  $Ca^{2+}$  was found (Table.2b). During winter, urine  $Ca^{2+}$  level increased with time, whereas during summer the reverse was true. Excretion of  $Ca^{2+}$  through urine was higher in winter than that in summer (Fig.2g).

### Male goats

#### Plasma

There was a significant effect of fortnights on plasma  $Ca^{2+}$ , but seasons had no effect in changing its level (Table.2b). During winter, plasma  $Ca^{2+}$  did not vary with time, whereas during summer a consistent decrease in plasma  $Ca^{2+}$  was found with time (Fig.2h).

#### Faeces

Both seasons and fortnights had a significant influence on faecal  $Ca^{2+}$  level (Table.2b). However, the excretion of  $Ca^{2+}$  through faeces was higher in winter than that in summer. A consistent decrease in  $Ca^{2+}$  level was observed up to the last fortnight during both seasons (Fig.2i).

Adequate  $Ca^{2+}$  nutrition depends not only on sufficient total dietary supplies, but also on the chemical forms in which it occurs in the diet and on the vitamin D status of the animal. The dietary Ca: P ratio can also be important. Actually, ruminants can tolerate a wider range of Ca: P ratio particularly when tissue vitamin D status is high. With excess amounts of Ca or P in the diet, the availability of certain trace elements may be decreased.  $Ca^{2+}$  of forages in comparison to requirements of various species are useful in determining the status of this element (McDowell, 1997).

In the present study soil  $Ca^{2+}$  content during both seasons remained unchanged. However, the soil  $Ca^{2+}$  level was above the critical level suggested by Breland (1976) for normal growth of plants. The results for soil  $Ca^{2+}$

reported here are similar to what have earlier been found by Espinoza *et al.* (1991) for soil  $\text{Ca}^{2+}$  content in central Florida and Cuesta *et al.* (1993) in north Florida, but were above the values reported by other workers (Salih *et al.*, 1988; Merkel *et al.*, 1990; Pastrana *et al.*, 1991; Rojas *et al.*, 1993; Tiffany *et al.*, 2000) and lower than that found by Tejada *et al.* (1987) and Pastrana *et al.* (1991).

$\text{Ca}^{2+}$  level in forage differed significantly in both seasons. In winter  $\text{Ca}^{2+}$  content was very high in forage plants than that in summer. However, the values in summer were not within the range of ruminant requirements both for growing and lactating animals (Reuter and Robinson, 1997). Similarly  $\text{Ca}^{2+}$  accumulation in forages above the critical values have earlier been found in a number of studies, *e.g.* (Espinoza *et al.*, 1991; Cuesta *et al.*, 1993; Tiffany *et al.*, 2000; Tejada *et al.*, 1987; Ogebe and Ayoade, 1995). Forage  $\text{Ca}^{2+}$  requirements of grazing ruminants is a subject of considerable debate as the requirement is influenced by the animal type and level of production, age, and weight (Tiffany *et al.*, 2000).

Feed  $\text{Ca}^{2+}$  content was below the range of the animal requirement during both seasons while water  $\text{Ca}^{2+}$  level seemed to be equally important with minor contribution in complementing the forage and feed  $\text{Ca}^{2+}$  concentration required by the ruminants. Naturally high salt concentration of drinking water decreases mineral supplementation intake.  $\text{Ca}^{2+}$  deficiency is rare in grazing animals with the exception of animals lactating large quantities of milk or those grazing on acid, sandy or organic soils in humid areas where the herbage consists mainly of quick-growing grasses devoid of legume species (Underwood, 1981). Although  $\text{Ca}^{2+}$  deficiency can be easily produced in young growing animals and lactating dairy animals fed native forage supplemented with concentrates, the deficiency is frequently reported in grazing beef cattle, even during lactation (McDowell and Valle, 2000).

Feed  $\text{Ca}^{2+}$  concentration was slightly higher in summer than that in winter, which was considered insufficient to satisfy the mineral requirements of the farm animals. There is a considerable difference in the availability of mineral element provided from different sources. A chemical analysis of a mineral element in feed or mineral mixture does not provide information on availability of an element for animals. The unbalanced mineral mixture which is extremely high in  $\text{Ca}^{2+}$  would likely be more detrimental to grazing animals than having no access to supplemental minerals, and actually contribute to a P deficiency (McDowell *et al.*, 1993)

In lactating animals, plasma  $\text{Ca}^{2+}$  concentration was not significantly different in both seasons. Plasma  $\text{Ca}^{2+}$  level during both seasons was found below the critical level (McDowell *et al.*, 1984). Although in sources, the  $\text{Ca}^{2+}$  level was sufficiently high to meet the requirements of ruminants, lower  $\text{Ca}^{2+}$  concentration in the plasma may have been due to greater excretion/lactation or interaction with other elements. In non-lactating animals, the plasma  $\text{Ca}^{2+}$  level was higher than that in lactating animals but no significant seasonal effect was found; it was below the critical level. In male goats, the plasma  $\text{Ca}^{2+}$  concentration was higher than that in lactating and non-lactating goats but lower than the critical level.

