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Contribution of Soil, Forages, Water, and Feed Toward Meeting Cobalt Requirements of Grazing Goats During Different Seasons

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Abstract: A study was carried out to evaluate the cobalt status of grazing goats on the basis of cobalt concentration in soil, dietary sources, plasma, milk, faeces, and urine as affected by seasons and animal class. It was found that only forage had seasonal variation with grater concentration in winter than that in summer. Soil and forage Co concentration were inadequate for the normal requirements for plants and animals during both seasons. Positive association was found between plasma Co levels and sources of Co consumed by animals. Higher plasma Co concentration was found in male goats as compared to that in other groups while fecal Co concentration was higher in lactating goats that in other classes during both seasons. Although the Co concentrations in forage, the principal dietary factor were deficient, but the plasma Co levels in all classes of goats were sufficiently high showing the contribution of feed and water in complementing the forage Co required by animals for normal body function. The overall Co status of goats may be considered adequate mainly due to feed supplement, since forage Co concentration was low to deficient.

Key words: Goats, grazing, cobalt, status, plasma, milk, soil, forage and water pasture

Introduction

Mineral deficiencies or excesses in soils and forages have been associated with inefficient animal production including low productive rates among grazing ruminants (McDowell *et al.*, 1984 and 1985). Analysis of soils and forages for mineral composition is important for obtaining mineral status of an area with a view to providing mineral supplement to grazing animals. Adequate information on soil characteristics and mineral concentrations of forages, feed, and water in Pakistan is lacking despite the importance of this country to livestock production. Like other nutrients cobalt deficiencies occur most frequently in grazing ruminants and is widespread throughout large areas of the world (Ogebe and McDowell, 1998). For the grazing livestock in the Pakistan, the only source of nutrients for animals may be forages. Often forages do not contain all the nutrients needed by grazing livestock, including cobalt. Cobalt is an essential and plays an important role in the animal's body (McDowell, 1997).

It has been reported that soil cobalt (Co) content varies between 0.2 and 18 mg kg^{-1} DM, approximately 10 to164 times greater than herbage Co content (Fleming and Parle, 1987). Soils of low Co status occur where the parent material is formed from granite, sandstone, quartzite or peat land (Fleming and Parle, 1987; Mac Naeidhe and Parle, 1990). Animal deficiencies are likely on soils of total Co content <6mg kg^{-1} DM (Rogers and Gately, 1992) or extractable Co content <0.025 mg kg^{-1} DM or when Mn content exceeds 500 ppm DM (Rogers and Gately, 1992). Soil contamination greatly increases the Co concentration found on plant analysis, thus limiting the usefulness of plant Co deficiencies.

The desirable Co content in pasture for livestock is 0.11 mg kg^{-1} dry matter (SCA, 1990). Co is not particularly toxic and livestock has been shown to tolerate dietary levels of about 30mg kg^{-1} dry matter, although it is generally recommended that dietary level should not exceed 10mg kg^{-1} dry matter (NRC, 1980) Signs of toxicosis are similar to those seen with Co deficiency and include anaemia, reduced fed intake and body weight loss.

Cobalt toxicosis is less likely occurs than its deficiency and toxic level appears to be at least 300 times greater than the requirements in most species (NRC, 1980). Cases of toxicity are often the result of accidental, over-supplementation to prevent Co deficiency. Dietary concentrations of 10mg Co kg^{-1} are considered safe (Gupta, 1998).

There is a poor correlation between total soil Co concentration and its availability to plants. Soils with high level of manganese oxide minerals strongly bind free soil Co to these services, leading to low availability of Co to plant (McKenzie, 1967 and 1975). Concentrations of Co are usually lower in grasses than in other forages but these differences may be small on soils of low available Co (Minson, 1990). Increased Co uptake by pasture plants may occur on water logged soils (Adams and Honeysett, 1964).

Cobalt is essential to ruminants through its participation in the ruminal synthesis of vitamin B12. This metabolic process, unique to ruminants, allows us to virtually ignore the dietary supplementation of B-vitamins in animals. In fact, since cobalt in poorly stored in body tissues, cobalt status is commonly assessed via vitamin B12 concentrations, in addition to cobalt inself. Multiple cobalt sources are utilized in mineral formulations, including carbonate, chloride, and sulphate. Some signs of cobalt deficiency include, loss of appetite, leading to weight loss, listlessness, diarrhea, anemia (McDowell, 1997).

