TEMPORAL VARIABILITY IN THE TRANSFER OF SODIUM FROM SOIL AND DIETARY SOURCES TO GRAZING LIVESTOCK IN A SEMI-ARID RANCH, PUNJAB, PAKISTAN

ZAFAR. I. KHAN^{1*}, M. ASHRAF², M. DANISH³ AND KAFEEL AHMAD¹

^{1*}Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan. ²Department of Botany, University of Agriculture, Faisalabad, Pakistan ³Department of Chemistry, University of Sargodha, Sargodha, Pakistan ¹*Corresponding Author: drzafar10@hotmail.com

Abstract

This investigation was carried out in the semi-arid region of Punjab, Pakistan to determine the sodium status in plant forages and grazing sheep therein, alongwith assessing the soil samples. From the pasture soil, forage, feed, water and animal samples (Blood plasma, milk, faeces, and urine) were collected fortnightly during winter and summer seasons. These samples were analyzed for sodium concentrations in plant forages which were found below those recommended for optimum ruminant production during both seasons of the year. Seasons did not affect soil, forage and plasma Na⁺ concentrations. These samples were lower than the required range in relation to Na levels for ruminants. The fecal, urine, and milk Na⁺ losses were found to be responsible for low plasma Na⁺ status in different goat classes. From these results it is concluded that high levels of this element should be supplied in the feed supplements used at different times of the year to prevent the sodium deficiency in grazing goats in this ranch.

Introduction

Forages are mostly used as the main source of different minerals and other nutrients for grazing livestock and these are usually low in many essential minerals. Many naturally occurring deficiencies in grazing animals have been considered because of the nature of the soil and other properties (McDowell, 1997; McDowell *et al.*, 1983). Pastures are often considered to be approprate for providing nutrition to sustain different livestock forms and the quality of various forages is often inadequate for growing animals whose physiological demands are higher (Chambliss *et al.*, 1991; Serra *et al.*, 1987). The chemical composition of different forages is dependent on many factors and very low relationship has been reported between soil composition and mineral constituents of various forages (Reid & Horvath, 1980). Pasture mineral concentrations vary to a larger extent among plant species and the potential incidence and existence of mineral imbalances is often greater in different areas of the world and minerals most likely to be found in deficient amounts are P, Na, Mg and Cu (Little, 1982).

The macro-minerals, sodium, potassium, calcium, chlorine, magnesium and sulphur all are very important for grazing livestock (Underwood, 1981; Masters *et al.*, 1993). In many different countries of the world constraints in livestock production and improvement are due to naturally occurring deficiencies and excesses of one or more of these essential minerals found in forages consumed by the grazing livestock (Masters *et al.*, 1993; Islam *et al.*, 2003). Although deficiencies of all these elements results in low productivity of grazing livestock, but these imbalances occurs rarely in nature. By contrast sodium deficiency occurs in ruminants in many parts of the world and is wide and is found in many regions of the world. It has been reported that annual rainfall and distance from the sea level are most important factors to contribute to the incidence of sodium deficiency in grazing livestock (McDowell *et al.*, 1984; Prabowo *et al.*, 1990). Forages normally do not contain sufficient quantities of Na to meet the requirements of grazing ruminants throughout the year. This inadequacy is easily overcome by the practice of providing common salt *ad-libitum*. Nevertheless, most grazing livestock either receive insufficient salt or have only very limited access to salt at certain times of the year. Na in addition to K and Cl, play very important role in regulating many metabolic functions. It functions as an electrolyte in body fluids and is specifically involved at a cellular level in water metabolism, nutrient uptake and transmission of nerve impulses (McDowell, 1985). Deficiency of sodium is a recognized problem in semi-arid regions of Punjab, Pakistan, and salt supplements are not usually provided to grazing ruminants regularly throughout the year (Khan *et al.*, 2005, 2006).

The studied region of the Punjab, Pakistan contains vast areas of both semi-arid environments. These areas or pastures are used for grazing animals and support number of herds of goats and sheep in addition to cattle and buffaloes. Although there are some areas, which are canal, irrigated, but little fertilizer is used to promote pasture production for ensuring the availability at optimum level of various minerals to the grazing livestock. Mineral and other feed supplements are often inadequately provided to the grazing animals to maintain their live weight for economic reasons. Under such conditions, grazing livestock may be susceptible to mineral deficiencies. The objective of this study was to determine the sodium level in soil, forage and goats grazing therein, keeping in view the importance of this mineral for the grazing animals and to formulate mineral supplements with high bioavailability of this essential element. This information would be used for different goat producing regions during winter and summer seasons in Pakistan and other developing countries with similar ecosystems.

