Asian-Aust. J. Anim. Sci.
Vol. 20, No. 3 : 393 - 398
March 2007

www.ajas.info

Influence of Soil and Forage Minerals on Buffalo (*Bubalus bubalis*) Parturient Haemoglobinuria

M. Z. Akhtar, A. Khan*, M. Sarwar[1] and A. Javaid[1]

Department of Veterinary Pathology, University of Agriculture, Faisalabad-38040, Pakistan

ABSTRACT : The present study was carried out to investigate the serum minerals profile in buffaloes (*Bubalus bubalis*) suffering from parturient haemoglobinuria (PHU) along with minerals profile of soils and fodders from the disease prone areas and their interrelationships. Serum samples were collected from 60 each of healthy and PHU affected buffaloes randomly selected from field cases. Serum samples were collected from each animal. Fifty composite soil samples were collected where PHU was prevalent. Fifty samples of fodders including leaves and stems being fed to the diseased buffaloes were collected. The difference in the levels of calcium and potassium between upper and lower soil surface of disease prone areas under study were statistically non-significant. The mean values of phosphorous, copper, iron, selenium and molybdenum in upper soil surface were significantly ($p<0.05$) higher than in lower soil surface. None of the fodders offered to the diseased animals met the dietary requirements of phosphorus and copper whereas none of the fodders was deficient in potassium, iron and selenium rather were having excess of potassium, iron and selenium. The concentration of calcium was adequate in lucerne, berseem, sarson and sorghum, while maize, sugarcane and wheat straw did not meet the required levels for dairy animals. Molybdenum contents in all fodders were adequate to meet the dietary requirements of the dairy buffaloes. Serum phosphorus, copper and selenium were significantly ($p<0.001$) lower whereas potassium, iron and molybdenum in buffaloes suffering from PHU were significantly ($p<0.001$) higher than in healthy buffaloes. It was concluded that phosphorous deficient soils play a major role by transferring this deficiency to plants and ultimately reaching to animals where hypophosphataemia is a consistent finding. (**Key Words** : Parturient Haemoglobinuria, Buffaloes, Minerals Profile (Soil and Plants), Hypophosphataemia, Hypocupraemia, Hypermolybdenaemia)

INTRODUCTION

Parturient haemoglobinuria (PHU) is one of the major and economically important diseases of dairy animals (Pirzada and Hussain, 1998). It is an acute disease of high yielding buffaloes and cows characterized by hypophosphataemia, intravascular haemolysis, haemoglobinuria and anaemia (Radostits et al., 2000). The exact aetiology and pathogenesis of PHU is not known as variety of aetiological factors has been reported to be associated with the disease in different parts of the world. Nonetheless, hypophosphataemia is documented consistently in the affected animals (Chugh et al., 1998).

Minerals in addition to vitamins are involved in tissue defense mechanisms against free radical damage to biological systems. Several metalloenzymes which includes glutathione peroxidase (Se), catalase (Fe), and superoxide dismutase (Cu, Zn and Mn) are also critical in protecting the internal constituents from oxidative damage (Han et al., 2004; 2006). Copper deficiency is considered to be an important aetiological factor in New Zealand as copper supplementation reduced the incidence of post-parturient haemoglobinuria. Copper deficiency reduces the activity of the copper-containing superoxide dismutase, which is a part of the erythrocyte protective mechanism against oxidative stress (Jubb et al., 1993). Post-parturient haemoglobinuria has also been associated with low selenium status as determined by glutathione peroxidase activity in whole blood of cattle (Ellison et al., 1986).

Pakistan is a land of diversified soils having different agro-climatic regions. The quality and quantity of nutrients of forage mainly depend on irrigation. Mineral availability, particularly trace elements, varies to a very great extent from soil to plants and animals. Micronutrients are depleted from light textured and calcareous soils, particularly when

* Corresponding Author: A. Khan. Tel: +92-41-9200161-170 (Ext. 3119), Fax: +92-41-9200764, E-mail: ahrar1122@yahoo.com

[1] Institute of Animal Nutrition and Feed Technology, University of Agriculture, Faisalabad-38040, Pakistan.

