PARALYTIC DISORDERS ASSOCIATED WITH PHOSPHORUS DEFICIENCY IN BUFFALOES

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ABSTRACT

An investigation was carried out during the month of May to August 2001 to diagnose hind limbs paralysis in buffaloes of Buner area of NWFP. Serum concentrations of calcium (Ca) and phosphorus (P) in 40 buffaloes (29 affected and 11 normal) from six different villages were measured together with feed and soil samples analysed for different macro and micro minerals. Serum P level was lower ($P<0.001$) in affected buffaloes and averaged 3.05 mg/100 ml of serum against 6.73 mg/100 ml found in the normal buffaloes. Serum Ca level was in normal range and did not differ between affected and normal buffaloes (12.16 and 12.93 mg/100 ml, respectively). Range grass hay, the main feed offered to the animals during dry season of high disease incidence, was extremely low in P (0.10mg/100g) and sodium (0.03g/100g), with adequate level of Ca (0.41 g/100 g). Concentrations of potassium (K) and magnesium (Mg) in range grass hay were very high and averaged 5.0 and 0.34g/100 g, respectively. Copper (Cu), manganese (Mn) and iron (Fe) contents in the hay were 28.50, 113.0 and 242.0 μg/g, respectively and were in excess of the recommended dietary requirements for cattle. However, zinc (Zn) concentration (42.33 μg/g) in the hay was marginally adequate to meet the dietary requirement of dairy cattle. The mineral profile of hay was in close agreement with that of soil in the area. The results concluded that deficiency of P in hay, which might have resulted due to low P in soil, ascribed low serum P in buffaloes and might be the major cause of the disease. The imbalance of other minerals in hay and soil might have also reduced the bioavailability of P to plants and animals. *In vitro* matter digestibility (38.1%), crude protein (5.63% in DM), and metabolizable energy (1.32 Mcal/kg DM) values of the hay offered to animals were below the standard requirements and caused general emaciation of the buffaloes during dry seasons. Correction of the hind limbs paralysis in buffaloes would require both P supplementation and improvement in nutritive value of range grass hay. Feeding of molasses-urea blocks fortified with deficient minerals is suggested as appropriate strategic supplement for correcting the health disorder in buffaloes of the Buner area.

Key words: Phosphorus-deficiency, minerals, buffaloes, paralytic disorder.

INTRODUCTION

Mineral deficiency and imbalance in livestock is a wide spread problem in the tropical and subtropical areas including Pakistan (McDowell, 1992; Hanjra *et al*., 1996). The problem appears to be more prevalent in the hilly regions of Pakistan, particularly in North West Frontier Province (NWFP), where fluctuating nutrient supply and myths-based poor management are the characteristic features of mountainous livestock farming system. Mineral composition of soil and plants in hilly areas is highly variable due to large differences in geophysical conditions. Many grass species during early vegetative stage may be adequate in nutrients including minerals but are available for a short period and for a greater part of the year livestock subsist on mature grasses. Shortage of green forages is mostly experienced during summer (May to August) and winter (November to February) seasons and animals are mostly fed mature range grass hay and crop residues. Most of the farmers cannot afford to feed expensive concentrate supplements.

Feed availability situation in the hilly regions of Pakistan does not justify keeping high nutrient demanding livestock such as buffaloes and crossbred cows. Yet a continuos increase in buffalo population has been observed during the last two decades in these areas of NWFP (GOP, 1998). This has caused several health and reproductive problems in these animals. Recently, farmers from Buner district of NWFP reported occurrence of hind leg paralysis in livestock, especially in milking and pregnant buffaloes during dry season when no green fodder was available. Similar problem was also reported from other dry areas of NWFP. A colloquial term "shalley" means paralysis, is used by the local farmers to describe the condition in animals and is characterized by progressive weakness, normal or depraved appetite, stiff joints and

disinclination to walk. The condition is occasionally preceded by haemoglobinuria without pyrexia.

In response to farmer's concern, the present investigation was initiated to explore relationship of blood profile of Ca and P in affected buffaloes with occurrence of this paralytic disorder in buffaloes. Mineral composition of soil and feed was also determined to help diagnosis.