Materials and Methods

Sample collection: Soil, forage, feed, water and animal samples were taken eight times from the farm "Livestock Experimental Station" in the Province Punjab, Pakistan during the year (four times each during summer and winter). Composite soil and forage samples were collected at three sites from the pasture.

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

Animal samples were taken from lactating, on-lactating and male goats, respectively. Blood plasma, milk, feces and urine samples from lactating, plasma, feces and urine from non-lactating and plasma and feces from male goats were taken concurrently with the soil and forage samplings.

Blood samples were collected by jugular vein punctured with a syringe and needle, then drawn by vacuum into evacuated tubes containing lithium heparin as an anticoagulant. The plasma was separated by centrifugation and harvested into polyethylene tubes and frozen at -20° C for subsequent analysis for sodium. Fecal samples were collected from the rectum of the animals manually and urine samples

collected *via* manual stimulation of the vulva of female animals after which 10 ml aliquot were transferred to polyethylene tubes, acidified with 0.3 ml concentrated HCl, and frozen for subsequent analysis. The fecal samples were kept in open bags and allowed to dry in sun to constant atmospheric moisture (<30%). Milk samples were collected in 125 ml nalgene bottles using the first drawn milk. Milk samples were taken in plastic vials and stored frozen until analysis (Fick *et al.*, 1979).

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

Sample preparation: Air- and oven-dried soil samples were pulverized in a ceramic mortar to pass through a 2 mm sieve and were analyzed for Na concentration using a Mehlich-1 (Hesse, 1972; Rhue & Kidder, 1983) extraction procedure: 5 g of soil were added to 20 ml of 0.05 M HCl and 0.025 M H₂SO₄, shaken on reciprocating shaker for 30 min., and final volume was analyzed.

Water and urine samples were filtered into sterilized plastic beakers, and aliquots (each 1 ml) were used to prepare serial dilutions for analysis. Air and oven dried samples of forage, feed and feces were ground with a Wiley mill to pass through a 1mm mesh. Completely dried and ground samples of about 2 g each of forage, feed and feces were digested with nitric acid and perchloric acid mixture (3:1) at 250°C until the solution changed to colorless and thick white fumes appeared in the flask. The contents of the flask were washed with pure water and diluted to constant volume. The supernatant obtained from centrifugation was used for analysis (Koh & Judson, 1986). Direct dry or wet ashing of plasma and milk was not possible because of the high fat, protein and moisture as sputtering and swelling might result in loss of sample. Therefore, appropriate quantities of each plasma or milk sample was placed in a crucible after thawing. For pre-digest, the samples were pretreated with 50% HNO3 over an electric heater until smoking ceased to char the majority of organic matter, these samples were ashed for 6 h at 550 °C in a muffle furnace. The residues were dissolved in 1% HCl and transferred to a volumetric flask to make up a constant volume of 50 ml. The samples were poured into labelled plastic tubes suitable to fit the auto-sampler of atomic absorption spectrophotometer following Mpofu et al. (1995). All the samples were filtered through Whatman filter paper No. 42 and brought to the appropriate volume with double distilled water and stored in polyethylene tubes. The samples were analysed for Na concentration by atomin absorption spectrophotometry (Perkin-Elmer Model 5000).

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

Results

Soil: Data for soil Na⁺ exhibited that seasons had non- significant effect (p>0.05) on its concentration but sampling intervals showed significant effect (p<0.001) on soil Na⁺ (Table 1). Low level of Na was found at late sampling periods during both seasons with no markedly variation at different sampling intervals. However, there was no significant statistical difference between the seasons with respect to soil Na⁺ within the season (Fig. 1a). Oveall, fluctuation soil Na at all sampling periods were very inconsistent.

recu at univerent sampling periods during winter and summer seasons.								
Source of	Degree of	Mean Squares						
variation S.O.V.	freedom	Soil	Forage plants	Water	Feed			
Season (S)	1	108.18 ^{ns}	189760.20**	508.45 ^{ns}	3456785.90*			
Error	8	89.14	89165.45	67.12	87342.65			
Fortnight (FN)	3	1128.34 ^{***}	46789.35**	12.24 ^{ns}	56794.47**			
S x FN	3	78.28 ^{ns}	8765.56 ^{ns}	18.56 ^{ns}	4532.44 ^{ns}			
Error	24	107.38	6579.70s	6.78	8743.48			

 Table 1. Analysis of variance of data for Na⁺ concentration in soil, forage plants, water and feed at different sampling periods during winter and summer seasons.