Received April 17, 2006; Accepted September 15, 2006

high yielding crops varieties are grown under intensive cropping system (McDowell and Valle, 2000).

The soil-plant-animals system is a complex system which has not been investigated adequately. Information is required on interrelationship of minerals among soils, plants, and animals. In view of the diverse aetiological factors associated with PHU, a comprehensive study is needed to elucidate the aetiopathology of this disease particularly in buffaloes. Therefore, the present investigation was undertaken to study the serum minerals profile in buffaloes suffering from PHU along with minerals profile of soils and fodders from the disease prone areas.

MATERIALS AND METHODS

Experimental animals

A total of 60 buffaloes (*Bubalus bubalis*) suffering from PHU were randomly selected from field cases occurring in Faisalabad, Toba Tek Singh and Jhang districts of Punjab province. Simultaneously, 60 clinically healthy buffaloes of similar description from the same localities were included for case control study. Affected buffaloes were from 1-8 parities having age 5-11 years and parturated normally. The disease was diagnosed clinically on the basis of specific signs such as haemoglobinuria and characteristic straining while defecating in buffaloes in early lactation or in advanced pregnancy (Digraskar et al., 1991). The possibility of other diseases causing a reddish discolouration of urine like babesiosis, leptospirosis and bacillary haemglobinuria was ruled out through laboratory tests.

Sample collection

Blood samples were collected from the jugular vein of each animal without anticoagulant. Serum was separated and stored in aliquots at -20°C until analysis for various minerals.

Fifty composite soil samples were collected from the fields, where from green fodder was being fed to diseased buffaloes. Soil samples were collected from randomly selected five sites in each field from 0-15 and 15-30 cm depth. These samples were mixed thoroughly and a composite sample about one kilogram was taken separately for each horizon. These samples were air-dried, crushed, passed through 2 mm sieve and preserved for mineral analysis (Chapman and Pratt, 1961).

Fifty samples of fodders including leaves and stems (about 1 kg each) being fed to the diseased buffaloes were collected, dried at 65°C in a forced draft oven for 48 h, ground and preserved in airtight containers for mineral analysis (Chapman and Pratt, 1961).

Preparation of samples for mineral analysis

Minerals were extracted from soil using the Mehlich-1 extracting solution method (0.05 N HCl+0.025 N H_2SO_4) following the method described by 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. 42). Clear supernatant was obtained by centrifugation for 5 minutes at 180 rpm. The supernatant was stored in plastic bottles for further analysis.

One gram of the dried plant sample was taken in a 50 ml conical flask, and kept overnight after adding 5 ml concentrated nitric acid (HNO_3) and 5 ml perchloric acid ($HClO_4$). Next day, again 5 ml nitric acid was added to each sample. All the samples were digested on hot plate at 250 °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 minerals (AOAC, 1990).

All serum samples were prepared by wet digestion following the procedure of Richards (1968). Briefly, 0.5 ml serum sample was digested with 10 ml concentrated nitric acid in a 100 ml digestion flask first at low temperature for about 15-20 minutes till the contents were clear and then with 5 ml perchloric acid for 15 minutes. The solution in the flask was heated vigorously till 2-3 ml colourless material was left. After cooling, the contents were diluted up to 20 ml with redistilled water in a volumetric flask and preserved for the analysis of minerals.

Mineral analysis

Calcium, copper, iron, molybdenum and selenium were determined by using an atomic absorption spectrophotometer (Varian Spectr AA-5) and the analysis of phosphorus was done with the help of spectrophotometer (Philip Model 1100). Potassium levels were determined with flame photometer (Jenway PFP-7). The concentrations of elements in the diluted samples were measured and the final quantities were computed by comparison of sample reading with standard curves.

Statistical analysis

The data thus obtained were subjected to analysis of variance. Pearson correlations were calculated using Minitab statistical package (Anonymous, 1986). The level of significance was $p<0.05$.