Plasma  $\text{Ca}^{2+}$  values were lower than those reported in the previous studies conducted in eastern Venezuela (Velasquez, 1979) and similar to those values reported for Guatemala (Knebusch *et al.*, 1988). However the homeostatic regulation of plasma  $\text{Ca}^{2+}$  by the parathyroid hormone, active form of vitamin D and thyrocalcitonin prevent wide fluctuation in blood  $\text{Ca}^{2+}$  concentration (McDowell, 1992).

High incidence of mean plasma  $\text{Ca}^{2+}$  below critical level indicated that goats have very severe  $\text{Ca}^{2+}$  deficiency during the summer and winter seasons. Animals have a hormonal mechanisms which maintain blood  $\text{Ca}^{2+}$  within narrow limit, by adjusting the proportion of dietary  $\text{Ca}^{2+}$  absorbed, and when dietary  $\text{Ca}^{2+}$  is inadequate, they meet their  $\text{Ca}^{2+}$  requirements by reabsorbing  $\text{Ca}^{2+}$  from body reserves in the skeleton (Rowlands, 1980). Black *et al.* (1973) reported that plasma/serum  $\text{Ca}^{2+}$  concentration may be directly affected by dietary  $\text{Ca}^{2+}$  intake. Steevens *et al.* (1971) found that blood plasma  $\text{Ca}^{2+}$  is more affected by amounts of P and Mg in the diet than by  $\text{Ca}^{2+}$  itself. Plasma  $\text{Ca}^{2+}$ , however, is influenced only by severe deficiency, and  $\text{Ca}^{2+}$  dietary level may be a more adequate criterion in assessing status of  $\text{Ca}^{2+}$  (CMN, 1973).

The phenomenon of low  $\text{Ca}^{2+}$  in plasma of lactating goats can be explained since milk contains large amount of  $\text{Ca}^{2+}$  and can be withdrawn from bones under the action of parathormone during the period of increased needs such as in early lactation (Bath *et al.*, 1978). Milk  $\text{Ca}^{2+}$  concentrations were lower than that reported by Underwood (1981). Mean milk  $\text{Ca}^{2+}$  concentration found in this study were nearly similar to those values reported in Florida (Cuesta *et al.*, 1993).

The vitamin D status of the animal and dietary Ca: P ratio can also be important. In animals, the  $\text{Ca}^{2+}$  homeostasis is maintained by its increased absorption in the duodenum by active and passive transport and excretion of excessive amount through faeces and urine to prevent from toxicity and other disorders in the animal body (Hegsted, 1973).

Faecal  $\text{Ca}^{2+}$  concentration in lactating goats was significantly higher in winter than that in summer. While in non-lactating animals, no significant difference was found in faecal  $\text{Ca}^{2+}$  level. In male goats, faecal  $\text{Ca}^{2+}$  level was higher in winter than that in summer. However,  $\text{Ca}^{2+}$  faecal excretion was higher in lactating followed by non-

lactating and male goats respectively. Faecal  $\text{Ca}^{2+}$  level in different groups of animals reflected the  $\text{Ca}^{2+}$  content of the sources used.

Urine  $\text{Ca}^{2+}$  concentration in lactating animals differed significantly during both seasons being higher in winter than that in summer. While in non-lactating animals, it did not vary significantly. However, it was slightly higher in winter than that in summer. Urine  $\text{Ca}^{2+}$  levels also showed dietary reflection. In case of lactating and male animals, there is an inverse relationship in plasma  $\text{Ca}^{2+}$  and excreta  $\text{Ca}^{2+}$ , while in non-lactating this trend was not followed. This may be due to any physiological factor. Clinical signs of borderline  $\text{Ca}^{2+}$  and P deficiencies are not easily distinguishable from other deficiencies. An inadequate intake of  $\text{Ca}^{2+}$  may cause weakened bones, slow growth, low milk production and tetany (convulsions) in severe deficiencies (McDowell, 1992).