*,** = Significant at 0.05, 0.01 and 0.001 levels, respectively.

ns = Non-significant



Fig. 1. Na^+ concentration in (a) soil, (b) forage plants, (c) water and (d) feed at different fortnights during winter and summer seasons.

(Mean with the same letters do not differ significantly at p<0.05).

Forage plants: Na⁺ concentrations of all collected forages showed significant effect (p<0.01) of season and sampling periods on it (Table 1). Forage Na⁺ concentration in winter was markedly higher than that in summer season with inconsistend decrease or increase during both seasons of sampling (Fig. 1b).

Water: Both seasons or sampling periods had non-significant seasonal (p<0.01) and nonsignificant effects of sampling intervals (p>0.05) on water Na⁺ concentration (Table 1). During winter the Na⁺ concentration in water was found to be higher than that during summer with irregular changes in winter and consistent changes during summer at different sampling periods (Fig. 1c). **Feed:** Both sampling periods and seasons affected (p<0.001) significantly Na concentration in feed supplement (Table 1). Higher amount of Na⁺ was found to be present in feed during winter than that in summer. However, Na concentration in feed supplement showed a consistent pattern of decrease with time during both the seasons (Fig. 1d).

Animal samples

Lactating goats

Plasma: Plasma Na⁺ affected significantly (p<0.001) by sampling periods and nonsignificantly (p>0.05) by the seasonal changes (Table 2a, Fig. 2a). Overall, during winter plasma had more Na⁺ as compared to that in summer with inconsistent decrease or increase with sampling times during both seasons.

Faeces: Analysis of variance of data for fecal Na showed that the fecal Na⁺ level was affected significantly (p<0.001) both by the seasons and fortnights (Table 2a). Higher Na⁺ in faeces was found during summer than that in winter. A sharp decrease in fecal Na level was found with time during winter. In contrast, during summer, a consistent increase in fecal Na⁺ was observed with time (Fig. 2b).

Urine: Non- significant effect of season (p>0.05) or sampling periods was found on urine Na level (Table 2a). Urine Na⁺ level exhibited irregular changes during winter at different fortnights in contrast to during summer (Fig. 2c).

Milk: Analysis variance of data for milk Na^+ revealed significant seasonal (p<0.001) and non-significant fortnight effects (p>0.05) on milk Na^+ level (Table 2a). During winter, milk Na^+ remained unchanged at the last three fortnights, whereas during summer the trend of consistent decrease was found (Fig. 2d). However milk had higher Na^+ during winter than that during summer.

Non-lactating goats

Plasma: Sampling periods and seasons had significant effect (p<0.05) of seasons on plasma Na⁺ (Table 2b). Plasma Na⁺ level was found higher in winter than that during summer (Fig. 2e). Overall plasma contained significantly higher Na during winter than that during summer with inconsistent increase or decrease during both seasons.

Faeces: Analysis of variance of data for fecal Na showed that seasons and fortnights had a no-significant effect (p>0.05) on fecal Na⁺ (Table 2b). During winter, a consistent decrease in fecal Na⁺ was found (Fig. 2f), whereas, a consistent increase in fecal Na⁺ was observed with time during summer with consistent increase or decrease during winter and summer. However, fecal Na⁺ was found to be similar during winter and summer.