RESULTS

Soil analysis

The difference in the concentrations of macro and micro-nutrients in upper (0-15 cm soil depth) and lower soil surface (15-30 cm soil depth) between districts was statistically non-significant. Therefore, an overall picture of

Table 1. Overall comparison of minerals of upper and lower soil surface of disease prone areas

Element	Depth		SEM	p-value
	0-15 cm (n = 25)	15-30 cm (n = 25)		
Calcium (mg/kg)	260.00^{a}	237.60^{a}	9.060	0.220
Phosphorus (mg/kg)	11.11^{a}	7.48^{b}	0.516	0.000
Potassium (mg/kg)	201.60	178.00	6.440	0.066
Copper (mg/kg)	3.34^{a}	2.96^{b}	0.093	0.043
Iron (mg/kg)	39.17^{a}	27.84^{b}	2.060	0.005
Selenium (mg/kg)	0.31^{a}	0.21^{b}	0.015	0.002
Molybdenum (mg/kg)	1.22^{a}	0.99^{b}	0.050	0.022

Figures (Mean±SD) with different superscripts in a row differ significantly ($p<0.05$). SEM = Standard error of mean.

macro and micro-nutrients was developed (Table 1). The difference in the levels of calcium and potassium between upper and lower soil surfaces were statistically non-significant. The mean values for phosphorus, copper, iron, selenium and molybdenum in upper soil surface were significantly ($p<0.05$) higher than those in lower soil surface (Table 1).

Among macro and micro-nutrients in upper soil surface, calcium was positively correlated with selenium ($r = 0.753$, $p<0.001$) whereas copper was negatively correlated with molybdenum ($r = -0.483$, $p<0.014$). In lower soil surface, calcium was positively correlated with potassium ($r = 0.426$, $p<0.034$) and selenium ($r = 0.588$, $p<0.002$) whereas, it was negatively correlated with molybdenum ($r = -0.419$, $p<0.037$).

Fodder analysis

The concentration of calcium in *Medicago sativa* (lucerne), *Trifolium alexandrinum* (berseem) and *Brassica compestris* (sarson) was significantly ($p<0.001$) higher than in *Zea mays* (maize), *Sorghum vulgare* (sorghum), *Saccharam officinarum* (sugarcane) and *Triticum aestivum* (wheat straw). The concentration of phosphorus in lucerne, sarson and maize was significantly ($p<0.001$) higher than in berseem, sorghum, sugarcane and wheat straw. The level of potassium in lucerne, berseem, maize and sarson was significantly ($p<0.001$) higher than in sorghum, sugarcane and wheat straw. The copper concentration in berseem, lucerne and sugarcane was significantly ($p<0.001$) higher than in sorghum, sarson, maize and wheat straw (Table 2).

Iron contents in lucerne, berseem and wheat straw were significantly ($p<0.001$) higher as compared to sarson, maize, sorghum and sugarcane. The concentration of selenium in lucerne and berseem was significantly ($p<0.001$) higher than in maize, sorghum, sugarcane and wheat straw. Selenium contents in sarson showed non-significant difference as compared to contents in leucerne, berseem, maize and sorghum. The concentration of molybdenum in lucerne and berseem was significantly ($p<0.001$) higher as compared to that in maize, sugarcane and wheat straw (Table 2).

Serum minerals profile

Serum phosphorus, copper and selenium were significantly ($p<0.001$) lower, whereas potassium, iron and molybdenum ($p<0.001$) were higher in buffaloes suffering from PHU than healthy buffaloes (Table 3). Calcium concentration did not vary between two groups.

DISCUSSION

Calcium

Level of calcium in serum of the PHU affected buffaloes and in soil was adequate. Dietary requirement of calcium for dairy cattle is 0.43-0.60% dry matter (DM) basis (NRC, 2001). The concentration of calcium was adequate in lucerne, berseem, sarson and sorghum, while maize, sugarcane and wheat straw did not meet the required levels meant for dairy animals (Table 2). The calcium level in plants varies with pH of soil, extent of liming, and concentration of Mg in the soil. Legumes have higher level of calcium than cereal grasses and maize (Georgievskii et al., 1982; Sarwar et al., 2005).