MATERIALS AND METHODS

Collection of blood, feed and soil samples

Blood samples (about 10 ml) from jugular vein were collected from 29 affected and 11 apparently normal buffaloes in six villages of Nagrai Union Council, of District Buner, NWFP. Blood in the tubes was allowed to clot, serum was separated through centrifugation at 3000 rpm for 5 minutes and stored at -4^{o}C until analysed. Samples of range grass hay (about 500 g) were collected from different farms in the study area. Core samples of soil at about 10 cm depth (Rashid, 1996) were also randomly collected from all the six villages. Owners of the diseased animals were interviewed and their perceptions of the problem were recorded.

Laboratory analysis

Calcium and inorganic phosphorus concentrations in the serum samples were analyzed with colorimetric method using diagnostic kits (Clonital, New Jersey, USA). Feed samples were digested with nitric acid and perchloric acid. Soil samples were extracted with AB-DTPA (Rayment and Higginson, 1992). Magnesium (Mg), potassium (K) and sodium (Na) were measured on flame photometer. The concentrations of copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe) in the feed and soil samples were measured on atomic absorption spectrophotometer. Nitrogen in feed and soil samples was analyzed with the Kjeldhal procedure of AOAC (1990). *In vitro* dry matter digestibility of the feed samples was determined with the method of Tilley and Terry (1967) and metabolizable energy contents were calculated from *in vitro* digestibility values, as suggested by MAFF (1984).

Statistical analysis

The data were analysed with analysis of variance using the general linear model procedure of SAS (2000). The main effect for blood profile of calcium and phosphorus included health condition (normal or diseased), production status (dry or lactating), location (6 villages from where samples were collected) and interaction of these. Means were compared with Duncan Multiple Range Test (SAS, 2000).

RESULTS

Blood profile of Ca and P

Blood profile of Ca and P in normal and affected buffaloes is summarized in Table 1. Phosphorus concentration in affected buffaloes was significantly lower ($P<0.001$) than that of normal buffaloes and averaged 3.05 and 6.73 mg/100 ml serum, respectively. Phosphorus levels in affected buffaloes dropped to a critically low level of 1.07mg/100 ml of serum, with a maximum concentration recorded as 4.68 mg/100 ml.

Table 1: Blood serum profile of calcium and phosphorus in healthy and buffaloes suffering from paralytic disorder

Parameters	Mean values (mg/100 ml serum)	Range (mg/100 ml serum)	CV (%)
Calcium			
Normal (healthy)	12.16[a]	8.79 - 18.02	27.02
Diseased	12.92 [a]	6.78 - 23.60	27.17
Phosphorus			
Normal (Healthy)	6.73[a]	5.62 - 9.96	26.90
Diseased	3.05[b]	1.07 - 4.68	28.37

Mean values with different letter for each parameter differ significantly ($P<0.001$).

Calcium concentration in blood was not different ($P>0.05$) between the normal and affected buffaloes and averaged 12.16 and 12.92 mg/100 ml serum, respectively. Both Ca and P levels were not influenced by location ($P>0.05$) and production status (lactating or pregnant) of the buffaloes. There was also no interaction of production status and health condition of the buffaloes.

Nutritive value and mineral composition of feed

Range grass hay was the major feed offered to the buffaloes during the period when the occurrence of the disorder was high in the area. Nutritive value of the hay was found poor, as indicated by low nitrogen contents (0.96% in DM), low *in vitro* DM digestibility of 38.1% that reflected in low metabolizable energy of 1.17 Mcal/Kg DM (Table 2).

Mineral composition of the hay along with recommended dietary requirements for a lactating cow are given in Table 2. The hay was found adequate in Ca (0.41 g/100g) and Zn (42.33 μg/g), deficient in P (0.13 g/100g) and Na (0.03g/100g) and high in Mg (0.34g/100g), K (5g/100g), Cu (28.50μg/g), Mn (113

μg/g) and Fe (242μg/g). Mineral composition of hay largely varied among the samples collected from different locations, as indicated by their wide range in Table 2.