Urine: Seasonal and sampling interval effect was found to be significant (p>0.05) on urine Na⁺ (Table 2b). Almost similar amount of urine Na⁺ was found to be present at different fortnight in winter. In contrast, in summer the urine Na⁺ decreased consistently with time of sampling (Fig. 2g), with inconsistent changes at different sampling periods during winter and consistent decrease during summer, respectively.

scasons.								
Source of	Degree of freedom	Mean squares						
variation S.O.V.		Plasma	Faeces	Urine	Milk			
Season (S)	1	765434.86 ^{ns}	1993961.25***	123452.57 ^{ns}	543255.45**			
Error	18	643212.391	141234.32	324532.87	9456.23			
Fortnight (FN)	3	23453.76 ^{***}	64321.43***	45674.63 ^{ns}	7654.54***			
S x FN	3	54321.45***	321453.32***	8943.87 ^{ns}	8234.54 ^{***}			
Error	54	2343.63	4476.63	4567.98	654.28			

Table 2a. Analysis of variance of data for Na⁻ concentration in blood plasma, faeces, urine and milk of lactating goats at different sampling periods during winter and summer

Table 2b. Analysis of variance of data for Na⁻ concentration in blood plasma, faeces, urine of non-lactating goats at that plasma and faeces of male goats at different sampling periods during winter and summer seasons.

Source of	Degree	Mean squares				
variation S.O.V.	of freedom	Plasma	Faeces	Urine	Plasma	Faeces
Season (S)	1	156432.97 _{ns}	754325.65 _{ns}	60464.56 ^s	6578.01***	32456.32 ^{ns}
Error	18	234563.86	13876.24	345654.5 3	234532.76	164673.22
Fortnight (FN)	3	4532.28***	354.88 ^{ns}	20546.65*	54367.86**	4567.12**
S x FN	3	1123.34*	14567.45**	6754.23 ^{ns}	26734.65 ^{**}	36576.55**
Error	54	367.65	1090.25	7645.80	1456.18	876.45

*,** = Significant at 0.05, 0.01 and 0.001 levels, respectively.

ns = Non-significant.

Male goats

Plasma: Non-significant effect of seasons and sampling periods was found (p<0.001) on plasma Na⁺ (Table 2b). A consistent decrease in plasma Na⁺ during winter and increase during summer (Fig. 2h). However, plasma Na⁺ in male goats was slightly higher non-statistically during summer than that during winter.

Faeces: Seasonal as well as sampling period effect (p<0.01) was found to be significant on fecal Na⁺ level (Table 2b), with consistent decrease and increase in fecal Na⁺ during winter and summer, respectively (Fig. 2i).

Discussion

Variation in Na⁺ content of measured samples could be due to feeding, soil properties, forage species, seasonal changes and some other factors. The availability of minerals in soils depends upon their effective concentration in soil solution (Reid & Horvath, 1980). In the present study, mean soil extractable Na contents in winter and summer seasons were not different. Soil Na⁺ levels were below the critical value reported by Rhue & Kidder (1983). Similar results have already been reported by Morillo *et al.*, (1989) for different regions of central Venezuela for wet and dry seasons of the year.



Fig. 2. Na⁺ concentration in different sample types of lactating, non-lactating and male goats at different fortnights during winter and summer seasons. (Mean with the same letters do not differ significantly at p<0.05).

Forage Na concentration was deficient because of being lower than the critical values recommended by NRC (Anon., 1985; Tudsri & Kaewkunya, 2002). It has been reported that the most prevalent mineral deficiencies for grazing animals in world were Na⁺and its deficiency has been reported in many developing countries eg., Nigeria (Ogebe *et al.*, 1995) and Colombia (Pastrana *et al.*, 1991).

Sodium concentrations in forages were below the levels recommended for optimal animal productivity and production. The distribution of sodium within the forages and its chemical form may affect bioavailability. To meet the need of highly productive animals, forage should contain more than 0.15% sodium (Anon., 1973). Na⁺ deficiency is more likely to occur in animal grazing tropical pasture species and these plants generally accumulate less Na⁺ than temperate species (Morris, 1980; Nasrulla *et al.*, 2003). Natural forages low in Na⁺ have been reported in numerous tropical countries throughout the world (McDowell, 1985).

Feed and water Na^+ contents was found to be in larger amounts to meet the requirements of grazing goats and in complimenting the forage Na^+ in this ranch. The deficient level of Na^+ in plasma found in lactating, non-lactating, and male goats is most likely to occur, during lactation, due to secretion of large quantity of Na^+ in milk and loss of Na^+ through exceretion in faeces and urine or due to low Na^+ content in pasture as found in this investigation. It may be possible that high level of K in forage has depressed the Na^+ level (McDowell & Valle, 2000). These findings are in agreement with those of Mpofu *et al.*, (1995) who similarly reported low level of Na^+ in blood plasma of animals. Significant Na^+ concentrations were found to be excreted through faeces in summer and through urine in winter.