Cobalt deficiency occurs in large ares of many countries and is largely, but not exclusively, restricted to grazing ruminants which have little or no access to concentrates. With the exception of P and Cu, Co deficiency is the most severe mineral limitation to grazing livestock (McDowell *et al.*, 1982). Cobalt deficiency signs are not specific and it is often difficult to distinguish between a deficiency and malnutrition due to low intake of calories and protein. However, Co deficient animals respond quickly to treatment, recovering appetite, vigor and weight (McDowell *et al.*, 1984). Cobalt sub-deficiencies or borderline states are extremely common and are characterized by low production rates unaccompanied by clinical manifestation or visible signs, sub-deficiencies of Co often go unnoticed, thereby resulting in great economic losses to the ruminant livestock industry (Latteur, 1962; Nicol *et al.*, 1983 and Rosbrook *et al.*, 1992).

Clinical signs of variable severity of Co deficiency have been encountered in different countries (Judson and McFarlane, 1998; Shallow *et al.*, 1989). It has been stated that a pasture Co level of 0.11 mg kg^{-1} DM is required to maintained optimum vitamin B-12 status of livestock (Underwood and Suttle, 1999). Optimum status is desirable not only to meet the requirement, for growth and reproduction but also to ensure immune competency and provide adequate reserves to cover periods of transients deficiency (Judson *et al.*, 2002). Recent evidence with cattle fed semi-synthetic diets suggests that the Co requirement for maximum growth of young male animals was 0.12 mg kg^{-1} Co and that an adequate level based on biochemical indicators of vitamin B-12 status was 0.20 mg kg^{-1} (Strangl *et al.*, 2000). The higher requirement may be in part to due to the feeding of high energy diets reported to depress vitamin B12 production in the rumen and favour the synthesis of analogues (SCA, 1990).

In different regions of the world like other mineral cobalt is also most likely lacking (McDowell *et al.*, 1983 and 1984). Bray (1983) stated that Co is among those elements most likely to be deficient in location of progressive farming where food choice of grazing animals is restricted to few plants growing in that area. Seasonal incidence of Co deficiency in livestock is thought to be due to the dilution of Co in rapidly growing spring pasture and to the ingestion of soil Co on dry autumn pastures or on short winter pastures. Pasture analysis that includes an estimate of soil contamination can be used to monitor how seasonal conditions can affect Co uptake (Judson and McFarlane, 1998). Many areas relay on Co misting of pasture as a means of preventing vitamin B-12 deficiency in livestock, but more importantly for the prevention of phalaris staggers. Al present, the rule of thumb in the basis to such treatment rather than measurement, which is now possible with recent development in analytical services. Soil, forage, and animal tissue fluids have been reported to be deficient limiting the livestock production in many regions of the world.

Gartenberg (1989 and 1990) while studying the mineral status of ruminant in different livestock producing regions found that cobalt was most likely mineral along with other minerals to limit livestock production in certain regions. Velasquez-Pereira *et al.* (1997) reported that soils, forages, and animal tissues were deficient in Co along with other minerals. The Co concentration in pastures was found to be affected by seasons of the year, with concentration higher in dry seasons. The stage of maturity did not affect the concentration of this element in plants. The information in relation to geographical zones indicates that Co is low in different zones of different regions and would therefore, be recommended for supplementation.

Co deficiency in livestock shows a marked year to year and seasonal variation (Lee, 1951). Annual fluctuations in Co availability have also been reported by Gardiner (1977) who noted an increased incidence of Co deficiency in livestock in seasons favouring lush pasture growth. This may be due to in part to less soil ingestion by livestock. Adventitious can be a significant, if not a major, source of Co ingested by grazing animal.