From the results, it was concluded that forage during summer and feed during both seasons were marginally deficient while plasma of lactating and non-lactating goats was moderately deficient in  $\text{Ca}^{2+}$  during both seasons. In male goats, marginal deficiency of plasma  $\text{Ca}^{2+}$  during winter and moderate deficiency during summer was found. Therefore,  $\text{Ca}^{2+}$  status of goats in this region needs supplementation with  $\text{Ca}^{2+}$  containing supplements having maximum bioavailability during both seasons.

## Magnesium

### Pasture samples

#### Soil

Soil  $\text{Mg}^{2+}$  concentration was affected significantly both by seasons or fortnights of sampling (Table.3). The amount of  $\text{Mg}^{2+}$  in soil was higher in winter as compared to that in summer except at the 2<sup>nd</sup> fortnight. During both seasons there was a consistent decrease in soil  $\text{Mg}^{2+}$  from 2<sup>nd</sup> fortnight to onwards. (Fig.3a).

#### Forage plants

Significant seasonal or fortnight effects were observed on forage  $\text{Mg}^{2+}$  level (Table.3), and concentration of forage  $\text{Mg}^{2+}$  during winter was markedly higher than that during summer. During winter a decreasing trend in  $\text{Mg}^{2+}$  level was found with time but during summer no significant variation in forage  $\text{Mg}^{2+}$  was found at different time intervals (Fig.3b).

#### Water

Effects of seasons or fortnights on water  $\text{Mg}^{2+}$  were found to be non-significant (Table-3). The water  $\text{Mg}^{2+}$  was slightly higher in winter as compared to that in summer. During winter, a consistent decrease in water  $\text{Mg}^{2+}$  was found up to fortnight 3 and it then slightly increased at the last fortnight. In contrast, during summer, the variation in water  $\text{Mg}^{2+}$  at different fortnights was non consistent (Fig.3c).

#### Feed

A marked sampling interval effect was found on feed  $\text{Mg}^{2+}$  level but seasons had no effect in changing the feed  $\text{Mg}^{2+}$  level (Table-3). An increase in feed  $\text{Mg}^{2+}$  up to fortnight 2 and then a consistent decrease up to the last fortnight was found during winter. In contrast, during summer, a consistent pattern of decrease in feed  $\text{Mg}^{2+}$  was observed at different fortnights with time (Fig.3d).

## Animal samples

### Lactating goats

#### Plasma

The sampling period had a significant effect on plasma  $\text{Mg}^{2+}$  but there was no significant seasonal effect on it (Table-4.4a).  $\text{Mg}^{2+}$  concentration in plasma remained almost uniform at all sampling intervals during winter. On the other hand, during summer a consistent decrease in plasma  $\text{Mg}^{2+}$  level was found with time (Fig.4a).

#### Faeces

Non-significant seasonal but significant effect of fortnights was found on faecal  $\text{Mg}^{2+}$  (Table.4a). During both seasons faecal  $\text{Mg}^{2+}$  decreased progressively with time. Over all excretion of  $\text{Mg}^{2+}$  through faeces was higher in winter than that in summer (Fig.4b).

#### Urine

Urine  $\text{Mg}^{2+}$  was not affected by the seasons, whereas the sampling intervals had a significant effect on its concentration (Table.4a). A consistent decrease in  $\text{Mg}^{2+}$  level was observed with time during summer. Whereas, during winter the urine  $\text{Mg}^{2+}$  level remained almost unchanged throughout the whole season.  $\text{Mg}^{2+}$  excretion via urine was more during winter than that during summer (Fig.4c).

### Milk

Both seasons and fortnights had no significant effect in changing the milk  $Mg^{2+}$  concentration of goats (Table.4a). However, milk contained higher  $Mg^{2+}$  concentration during summer than that during winter (Fig.4d). A consistent decrease in milk  $Mg^{2+}$  was found in winter with time. During winter, a sharp decrease in milk  $Mg^{2+}$  was observed from fortnight 2 to onwards, whereas during summer no consistent pattern of increase or decrease in milk  $Mg^{2+}$  was observed with time.

### Non-lactating goats

#### Plasma

Plasma  $Mg^{2+}$  level did not vary with seasonal change, but fortnights had a significant effect on plasma  $Mg^{2+}$  (Table-4b). Plasma  $Mg^{2+}$  contents were found almost uniform at all fortnights during winter (Fig.4e), whereas during summer the plasma  $Mg^{2+}$  levels were uniform at the last three fortnights of sampling.