Mineral status of soil

Mineral concentrations in soil samples of the affected area along with a standard composition of a normal soil are presented in Table 3. The concentration of macro-minerals viz. Ca, P, Na, K and Mg were 9.16, 1.28, 62.50, 78.00 and 242 μg/g, respectively. Trace elements levels as μg/g in the soil were Cu (3.23), Zn (12.84), Mn (3.08) and Fe (21.53). Nitrogen contents, as indicative of soil fertility, ranged from 0.17 to 0.29 and averaged 0.23 g/100g soil.

DISCUSSION

Low P level in the blood of affected buffaloes suggested that hypophosphataemia may be the main causal factor of the observed paralytic syndrome in the buffaloes. Normal blood concentration of inorganic P was reported as 6.0-6.69 mg/100 ml of serum in healthy buffaloes (Nagpal *et al*., 1968; Patil and Jone, 1970). In the present study, P level in the normal buffaloes averaged 6.73 mg/100 ml of serum, while mean serum P concentration in the affected buffaloes was 3.05 mg/100 ml with a minimum value of 1.07 mg/100ml. In line with the present findings, Nagpal *et al*. (1968) reported that in buffaloes suffering from P deficiency, the inorganic P contents in serum reduced markedly to

Table 2: Mean concentrations of nitrogen and minerals in range grass hay and standard dietary requirements (on dry matter basis)

Parameters	Range grass hay	*Recommended dietary* concentrations for milking cows	Difference (%)
	Mean (range)	Mean	
Nitrogen (g/100g)	0.96 (0.88 -.94)	1.92	- 50
In Vitro DM digestibility (%)	38.10 (36.5 -39.7)	-	-
Metabolizable energy (Mcal/Kg DM)	1.17 (1.11-1.22)	2.35	-
Calcium (g/100g)	0.41 (0.25 - 0.58)	0.43	0
Phosphorus (g/100g)	0.010 (0.011-0.010)	0.28	- 53
Sodium (g/100g)	0.03 (0.02 - 0.03)	0.18	- 83
Magnesium (g/100g)	0.34 (0.26 - 0.50)	0.20	+70
Potassium (g/100g)	5.00 (1.68 -11.45)	0.90	+455
Copper (μg/g)	28.50 (8.60 - 48.40)	10	+185
Zinc (μg/g)	42.33 (30.20-54.40)	40	+ 5
Manganese (μg/g)	113.00 (83 -142)	40	+183
Iron (μg/g)	242.00 (223 -253)	50	+384

* Source = NRC (1998)

Table 3: Nitrogen and minerals levels in soil of the study area (on dry matter basis)

Parameters	Soil of the study area	*Approximate composition of normal soil
	Mean (range)	Range
Nitrogen (g/100g)	0.23 (0.17-0.29)	0.10-0.50
Calcium (μg/g)	916 (683 -1306)	500-700
Phosphorus (μg/g)	1.28 (0.65 -2.52)	5-10
Sodium (μg/g)	62.50 (59.20-61.10)	0.05-0.23
Magnesium(μg/g)	78.00 (56-94)	150-200
Potassium (μg/g)	242.67 (123-364)	120-130
Copper (μg/g)	3.23 (1.80-4.14)	0.30-0.50
Zinc (μg/g)	12.84 (9.74-14.82)	0.90-1.50
Manganese (μg/g)	3.08 (2.08-4.60)	0.60-0.10
Iron (μg/g)	21.53 (15.30-25.20)	3.00-5.00

* Source: Rashid, A. (1996) and Shah, Z. (Personal communication).

a mean value of 1.76 mg /100 ml and ranged from 1.22 to 3.55 mg/100 ml. Unlike Ca, blood inorganic P is not regulated by homeostatic control mechanism and declines to a sub-optimum level when animals are fed P deficient diets (NRC, 1988). In the study area, buffaloes were fed P deficient hay containing 0.132g/100g phosphorus, as a main feed. To meet animal requirements of P from forage source only, a level of 0.28g/100g DM in the feed is required (NRC, 1988), thus P deficiency of 53% in the diet was calculated (Table 2). Inadequate P in the hay was in close conformity with the soil composition in the area (Table 3).