Diagnostic testing with plants Co has been used with some success. Ozanne *et al.* (1963) reported that critical Co concentration for some forages in pasture was 0.04 mg kg^{-1} dry matter. Grasses or not responsive to Co applications and hence are not appropriate indicators species. However, reliance on a critical concentration for Co in forages is of limited value since Co responses in these forages based pastures commonly only occur if nitrogen supply is deficient. It is preferable to test the animals for Co or vitamin B-12 adequacy than to predict adequacy from soil or plants Co assays. Selective grazing and the ingestion of soil limit the usefulness of pasture testing to predict the Co needs of the animal. Liver is the preferred tissue for testing since the vitamin is stored

in this organ. However blood and other animal fluid tests for vitamin B-12 and Co itself are usually conducted because of the ease of sampling (Judson *et al.*, 1995). In some animals, vitamin B-12 and Co concentrations in blood plasma are of value in assessing the adequacy of dietary Co but low concentration in particularly in cattle do not exclude the possibility that cattle are on a low but adequate Co diet (SAC, 1990). Because of the variability in plasma Co and vitamin B-12 concentrations between animals in the same group, it is usually therefore at least 10 animals from the suspect group may be sampled for assessment purposes (Clark and Ellison, 1993).

Like other minerals deficiency, Co affect reproduction of grazing animal at pasture, and excessive intakes of this element can also have an adverse effect on animal health, but more commonly encountered problems in animals have been associated with deficient level in dietary factors. Therefore, the purpose of this study is to examine the potential for soils, forages plants, feed, and water as well as tissue fluids of grazing animals of indicators of likely Co deficiencies of livestock, so that low-cost strategies for overcoming Co deficiency in goats in different grazing systems may be formulated.

Materials and Methods

The study was conducted, beginning January 2001, using a herd of goats consisting of three classes according to physiological conditions and gender, (lactating, non-lactating, and male), grazing pasture on the farm in southern Punjab. Average temperature during the experimental year was between 38 ± 5°C during summer and 15 ± 7°C during winter: relative humidity 48 ± 5% during summer and 80 ± 8% during winter. These animals were with variable degrees of cross breeding. Animals on this farm grazed predominately native grasses crop residues, and hay along with new improved varieties of forages, and additional supplementations with concentrates and minerals. For sampling of soils, forage, feed, and water three sites, within the pasture were selected for the collection of composite samples. Five composite samples of different dietary sources and soils were taken fortnightly from each site for four times during each sampling periods (winter and summer seasons). Forage and soil samples were collected at the same time and the site. The forages samples collected mostly were the principal improved forage species in addition to grasses, and crop residues in the pasture in which the experimental animals grazed and this collection of forages was done after careful observation of animal grazing pattern.

Soil samples were taken using a stainless steel auger at a depth of 15-20 cm. Samples were collected in plastic bags, dried at 60°C for 48h. Forage samples were collected at the grazing sites from where soil samples were collected. These samples were collected according to recommended plant sampling procedures (Primary Industries S.A. Soil and Plant Analysis, Services) for analysis of Co content. These samples were clipped at a height of 3 to 6 cm to simulate grazing height with stainless steel scissors randomly from the same time and place as the soils samples. Subsequently these samples were packed into paper bags. The three sub samples were making up each composite sample collected from an area approximately 70cm in diameter.

Composite feed and water samples were also collected by their respective techniques of collection. Water samples were taken from particular channel from where it was being supplied to animals and was stored in plastic bottles for analysis.

Samples of feed were picked at the feed mill or storage or feeding sites by using a grain sampler from the entire depth of each container of a particular diet. These samples were put in plastic bag with a Naphthalene moth boll to kill insect eggs and larvae. Under these conditions, samples maintain integrity for several months.

Fecal samples were collected from rectum at the time of defecating animals while the urine samples were taken by fixing the catheter in the urethra of animals. The samples were placed in plastic vials until analyzed. Milk samples were taken shortly after administration of intra muscular oxytocin injection to stimulate milk let down.

Blood samples from the jugular vein of the animals were collected into 10 ml heparinised tubes and cooled immediately, plasma was separated by centrifugation and stored at −20°C until analysis.

Soil samples were pulverized in a ceramic mortar to pass through a 2 mm sieve and were analyzed for Co concentration using a Mehlich 1 extracting procedures (Rhue and Kidder, 1983; Hesse, 1972), 5 g of soil was added to 20 ml of 0.05N HCl in 0.025 N H_2SO_4, and the filtrate was prepared.

Water and urine samples were filtered into a sterilized plastic beakers and were kept for Co determination from the original samples without processing with any chemical reagent following Fick *et al.* (1979).

Forage, Feed, and fecal samples were dried at 60°C for 48h and ground to find powder using a ring grinder and ash contents of these samples were determined by incineration at 300°C for 2h followed by 700°C for 5h. These samples for Co assay were digested with a mixture of nitric acid and perchloric acid. The residue was made to volume with glass distilled water to prepare solution for analysis (Judson *et al.*, 1997 and Fick *et al.*, 1979).