Milk Na⁺ concentration was higher during winter than that during summer in lactating goats. These values of milk Na⁺ were below the values found in earlier research

(Underwood, 1981). Similar values were reported by Cuesta *et al.*, (1993). However the results of the present investigation are not in agreement with the findings of above workers with respect to seasonal effect.

On the base of various analyses it can be concluded that the grazing animals at this specific ranch should be provided supplementation to enhance the Na⁺ for maximizing the livestock productivity.

References

- Anonymous. 1973. *Tracing and treating mineral disorders in dairy cattle*. Netherlands Committee on Mineral Nutrition, Centre for Agriculture Publishing and Documentation-Wageningen, The Netherlands.
- Anonymous. 1985. National Research Council. Nutrient requirements of domestic animals, Nutrient Requirements of Sheep (5th ed.) Natl. Acad. Sci., N.R.C., Washington, D.C.
- Chambliss, C.G., Sollenberger and L.E. Bahiagrass. 1991. The foundation of cow-calf nutrition in Florida. *Proceedings Beef Cattle Short Course*. Institute of Food and Agricultural Sciences, Univ. of Florida, Gainesville, FL., pp. 74-80.
- Cuesta, P.A., L.R. McDowell, W.E. Kunkle, F. Bullock, A. Drew, N.S. Wilkinson and F.G. Martin. 1993. Seasonal variation of soil and forage mineral concentrations in north Florida. *Commun. Soil Sci. Plant Anal.*, 24: 335-347.
- Duncan, D.B. 1955. Multiple range and multiple F-test. Biometrics, 11: 1-42.
- Fick, K.R., L.R. McDowell, P.H. Miles, N.S. Wilkinson, J.D. Funk and J.H. Conrad. 1979. *Methods of mineral analysis for plant and animal tissues* (2nd ed.). Dept. Anim. Sci., Univ. Florida, Gainesville.
- Hesse, P.R. 1972. A textbook of soil analyses. Chemical Publishing Co., NY.
- Islam, M.R., C.K. Saha, N.R. Sharker, M. Jahilil and M. Hasanuzzaman. 2003. Effect of variety on proportion of botanical fraction and nutritive value of different Napier grass (*Pennisetum puporeum*) and relationship between botanical fraction and nutritive value. Asian-Aust. J. Anim. Sci., 16: 177-188.
- Khan, Z.I., Altaf Hussain, Muhammad Ashraf and L.R. McDowell. 2006. Mineral Status of Soil and Forages in South Western Punjab, Pakistan. Asian-Australian Journal of Animal Sciences, 19(7): 915-923.
- Khan, Z.I., Altaf Hussain, Muhammad Ashraf, E.E. Valeem and Ijaz Javed. 2005. Evaluation of variation of soil and forage minerals in pasture in a semiarid region of Pakistan. *Pakistan Journal of Botany*, 37(4): 921-931.
- Koh, T.S. and G.T. Judson. 1986. Trace elements in sheep grazing near a lead-zinc smelting complex at port pirie, South Australia. *Bull. Environ. Contaim Toxicol.*, 37: 87-95.
- Little, D.A.1982.Utilization of minerals, pp. 259-283.i.: (Ed.): J.B. Hacker, Nutritional Limits to Animal Products from Pasture.Commonwealth Agricultural Bureaux,Farnham,Royal,UK.
- Masters, D.G., D.B. Purser, S.X. Yu, Z.S. Wang, R.Z. Yang, N. Liu, D.X. Lu, L.H. Wu, J.K. Ren and G.H. Li. 1993. Mineral nutrition of grazing sheep in northern China. I. Macro-minerals in pasture, feed supplements and sheep. *Asian-Australian Journal of Animal Sciences*, 6: 99-105.
- McDowell, L.R. 1985. Nutrition of Grazing Ruminants in Warm Climates. Academic Press New York, pp. 443.
- McDowell, L.R. 1997. *Minerals for Grazing Ruminants in Tropical Regions*. Extension Bulletin, Anim. Sci. Dept. Centre for Trop. Agric., Univ. Florida. pp. 81.
- McDowell, L.R. and G. Valle. 2000. Major minerals in forages. In: Forage Evaluation in Ruminant Nutrition. (Eds.): D.I., Givens, E. Owen, R.F.E. Oxford and H.M. Omed. pp. 373. CAB International, Wallingford, UK.
- McDowell, L.R., J.H. Conrad and G.L. Ellis. 1984. Mineral deficiencies and imbalances, and their diagnosis. In: Symposium on Herbivore Nutrition in Sub-Tropics and Tropics-Problems and Prospects (Eds.): F.M.C. Gilchrist and R.I. Mackie, pp. 67-88. Craighall, South Africa.