In the present study, the blood Ca level was not affected by the disease condition and remained the same in normal and affected buffaloes (12.16 and 12.92 mg/100 ml of serum, respectively). This supports the findings of Nagpal *et al*. (1968) and Malik and Gautam (1971), who reported that serum Ca level in hypophosphataemic buffaloes might not be necessarily in the normal range of healthy animals. Serum Ca concentrations observed in the present study were similar to those reported by Qureshi *et al.* (1999) in lactating buffaloes.

Both Ca and P are closely inter-dependent and for optimum metabolism, NRC (1988) has suggested a dietary Ca/P ratio of 1.4:1. In the present study, a Ca/P ratio of 3:1 was observed in the range grass, thus the deficiency of P may have limited the utilization of Ca in the body and may have induced secondary deficiency of Ca even in the presence of a normal blood Ca level in the affected buffaloes.

Both Ca and P are important for skeleton and muscle functions in the animal body and chronic P deficiency was reported to cause stiff joints in farm animals (Georgievskii, 1982; NRC, 1988). In the present study, features of the syndrome included disinclination to move due to stiff joints especially of the fore legs that were kept abducted, abnormal posture with straighten neck and prominent scapula accompanied by progressive emaciation with normal appetite. In some cases, as reported by farmers, haemoglobinuria preceded the above symptoms which further added to the severity of the disease. In severe cases, the buffalo preferred lying down which often led to death. Lactating buffaloes were found highly susceptible to the disease, followed by pregnant buffaloes, presumably due to high demand of Ca and P in such animals. The reason for absence of the disease in cows is not yet known. However, it can be assumed that cows kept in the area were mainly indigenous type with low productive performance (1-2 liter of milk/day) and may, therefore, have lower demand for P. It can also be argued that local cattle may have developed better adaptability to mobilize their mineral reserves as compared to buffaloes which are not native to the area but imported/brought from the plain areas, hence may not be able to utilize their body reserve of Ca and P. The possibility of species difference in homeostasis of P cannot be ruled out (Georgievskii, 1982).

Although, all the affected buffaloes in six villages had consistently sub-optimum concentrations of serum P, severity of the disease varied due to variable serum P concentration in affected buffaloes. Blood and Radostits (1989) stated that signs of hypophosphatemia might occur when normal blood P levels have fallen from the normal level of 4.5 mg/100 ml to 1.5 mg/100 ml of serum. In severe cases, P level may fall to 1 mg/100 ml. In the present study, serum P levels were in the range of 1.07-to 4.68 mg/100 ml, with an average value of 3.05 mg/dl in the affected buffaloes.

Farmers in the area reported that hind limbs paralysis in buffaloes was observed more frequently during feed scarcity periods of summer and winter and the syndrome subsided with the availability of green fodder. As stated earlier, consumption of P deficient hay as the sole feed may explain the prevalence of the disease during dry periods; the poor nutritive value of the hay was also presumably an accentuating factor. Sample analysis of the hay revealed low energy and low nitrogen contents (Table 2) that primarily contributed to debilitating condition of the affected buffaloes. Very low digestibility of the hay might have further reduced the bioavailability of P and other minerals in the affected animals. Low P level in diet can also influence the cellulolytic activity of rumen microbes (Bryant, 1973). It is, therefore, inferred that correction of paralytic disorder in buffaloes would require both P supplementation and up-gradation of feeding value of poor quality roughages to improve energy and protein supply to the animals.

An imbalanced proportion of different minerals in the range grass hay, as depicted in Table 2, may have also impeded their utilization due to antagonism phenomenon explained by Georgievskii (1982). The high K level of 5 g/100 g against the required level of 0.90 g/100g may have interfered P absorption and the same would be expected with excessive Fe concentration found in the hay. This may have further accentuated P deficiency in the buffaloes. Low dietary sodium as found in the hay, was reported to cause muscles weakness (Blood and Radostits, 1989), a factor that may have added to locomotion problem in the P deficient buffaloes. The hay contained excessive amount of Cu (28 μg/g). If high level of Cu in diet can cause haemolysis of erythrocytes (Georgievskii, 1982) then it could be assumed that excessive Cu intake may have contributed to the development of haemo-globinuria in some P deficient buffaloes. Blood and

Radostits (1989) reported that competition for absorption between P and Cu might result in P deficiency in animals.