Blood plasma supernatant was decanted into crucibles

and was first dried in a forced drought oven at 60°C for 6h, and then ashed in a muffle furnace at 550°C followed by acid digestion to prepare ash solution. (Mpofu *et al.*, 1999 and AOAC, 1990).

For all samples, glassware and crucibles were cleaned carefully by rinsing many times in double-distilled water, followed overnight immersion in 1% Acationox detergent, overnight immersion in 10% HCL and final rinsing in double-distilled water.

The Co was determined in the digests (prepared solution) by flameless atomic absorption spectrophotometer equipped with a graphite furnace and Zeeman background correction.

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

Results

Pasture Samples

Soil: Co^{2+} concentration was not affected significantly both by the seasons or sampling periods (Table 1). A high elevation in soil Co^{2+} was observed at the 1st fortnight during winter which thereafter decreased and remained so upto the last fortnight, whereas during summer the soil Co^{2+} concentrations remained statistically unchanged throughout the season (Fig. 1a).

Forage Plants: Mean squares from the analysis of variance of the data for forage Co^{2+} concentration revealed that both seasons and sampling periods had a significant effect on Co^{2+} concentration in forage but effect of sampling periods was not significant (Table 1). Higher values of forage Co^{2+} were found in winter than that in summer. During winter, the forage Co^{2+} level remained almost uniform except at the fortnight 2 where a very sharp depression in Co^{2+} concentration was recorded (Fig. 1b). During summer, no consistent pattern of increase or decrease in forage Co^{2+} with time was found.

Water: Effect of seasons or fortnights was found to be non-significant in relation to availability of Co^{2+} in water (Table 1) During winter, the Co^{2+} level at the last two fortnights was significantly higher than that at the initial two fortnights. On contrary, during summer a sharp increase in water Co^{2+} as fortnight 2 accompanied by a consistent decrease was found (Fig.1c).

Feed: Non-significant seasonal and sampling intervals effect was observed on feed Co^{2+} level (Table 1). During winter, no consistent pattern of increase or decrease of feed Co^{2+} was found at different fortnights. During summer, a consistent increase in feed Co^{2+} was observed with time (Fig. 1d).

Animal Samples

Lactating goats

Plasma: A non-significant sampling interval and seasonal effects on plasma Co^{2+} of lactating goats were observed (Table 2a). There was a consistent decrease in Co^{2+} concentration with time during winter, whereas during summer a non-consistent pattern of increase or decrease was observed (Fig.2a). Overall, plasma contained higher Co^{2+} in winter than that in summer.

Faeces: Fecal Co^{2+} concentration was not affected by the seasons or sampling periods (Table 2a). A non-consistent pattern of increase or decrease in fecal Co^{2+} concentration was found at different fortnights during both seasons (Fig. 2b). Generally, the excretion of Co^{2+} through faeces was higher in winter than that in summer.

Urine: Urine Co^{2+} concentration was below the detection limit.

Milk: No significant effects of seasons or fortnights were observed on milk Co^{2+} concentration (Table 2a). Non-consistent fluctuations in milk Co^{2+} level were observed at different fortnights of sampling during both seasons (Fig. 2d). Milk contained slightly higher Co^{2+} during winter than that during summer at all the fortnights except the 4^{th} one, where the reverse was true.

Non-lactating Goats

Plasma: Co^{2+} concentration in plasma did not change significantly during different seasons, but the sampling period showed significant effects on it (Table 2b). There was a consistent decrease in plasma Co^{2+} with time during both seasons (Fig. 2e). Plasma contained markedly higher Co^{2+} during winter than that during summer.

Faeces: Co^{2+} concentration in faeces was significantly affected by the fortnights, while seasons had no significant effects on it (Table 2b). Fecal Co^{2+} increased consistently from the 1^{st} to 3^{rd} fortnight during both seasons (Fig. 2f)

Urine: Urine Co^{2+} level was below the detection limit.