1120

- McDowell, L.R., J.H. Conrad, G.L. Ellis and L.K. Loosli. 1983. Minerals for grazing ruminants in tropical regions. *Extension Bulletin Anim. Sci. Dept., Univ. of Florida*.
- Morillo, D.E., L.R. McDowell, C.F. Chicco, J.T. Perdomo, J.H. Conrad and F.G. Martin. 1989. Nutritional Status of beef cattle in specific regions of Venezuela. I. Macro-minerals and forage organic constituents. *Nutr. Rep. Int.*, 39: 1247-1262.
- Morris, J.G. 1980. Assessment of sodium requirements of grazing beef cattle: a review. J. Anim. Sci., 50: 145-152.
- Mpofu, I.D.T., L.R. Ndlova and N.H. Casey. 1995. The calcium, phosphorus, sodium, potassium and magnesium status of cattle in the Sanyati and Chinamhora communal grazing areas. J. Zimbabwe Soc. Anim. Prod., 7: 157-162.
- Nasrullah Niimi, M. Akashi and R.O. Kawamura. 2003. Nutritive evaluation of forage plants in South Sulaweshi, Indonesia. *Asian-Aust. J. Anim. Sci.*, 16: 693-701.
- Ogebe, P.O. and J.A. Ayoade, L.R. McDowell, F.G. Martin and N.S. Wilkinson. 1995. Mineral concentrations of forages and soils in Benue State, Nigeria. I. Macrominerals and forage *in vitro* organic matter digestibility and crude protein concentrations. *Commun. Soil Sci. Plant Anal.*, 26: 1989-2007.
- Pastrana, R., L.R. McDowell, J.H. Conrad and N.S. Wilkinson. 1991. Macromineral status of sheep in the Paramo region of Colombia. *Small Rumin. Res.*, 5: 9-21.
- Prabowo, A., L.R. McDowell, N.S. Wilkinson, C. J. Wilcox and J. H. Cornad. 1990. Mineral status of grazing cattle in South Sulawesi, Indonesia; I. Macrominerals. Am. J. Anim. Sci., 4: 111-120.
- Reid, R.L. and D.J. Horvath. 1980. Soil chemistry and mineral problems in farm livestock: a review. *Anim. Feed Sci. Tech.*, 5: 95-167.
- Rhue, R.D. and G. Kidder. 1983. Analytical procedures used by the IFAS extension soil laboratory and the interpretation of results. Soil Sci. Dept., Univ. Florida, Gainesville.
- Sanchez, P.A. 1976. *Properties and Management of Soils in Tropics*, John Wiley and Sons, NY., 97-101.
- Serra, A.B., S.D. Serra, E.A. Orden, L.C. Cruz, K. Nakamura and T. Fujihara. 1987. Variability in ash, crude protein, detergent fiber and mineral content of some minor plant species collected from pastures grazed by goats. *Asian-Aust. J. Anim. Sci.*, 10: 28-34.
- Steel, R.G.D. and J.H. Torrie. 1980. *Principles* and *procedures of statistics*. A *Biometrical approach* (2nd Ed.). Mcgraw Hill Book Co. New York.
- Tudsri, S. and C. Kaewkunya. 2002. Effect of *Leucaena* row spacing and cutting intensity on growth of *Leucaena* and three associated grasses in Thailand. *Asian-Aust. J. Anim. Sci.*, 15: 986-991.
- Underwood, E.J. 1981. *The Mineral Nutrition of Livestock*. Commonwealth Agricultural Bureaux, London.

(Received for publication 10 March 2007)