#### Faeces

Non-significant seasonal effects and significant of sampling intervals were observed on faecal  $Mg^{2+}$  concentration (Table-4b).  $Mg^{2+}$  level did not vary at all fortnights during winter, and at the last three fortnights during summer. The higher faecal  $Mg^{2+}$  was found at the first fortnight during summer (Fig.4f).

#### Urine

No seasonal but significant fortnight effect on urine  $Mg^{2+}$  level was observed (Table.4b). During winter,  $Mg^{2+}$  level remained almost unchanged at different fortnights except at the last fortnight where its level was greatly reduced. In contrast, during summer, a consistent decrease in  $Mg^{2+}$  level was found with time (Fig.4g).

### Male goats

#### Plasma

Both seasonal or sampling interval effects were non-significant on plasma  $Mg^{2+}$  level (Table.4b). During winter, a slight increase in plasma  $Mg^{2+}$  was observed up to the 3<sup>rd</sup> fortnight. Whereas during summer, the plasma  $Mg^{2+}$  decreased consistently with time (Fig.4h).

#### Faeces

Faecal  $Mg^{2+}$  levels were not affected both by the seasons or fortnights (Table.4b). However, concentration in faeces was markedly higher in winter than that in summer. The fecal  $Mg^{2+}$  level remained almost unaffected at all fortnights during both seasons (Fig.4i).

The practical important of  $Mg^{2+}$  is its relationship to the serious metabolic disorders grass tetany. Grass tetany is a complex ruminant metabolic disorder that is affected by forage species and mineral composition, soil properties, fertilizer practices, season of the year, temperature, animal species, breed and age (McDowell and Valle, 2000)

In the present study there was a significant seasonal effect on mean soil  $Mg^{2+}$  concentration so that  $Mg^{2+}$  content in soil was higher in winter than that in summer. These concentrations were above the requirement of plant growth (Rhue and Kidder, 1983). The soil  $Mg^{2+}$  concentration higher than that reported earlier in north Florida (Cuesta *et al.*, 1993; Tiffany *et al.*, 1998, 1999, 2000). Forage  $Mg^{2+}$  concentrations differed significantly during winter and summer. The high forage  $Mg^{2+}$  level in winter was above the requirement of ruminants suggested by National Research Council (1985), whereas in summer the forage  $Mg^{2+}$  was below the critical level. Similar low values in summer and adequate values for winter forage  $Mg^{2+}$  were reported by Salih *et al.* (1983) in Florida.

Feed and water  $Mg^{2+}$  concentrations showed no significant seasonal effect. Higher level of  $Mg^{2+}$  in feed and water was, however, found in winter. Feed  $Mg^{2+}$  was slightly above the requirement level of animals during both seasons of the year. Despite higher  $Mg^{2+}$  level in feed its biological availability is very low perhaps due to certain unknown inter- actions of various elements and conditions of rumen of the animals. According to Dua and Care (1995) the dietary  $Mg^{2+}$  availability to stock is markedly affected by other dietary components, especially  $K^+$ . High dietary levels of  $K^+$  and N will inhibit  $Mg^{2+}$  absorption from the rumen.  $Ca^{2+}$  and soluble carbohydrates may respectively increase and decrease dietary  $Mg^{2+}$  requirements of livestock, whereas raised dietary P levels appears to lower the requirements for both  $Ca^{2+}$  and  $Mg^{2+}$  (Judson and McFarlane, 1998). Mean plasma  $Mg^{2+}$  levels in lactating goats was not affected significantly during winter and summer. These levels were generally lower than the known critical level (McDowell *et al.*, 1983). In non-lactating goats the plasma  $Mg^{2+}$  levels were close to the critical level during both seasons with no seasonal effect. In contrast, in male goats the plasma  $Mg^{2+}$  levels were above the

critical level and higher than that in non-lactating and lactating goats. Seasonal influence in all cases was not prominent. The plasma  $Mg^{2+}$  levels of lactating goats were lower than those in non-lactating and male goats. This may have been due to large amount of  $Mg^{2+}$  secreted through milk in lactating goats. Mean plasma  $Mg^{2+}$  values were lower than those reported in previous studies conducted in Venezuela (Rojas *et al.*, 1993) and in Indonesia (Prabowo *et al.*, 1990). In view of Miller *et al.* (1972) plasma  $Mg^{2+}$  concentration is controlled to some extent by homeostatic mechanism. For lactating goats  $Mg^{2+}$  concentration was deficient during both seasons, although source of  $Mg^{2+}$  (forage, feed and water) had higher  $Mg^{2+}$  level above the requirements of ruminants. The depression in plasma  $Mg^{2+}$  may have been due to the fluctuations and other dietary interactions, or other factors affecting requirements (Hannam *et al.*, 1990; Shallow *et al.*, 1989; Baumgurtel and Judson, 1998).