Phosphorus

Dietary requirement of phosphorus for dairy cattle is 0.31-0.40% DM basis (NRC, 2001). None of the fodders

Table 2. Mineral composition of fodders (on dry matter basis) used to fed to haemoglobinuric buffaloes

Fodders	Calcium (%)	Phosphorus (%)	Potassium (%)	Cupper (mg/kg)	Iron (mg/kg)	Selenium (mg/kg)	Molybdenum (mg/kg)
Berseem	1.51^{a}	0.16^{a}	2.09^{a}	8.77^{a}	138.83^{a}	0.61^{a}	4.29^{a}
Sarson	1.18^{b}	0.20^{b}	1.93^{a}	6.91^{b}	61.22^{b}	0.56^{ab}	3.78^{ab}
Maize	0.40^{c}	0.19^{b}	2.05^{a}	5.75^{ab}	57.17^{b}	0.48^{bc}	3.37^{b}
Sorghum	0.45^{c}	0.11^{c}	1.72^{ab}	7.41^{c}	63.14^{b}	0.51^{b}	3.87^{ab}
Sugarcane	0.32^{c}	0.12^{c}	1.53^{b}	7.98^{a}	63.75^{b}	0.39^{c}	3.13^{b}
Lucerne	1.96^{d}	0.20^{b}	2.36^{a}	8.53^{a}	178.00^{c}	0.69^{a}	4.48^{a}
Wheat straw	0.27^{c}	0.07^{d}	1.28^{c}	4.05^{d}	104.75^{d}	0.15^{d}	1.93^{c}
SEM	0.085	0.006	0.062	0.207	5.980	0.022	0.125
p-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Figures (Mean±SD) with different superscripts in a column differ significantly ($p<0.001$). SEM = Standard error of mean.

Table 3. Serum minerals profile in healthy and PHU affected buffaloes

Parameters	Healthy	Haemoglobinuric	SEM	p-value
Calcium (mg/dl)	9.83^a	9.86^a	0.105	0.907
Inorganic phosphorus (mg/dl)	5.41^a	1.88^b	0.172	0.000
Potassium (m mol/L)	4.46^a	13.75^b	0.433	0.000
Copper (μg/dl)	118.36^a	65.38^b	2.480	0.000
Iron (μg/dl)	161.18^a	217.59^b	2.85	0.000
Selenium (μg/dl)	18.42^a	12.27^b	0.445	0.000
Molybdenum (μg/dl)	54.82^a	171.53^b	6.530	0.000

Figures (Mean±SD) with different superscripts in a row differ significantly ($p<0.001$). SEM = Standard error of mean.

offered to the diseased animals met the dietary requirement of phosphorus in the present study (Table 2). During soil analysis it was noted that these fodders were grown on phosphorus deficient soils (Table 1). Therefore, it was not astounding to find hypophosphataemia in PHU affected buffaloes (Table 3). Nagpal et al. (1968) related extremely low inorganic phosphorus in the sera of buffaloes suffering from haemoglobinuria to low to medium phosphorus contents of soils in Indian Punjab and feeding of Berseem and Brassica which contribute to hypophosphataemia. Molybdenum content is high in the soils in Indian Punjab. The fodders in particular berseem grown on such soils have high molybdenum contents. The excess of this element reduces the phosphorus contents of the body by interfering with its absorption from the gastrointestinal tract and also increased phosphorus elimination through urine which may lead to hypophosphataemia (Dhillon et al., 1972) as has been observed in PHU affected buffaloes.

Haemoglobinuria mainly due to haemolytic anaemia has been associated with dietary phosphorus deficiency (Stockdale et al., 2005). It has been suggested that phosphorus deficient soils and drought conditions are responsible for low herbage phosphorus concentrations. Phosphorus deficient soils are most common in dry tropical regions. High levels of calcium, iron, and aluminum in soil form insoluble complexes with phosphorus, making it unavailable for plant use, results in low phosphorus contents in plants and hypophosphataemia in animals which could be predisposing cause for haemoglobinuria. Dry mature forages and drought damaged forages may be low in phosphorus even on soils with acceptable phosphorus contents (Radostits et al., 2000). Moreover, heavy drainage of phosphorus through milk particularly in high producing animals leads to hypophosphataemia if their ration is also deficient in phosphorus (Bhikane et al., 1995).