The above observations suggested that deficiency of P in the range grass hay together with imbalance of macro and micro minerals in the hay were responsible for the low serum P levels that caused the paralytic syndrome in the local buffaloes.

Mineral composition of hay was apparently related to mineral profile of the soil. As found in the hay, the soil samples collected from the affected area were also found low in P but were adequate in Ca. The concentrations of K, Mg, Mn, Cu and Fe were found in excess of that reported in normal soil (Table 3). McDowell (1992) reported that generally, mineral composition of plants and soil of the same area are positively correlated. Thus, it could be argued that deficiency of P and imbalance of minerals in the soil were the root cause of the paralytic problem in buffaloes of Buner area of NWFP.

It is suggested that strategies to supplement P and or improve its bioavailability in animals through minimizing mineral imbalances and better utilization of local forages need to be developed. Molasses-urea blocks fortified with deficient minerals that also simultaneously improve fiber digestion could be a suitable approach to control the paralytic disorder in buffaloes of Buner area of NWFP. However, the real value of this strategy as yet remains to be tested.

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REFERENCES

AOAC, 1990. Official Methods of Analysis (13th ed). Hoerwitz, W. (de). Association of Analytical Chemists. Washington D.C., U.S.A.

Blood, D.C. and O.M. Radostits, 1989. Veterinary Medicine. Balilliere Tindall, London.

Bryant, M.P., 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. Fed. Proc., 32: 1809-1812.

Georgievskii, V.I., 1982. The physiological role of macro elements. In: Mineral Nutrition of Animals. Georgievskii, V.I., B.N. Annekov, and V.T. Samokhin (authors). Butterworth, London.

GOP, 1998. Livestock Census –1996, NWFP. Vol-II, Part-I. Agric. Census Org. Statistics Div. Govt. Pakistan, Gulberg, Lahore.

Hanjra, S.H., A. Iqbal and J. M. Hayat, 1996. Mineral deficiencies in grazing sheep in Pakistan. In: Mineral Problems in sheep in Northern China and Other Regions of Asia. (Editors: Masters D.G., Yu Shunxiang, Lu De-Xun and White, C.L.), ACIAR Proceedings No.73, ACIAR, Canberra, Australia. pp: 61-67.

MAFF, 1984. Energy Allowances and Feeding Systems for Ruminants. Ministry of Agric., Fisheries and Food, London Her Majesty's Stationery Office. pp: 71-73

McDowell, L.R., 1992. Minerals in Animal and Human Nutrition. Academic Press Inc. San Diego, California, USA.

Malik, K. S. and O. P. Gautam, 1971. Haemoglobinuria in buffaloes. Hariana Agric. Univ. J. Res., 1: 109-113

Nagpal, M., O. P. Gautam and R. L. Gulati, 1968. Haemoglobinuria in buffaloes. Indian Vet. J., 45: 1048-1058

NRC, 1988. Nutrient Requirements of Dairy Animals. National Research Council, National Academy Press, Washington, D. C.

Patil, B. D. and D. I. H. Jones, 1970. The mineral status of some temperate herbage varieties in relation to animal performance. In: Proceedings of the11th International Grassland Congress, Surfers Paradise, Australia. pp: 726-730.

Qureshi, M. S., M. M. Siddiqui, S. Khattak, G. Habib, H. A. Samad, H. A. Shamroz, and N. Bano, 1999. Reproductive-nutrition interaction in dairy buffaloes. III. Effect of macro minerals intake and blood profile. Egyptian J. Dairy Sci., 28 (1): 109-119.

Rashid, A., 1996. Soils: Basic concepts and principles. In: Soil Science (Editors: A. Rashid and K.S. Memon). National Book Foundation, Islamabad. pp: 3-23.

Rayment, G. E and F. R. Higginson, 1992. Australian Laboratory Handbook of Soil and Water: Chemical Methods. Inkatta Press, Melbourne, Australia.

SAS. 2000. SAS/Stat guide for personal computers. SAS Inst. Inc., Cary, N. C. USA.

Tilley, J. M. A. and R. A. Terry, 1967. A two-stage technique for the *in vitro* digestion of forage crops. J. British Grassland Soc., 18: 104-108.