Faecal  $Mg^{2+}$  levels in all classes of goat were higher, although not significant in winter than that in summer, which reflected the  $Mg^{2+}$  contents of the source used. Urine  $Mg^{2+}$  concentration in lactating and non-lactating goats was found to be higher in winter showing the dietary intake (Caple and Halpin, 1985).

Milk  $Mg^{2+}$  concentration of late lactation in summer was slightly higher than that in winter corresponding to early lactation. Milk  $Mg^{2+}$  values were below the critical level (Underwood, 1981), showing high absorption in this season. Similar low values of  $Mg^{2+}$  in milk of cattle were reported by Cuesta *et al.* (1993) and Merkel *et al.* (1992) in Florida.

In the present study it was found that  $Mg^{2+}$  availability was not within the range of ruminant requirements and more excretion through faeces and urine resulted in less availability or absorption of this element. As the rumen-reticulum is the major site of  $Mg^{2+}$  absorption in ruminants, with the large intestine playing a major role under some circumstances. In ruminants, the absorption rate of  $Mg^{2+}$  varies from 5 to 30%. Low absorption is caused by high K content (due to higher negative electrical potential in rumen fluid against blood), low fibre content in feed, and high pH in rumen fluid (due to intake of protein rich feeds) (Meyer, 1976). Young, lush green pastures would be detrimental to  $Mg^{2+}$  absorption by having high concentration of protein and  $K^+$  and being low in fibre, which would increase the passage rate and thereby reduce absorption.

In conclusion, forages tended to be deficient marginally in  $Mg^{2+}$  during summer and forage and feed  $Mg^{2+}$  levels were ineffective in raising the plasma  $Mg^{2+}$  levels in all classes of goats except in male animals during winter. The plasma  $Mg^{2+}$  levels were likely to be deficient marginally in lactating and non-lactating goats during winter and summer and in male animals only during winter. Therefore, supplementation is needed with mixture containing  $Mg^{2+}$  or by other safe and practical means of raising the  $Mg^{2+}$  intake of animals sufficient to maintain normal plasma value during both seasons and to prevent losses from lactation tetany.

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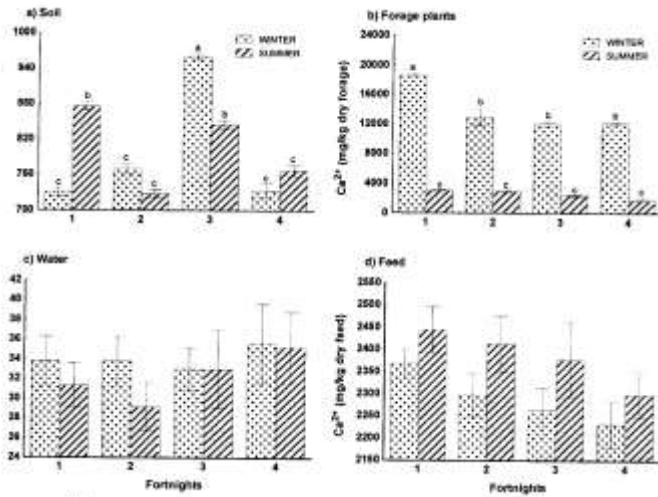


Fig. 1.  $\text{Ca}^{+2}$  concentration in (a) soil, (b) forage plants, (c) water and (d) feed at different fortnights during winter and summer seasons at goat ranch. (Means with the same letters do not differ significantly at  $P \leq 0.05$ ).