Potassium

Dietary requirement of potassium for dairy cattle is 0.80% DM basis (NRC, 2001). As the concentration of potassium in soil was high, none of the fodders offered to the diseased animals was deficient in potassium (Table 2). The serum potassium concentration in PHU affected buffaloes was significantly higher than that in healthy buffaloes. The increase in potassium could be attributed to cell membrane damage, probably as a result of hypoxia that results in efflux of intracellular K^+ to the extracellular fluid, and necrosis of large mass of tissues, especially muscles, may release a large amount of K^- and produce hyperkalemia (Singh et al., 1999; Latimer et al., 2003).

Copper and molybdenum

Dietary requirement of copper and molybdenum for dairy cattle is 10 mg/kg (NRC, 2001) and 0.2-7 mg/kg (Church, 1988), respectively. None of the fodders offered to the diseased buffaloes met the dietary requirement of copper whereas molybdenum contents in all fodders were adequate (Table 2). In the present study, the copper contents in both soil surfaces were high. Similar results have been reported by Ogebe and McDowell (1998). Copper contents vary with the plant species, soil type, vegetative stage and the use of copper containing fertilizers (Georgievskii et al., 1982). In both soil surfaces, the molybdenum contents were adequate for plant growth. The findings of the present study were in agreement with those of Rashid and Memon (2001). They reported that there are adequate quantities of molybdenum in all soils of Pakistan because of alkaline in nature.

The concentration of serum molybdenum and copper were significantly higher and lower in the PHU affected buffaloes than in healthy buffaloes, respectively (Table 3). Significantly decreased copper in PHU affected buffaloes in the present study could be attributed to a three-way interaction between copper, molybdenum and sulfur. As reported by Suttle (1991), this interaction can occur with concentrations of molybdenum and sulfur that are naturally present in feedstuffs and are involved in the formation of thiomolybdates in the rumen. Sulfides are produced by the rumen microorganisms via reduction of sulphate and also degradation of sulfur amino acids. These sulfides react with molybdate to form thiomolybdates that bind with copper lead to form highly insoluble complexes that do not release copper even under acidic conditions, and render it unavailable for the animals, resulting in copper deficiency (Allen and Gawthorne, 1987).

Iron

Dietary requirement of iron for dairy cattle is 35 mg/kg DM basis (NRC, 2001). None of the fodders offered to the diseased animals was deficient in iron rather were having excess in the present study (Table 2). Higher iron contents in lucerne, berseem and wheat straw have also been reported by Malik and Chughtai (1979). Iron contents in plant vary with the species (leguminous grasses contain more Fe than cereal grasses), vegetative stage (decreases with age), soil type and environmental pollution (Sarwar and Hasan, 2001). Iron contents in both soil surfaces were high. Similar findings have been reported by Ranjha et al. (1987), Baig et al. (1990) and Khan et al. (2006). These results are supportive to the report of McDowell et al. (1984) in which it was indicated that iron deficiency is rare in grazing animals due to generally adequate contents in soils and forages.

In the present study, the serum iron concentration was significantly higher in haemoglobinuric buffaloes as compared to healthy buffaloes. The higher concentration of serum iron could be due to acute intravascular haemolysis (Latimer et al., 2003).

Selenium

Dietary requirement of selenium for dairy cattle is 0.10 mg/kg DM (NRC, 2001). None of the fodders offered was deficient in selenium rather were having excess (Table 2). Among crops brassicas and legumes contain higher selenium than other crops. The contents of selenium in both soil surfaces were adequate (Table 1). These findings are in accordance with those of Tisdale et al. (1993) who reported that high pH, and calcareous soils in regions of low rainfall are usually high in selenium. Though the concentration of selenium in PHU affected buffaloes was significantly lower than in the healthy buffaloes (Table 3), this level was within the normal dietary requirement. Blood levels of selenium are variable, depending upon dietary intake (Tian et al., 2006). The low levels of selenium in buffaloes suffering from haemoglobinuria could be due the progressive loss of appetite and decreased feed intake (Singari et al., 1989).