Male Goats

Plasma: Plasma Co^{2+} concentration did not vary significantly during different seasons, or fortnights. (Table 2b). A progressive increase in plasma Co^{2+} was

Table 1: Analysis of variance of data for Co^{2+} 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	Mean squares			
		Soil	Forage plants	Water	Feed
Season (S)	1	0.000003^{ns}	0.029**	0.0001^{ns}	1.85^{ns}
Error	8	0.00002	0.10003	0.00003	1.13
Fortnight(FN)	3	0.00002	0.008**	0.00004^{ns}	0.49^{ns}
Sx FN	3	0.00002***	0.004**	0.00007^{ns}	0.454^{ns}
Error	24	0.00002	0.001	0.00004	0.740

,*- Significant at 0.01and 0.001 levels, respectively
ns = non-significant

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

Source of variation	Degree of freedom	Mean squares			
		Plasma	Faeces	Urine	Milk
Season (S)	1	0.116^{ns}	2.81^{ns}	Below	0.00001^{ns}
Error	18	0.088	1.70	detection	0.003
Fortnight(FN)	3	0.009^{ns}	0.31^{ns}	limit	0.0009^{ns}
Sx FN	3	0.004^{ns}	0.12^{ns}		0.0026^{ns}
Error	54	0.006	0.26		0.002

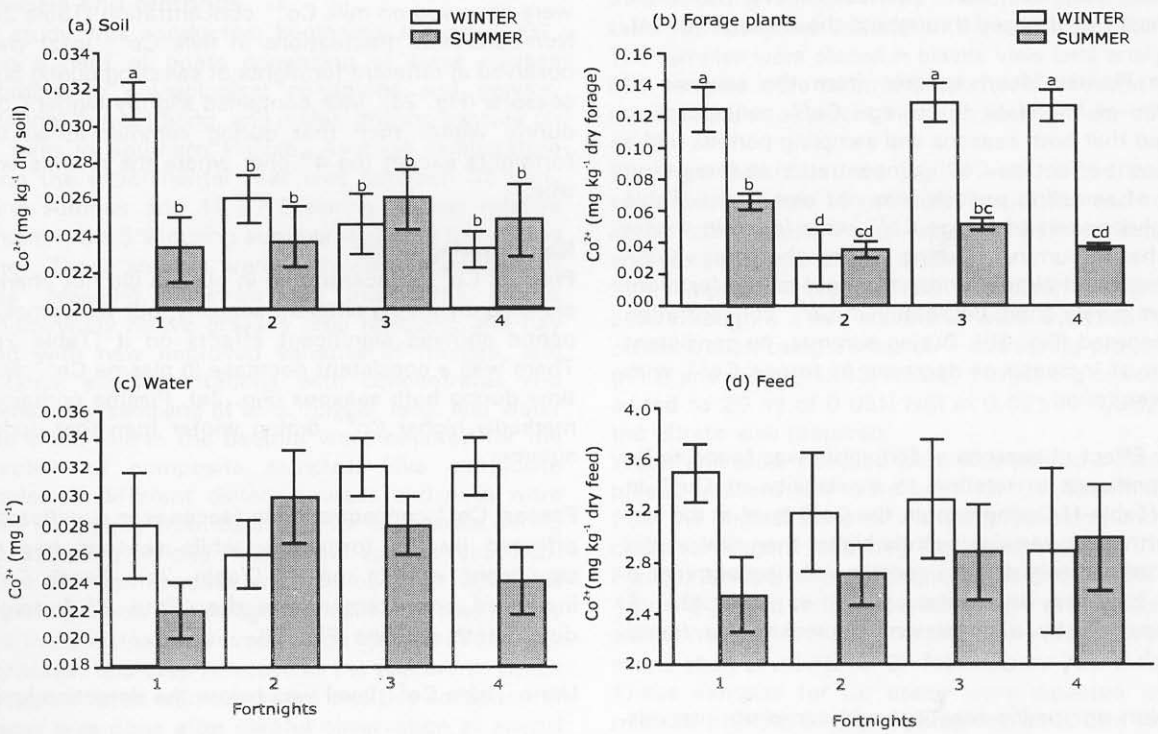

Fig.1: Co^{2+} concentration in (a) soil, (b) forage plants, (c) water and (d) feed at different fortnights during winter and summer seasons (goat farm)
(Means with the same letters do not differ significantly at $P<0.05$)