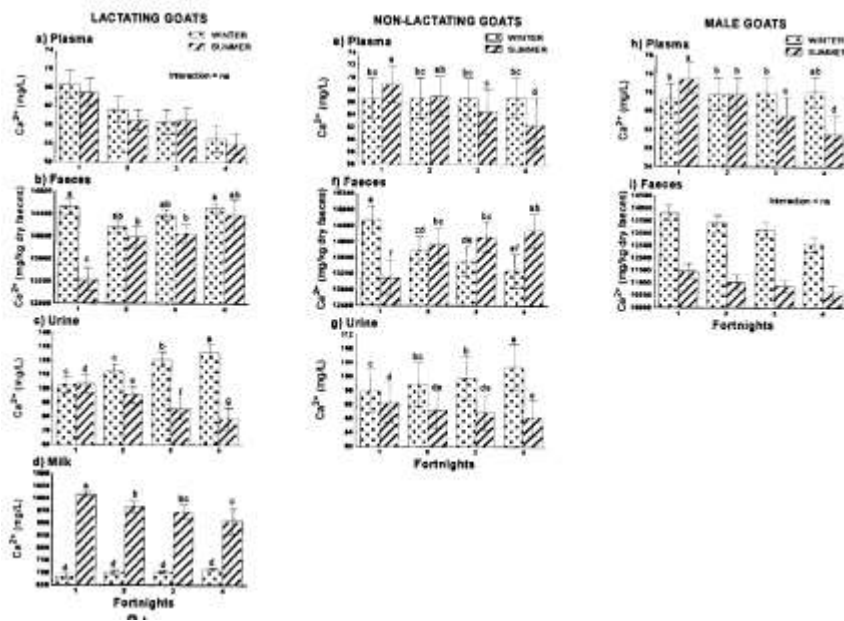


Fig. 2.  $\text{Ca}^{+2}$  concentration in different sample types of lactating, non-lactating and male goats at different fortnights during winter and summer seasons. (Means with the same letters do not differ significantly at  $P \leq 0.05$ ).

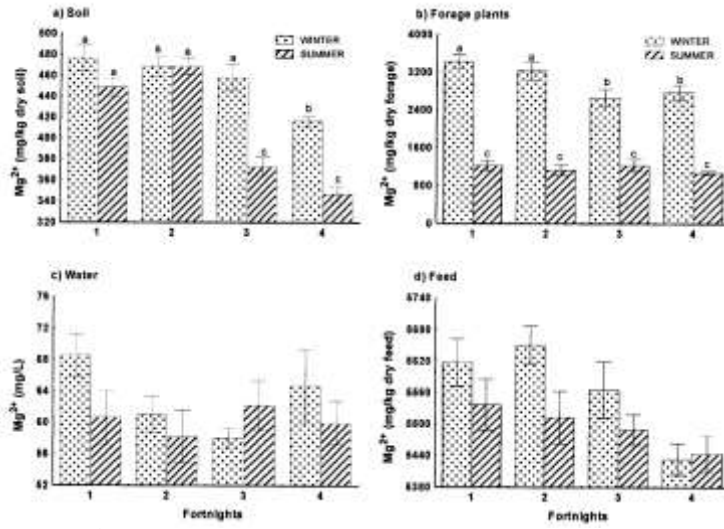


Fig. 3. Mg<sup>2+</sup> concentration in (a) soil, (b) forage plants, (c) water and (d) feed at different fortnights during winter and summer seasons at goat ranch. (Means with the same letters do not differ significantly at P<0.05).

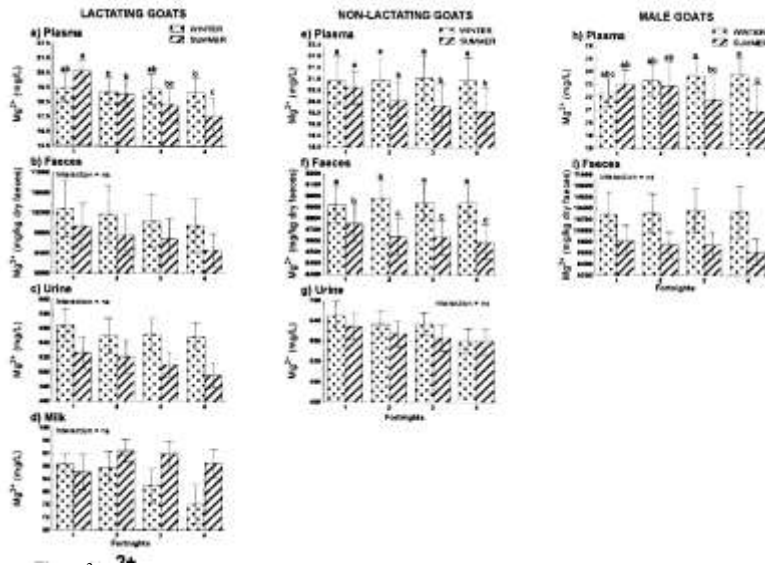


Fig 4. Mg<sup>2+</sup> concentration in different sample types of lactating, non-lactating and male goats at different fortnights during winter and summer seasons. (Means with the same letters do not differ significantly at P<0.05).