Implications

It was concluded from the present study that all minerals in relation to plants requirements were adequate in the both soil surfaces except phosphorous that was deficient in lower soil surface. None of the fodders offered to the diseased animals met the dietary requirements of phosphorus and copper. Serum phosphorus, copper and selenium were significantly ($p<0.001$) lower whereas potassium, iron and molybdenum in buffaloes suffering from PHU were significantly ($p<0.001$) higher than in healthy buffaloes. It was concluded that phosphorous deficient soils play a major role by transferring this deficiency to plants and ultimately reaching to destination i.e., animals where hypophosphataemia is a consistent finding. Other significant findings were hypocupraemia, hypermolybdenaemia, and haemoglobinuria.

REFERENCES

Allen, J. D. and J. W. Gawthorne. 1987. Involvement of the solid phase of rumen digesta in the interaction between copper, molybdenum and sulphur in sheep. Br. J. Nutr. 58:265-276.

Anonymous. 1986. MSTAT-C Version 4.00/EMRevised 7/1/86 by Dept. of Crop and Soil Sciences, Michigan State University, USA.

AOAC. 1990. Official Methods of Analysis. Association of Official Analytical Chemists. 15th ed. Arlington, Virginia, USA.

Baig, M. B., M. S. Zia, M. I. Nizami and M. Salim. 1990. Micronutrient status of selected benchmark soils in Punjab and their relationship with soil properties. Pak. J. Agri. Sci. 27:39-42.

Bhikane, A. U., M. S. Ali, B. W. Narladkar and S. B. Kawitkar. 1995. Post-parturient haemoglobinuria in a crossbred cow and its treatment. Indian Vet. J. 72:734-736.

Chapman, H. D. and P. F. Pratt. 1961. Methods of Analysis for Soils, Plants, and Waters. Division of Agricultural Sciences. University of California.

Chugh, S. K., R. M. Bhardwaj and M. M. Mata. 1998. Lowered antioxidant status of red blood cells in post-parturient haemoglobinuria of buffaloes. Vet. Res. Commun. 22:385-388.

Church, D. C. 1988. The Ruminant Animal Digestive Physiology and Nutrition. Prentice Hall, Englewood Cliffs, New Jersey.

Dhillon, K. S., J. Singh and R. Bajwa. 1972. Treatment of haemoglobinuria due to molybdenum induced phosphorus deficiency in buffaloes. India J. Anim. Sci. 42:996-998.

Digraskar, S., B. Singh and B. B. Deshpande. 1991. Epidemiology and clinico-pathology of haemoglobinuria in buffalo (*Bubalus bubalis*). Livestock Advisor 16:32-38.

Ellison, R. S., B. J. Young and D. H. Read. 1986. Bovine post-parturient haemoglobinuria: Two distinct entities in New Zealand. New Zealand Vet. J. 34:7-10.

Georgievskii, V. I., B. N. Annenkov and V. T. Samokhin. 1982. Mineral Nutrition of Animals. Butterworths, London.

Han, B., S. S. Yoon, J. Su, H. R. Han, M. Wang, W. Qu and D. Zhong. 2004. Effects of selenium, copper and magnesium on antioxidant enzymes and lipid peroxidation in bovine fluorosis. Asian-Aust. J. Anim. Sci. 17:1695-1699.

Han, B., S. S. Yoon, P. F. Wu, H. R. Han and L. C. Liang. 2006. Role of selenium in alteration of erythrocyte parameters in bovine fluorosis. Asian-Aust. J. Anim. Sci. 19:665-712.

Jubb, K. V. F., P. C. Kennedy and N. Palmer. 1993. Pathology of Domestic Animals. 4th Ed. Academic Press, Inc. New York, USA.

Khan, Z. I., A. Hussain, M. L. Ashraf and R. McDowell. 2006. Mineral status of soils and forages in Southwestern Punjab-Pakistan: Micro-minerals. Asian-Aust. J. Anim. Sci. 19:1139-1158.