Table 2b: Analysis of variance of data for Co^{2+} concentration in blood plasma, faeces,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	Mean squares				
		Non- lactating goats			Male goats	
		Plasma	Faeces	Urine	Plasma	Faeces
Season (S)	1	0.240^{ns}	0.010^{ns}	Below	0.026^{ns}	1.41^{ns}
Error	18	0.196	2.26	detection	0.1582	0.98
Fortnight(FN)	3	0.044^{***}	1.17^{***}	limit	0.005^{ns}	0.05^{ns}
Sx FN	3	0.002^{ns}	0.09		0.0060^{***}	0.61^{ns}
Error	54	0.004	0.16		0.0031	0.36

*** = Significant at 0.001 level, respectively
ns = non-significant

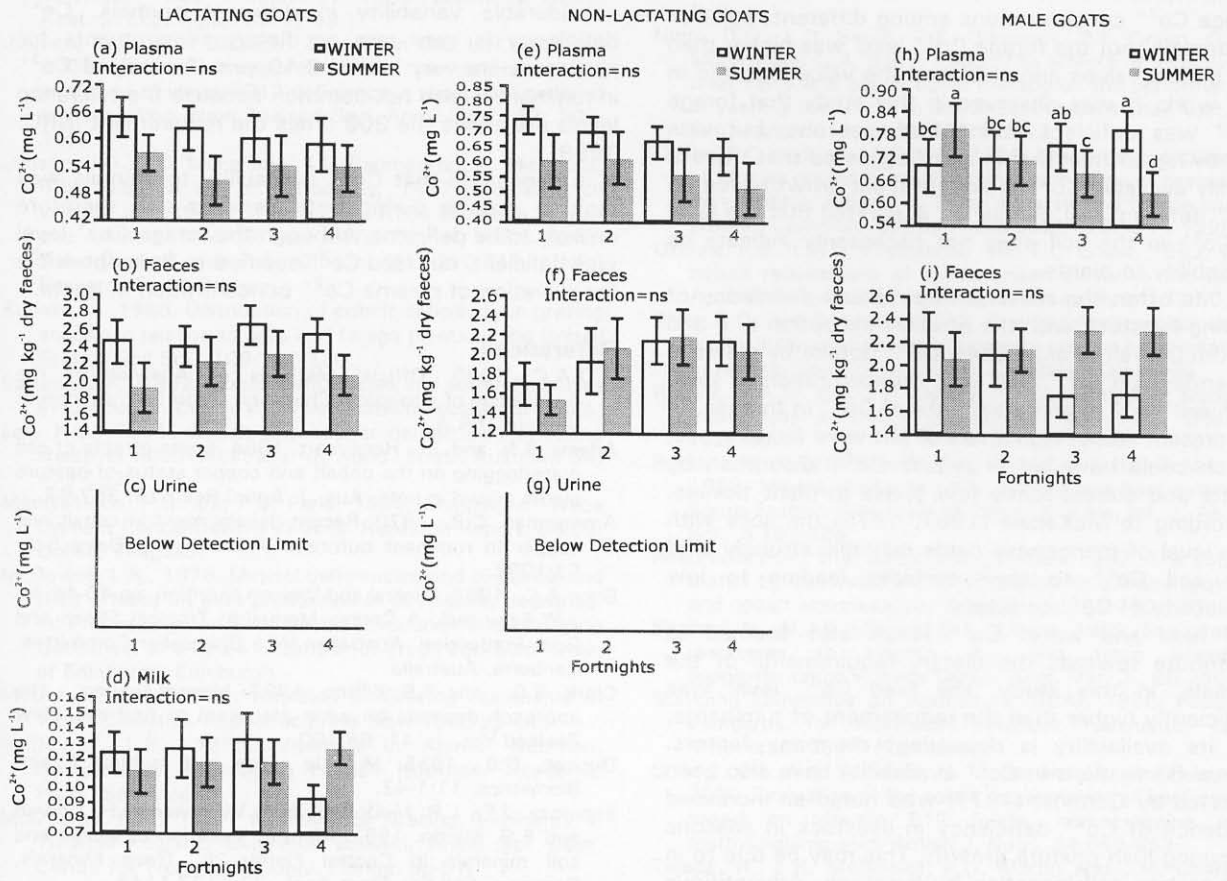

Fig.2: Co^{2+} concentration in goats different sample types of lactating, non-lactating and male at different fortnights during winter and summer seasons
(Means with the same letters do not differ significantly at $P<0.05$)

observed with time during winter (Fig. 2h). Conversely, during summer, there was a consistent decrease in plasma Co^{2+} with time.