Latimer, K. S., E. A. Mahaffey and K. W. Prasse. 2003. Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology. 4th ed. Iowa State Press, Ames, Iowa.

Malik, M. Y. and M. I. D. Chughtai. 1979. Chemical Composition and Nutritive Value of Indigenous Feedstuffs. Pak. Assoc. for the Advancement of Sci., Lahore, Pakistan.

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 (Ed. F. M. C. Gilchrist and R. I. Mackie), Craighall, South Africa.

McDowell, L. R. and G. Valle. 2000. Major mineals in forages. In: Forage Evaluation in Ruminant Nutrition (Ed. D. I. Givens, E. Owen, R. F. E. Oxford and H. M. Omed). p. 373. CAB International, Wallingford, UK.

Nagpal, M., O. P. Gautam and R. L. Gulati. 1968. Haemoglobinuria in buffaloes. Indian Vet. J. 45:1048-1059.

NRC. 2001. Nutrient Requirements of Dairy Cattle, 7th ed. National Academy Press, Washington, DC.

Ogebe, P. O. and L. R. McDowell. 1998. Mineral concentrations of forages grazed by small ruminants in the wet season in Benue State, Nigeria. II. Trace minerals and forage crude protein. Commun. Soil Sci. Plant Anal. 29:1211-1220.

Pirzada, W. H. and S. Z. Hussain. 1998. Parturient haemoglobinuria in buffaloes: a review. Trop. Anim. Health Prod. 30:209-215.

Radostits, O. M., C. C. Gay, D. C. Blood and K. W. Hinchcliff. 2000. Veterinary Medicine: a Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9th ed. Saunders, London.

Ranjha, A. M., M. B. Baig, I. Iqbal, A. Jabbar and M. B. Tahir. 1987. Micronutrient status of soils in the Thikriwala project area of Faisalabad district. Pak. J. Sci. Ind. Res. 30:100-102.

Rashid, A. and K. S. Memon. 2001. Soil Science. National Book Foundation, Islamabad.

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.

Richards, L. A. 1968. Diagnosis and Improvement of Saline and Alkaline Soils. 1st Ed. Agri. Handbook No. 60. IBH Pub. Co. New Delhi, India.

Sarwar, M. and Z. U. Hasan. 2001. Nutrient Metabolism in Ruminants. Friends Science Publishers, Faisalabad, Pakistan.

Sarwar, M., M. A. Khan, M. U. Nisa and N. A. Touqir. 2005. Influence of berseem and lucerne silages on feed intake, nutrient digestibility and milk yield in lactating Nili buffaloes. Asian-Aust. J. Anim. Sci. 18:475-478.

Singari, N. A., R. M. Bhardwaj and S. K. Chugh. 1989. Erythrocytic reduced glutathione instability in post parturient haemoglobinuria of buffaloes. Indian Vet. J. 66:405-409.

Singh, S. P., N. Kumar, K. B. Sharma and R. Kumar. 1999. Serum and CSF mineral profile of Himalayan yak (*Bos grunniens*) in their natural habitat. Asian-Aust. J. Anim. Sci. 12:189-191.

Stockdale, C. R., T. E. Moyes and R. Dyson. 2005. Acute post-parturient haemoglobinuria in dairy cows and phosphorus status. Aust. Vet. J. 83:362-366.

Suttle, N. F. 1991. The interactions between copper, molybdenum and sulphur in ruminant nutrition. Annu. Rev. Nutr. 11:121-140.

Tian, J. Z., M. S. Yun, C. S. Kong, L. G. Piao, H. F. Long, J. H. Kim, J. H. Lee, J. S. Lim, C. H. Kim, Y. Y. Kim and In K. Han. 2006. Effects of different products and levels of selenium on growth, nutrient digestibility and selenium retention of growing-finishing pigs. Asian-Aust. J. Anim. Sci. 19:61-66.

Tisdale, S. L., W. L. Nelson, J. D. Beaton and J. L. Havlin. 1993. Soil Fertility and Fertilizers. 5th ed. Macmillan Publishing Co. New York, USA.