Faeces: Fecal Co^{2+} level did not change significantly during both seasons or sampling periods (Table 2b). During winter, a consistent decrease and during summer a consistent increase in fecal Co^{2+} concentration was found up to the 3rd fortnight, while

the concentration at fortnight 4 during both seasons was almost equal to that at fortnight 3 (Fig. 2i)

Discussion

In the present study mean extractable soil Co^{2+} concentration was deficient based on the critical level of 0.1mg kg^{-1} (Kubota, 1968). These soil Co^{2+} values were not adequate compared to the requirement of plant growth. Similar low level of soil Co^{2+} has earlier been reported.

Forage Co^{2+} levels were deficient for ruminants during both seasons because these were lower than the critical level (NRC, 1980). Similar Co^{2+} deficient forages were found in Nicaragua by Velasquez-Pereira *et al.*, (1997), in Florida, USA, Espinoza *et al.* (1991). Rojas *et al.* (1993) found marginal to deficient Co^{2+} level. Tejada *et al.* (1987) did not find differences in forage Co^{2+} concentrations among different region in Guatemala, but the forage Co^{2+} level was higher than the critical values and also than the value reported in this work. It was observed in this study that forage Co^{2+} was deficient during both seasons, but was slightly higher than that in soil. Suggested that there is readily available Co^{2+} in soil for plant growth even on Co^{2+} deficient soil. Similarly, illustrated that the level of Co^{2+} in the soil does not necessarily indicate its availability to plants.

Co^{2+} is often the most severe mineral deficiency of grazing livestock with the possible exception of P and Cu (McDowell *et al.*, 1984). Co^{2+} uptake by plants is dependent on Co^{2+} and Mn^{+2} concentration in soils. High soil Mn^{2+} depresses uptake of Co^{2+} in forages. In the present study, high levels of Mn were found in soil, which could have led to reduce Co^{2+} absorption by plants and subsequently low levels in plant tissues. According to McKenzie (1967, 1975) the soils with high level of manganese oxide minerals strongly bind free soil Co^{2+} to their surfaces leading to low availability of Co^{2+} to plants.

The feed and water Co^{2+} levels also seemed to contribute towards the dietary requirements of the animals. In this study, the feed Co^{2+} level was sufficiently higher than the requirement of ruminants, but its availability is dependent on many factors. Annual fluctuations in Co^{2+} availability have also been reported by Gardiner (1977) who noted an increased incidence of Co^{2+} deficiency in livestock in seasons favouring lush pasture growth. This may be due to in part to less soil ingestion by livestock. Adventitious intake of soil can be a significant, if not a major, source of Co^{2+} ingested by grazing animals. The organic constituents of the diet can have a major impact on the amounts of minerals needed and tolerated. A good illustration is the relationship between Vitamin B_{12} and Co^{2+} (McDowell, 1976).

The sufficient amounts of Co^{2+} in forage and feed sources is not considered a criterion of adequacy, because of interrelationships between Co^{2+} and other dietary factors in feed, such as Fe, Cu, Se, and Mo (Ammerman, 1970). Mean plasma Co^{2+} concentrations in all classes of goats showed a positive association with the dietary intake in forage and feed Co^{2+}. Plasma Co^{2+} level in male animals was higher than that in non-lactating and lactating animals during winter and lactating goats had lower plasma Co^{2+} levels than other two groups during both seasons. This low level of plasma Co^{2+} in lactating goats may be due to its higher secretion in milk and excretion through faeces during both seasons. As in the present study high plasma Co^{2+} above critical value of 0.2 ng/ml has earlier been reported in the plasma of cattle by Mpofu *et al.* (1999) in Zimbabwe, which showed dietary reflection. Plasma Co^{2+} of about 0.2 ng/ml is an indicative of a Co^{2+} deficiency, although there is considerable variability in different animals. Co^{2+} deficiency is very rare as dietary requirements for ruminants are very low at 0.10ppm. Toxicity of Co^{2+} in ruminant is also not common because the tolerance levels are above the 300 times the requirement (NRC, 1989).

It is concluded that Co^{2+} availability to animals was high in plasma during both seasons and therefore unlikely to be deficient. Although the forage Co^{2+} level was deficient, but feed Co^{2+} seemed to be responsible for elevation of plasma Co^{2+} concentration in goats.

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