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Research Article

STUDIES ON THE EFFECT OF DIETARY SODIUM FLUORIDE ON SERUM MINERALS AND ALKALINE PHOSPHATASE ACTIVITY IN BUFFALO CALVES

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ABSTRACT

An experiment was carried out to evaluate the effect of high dietary fluoride intake on serum minerals and alkaline phosphatase activity (ALP) in male murrah buffalo calves of 6-8 months age. Eight calves (4 in each group) were selected from the Murrah herd according to their body weight. A normal farm ration was fed in control group while in another group sodium fluoride was added to the ration so as to make ration containing 60 ppm fluoride on dry matter basis. The animals were fed for a period of 3 months and a metabolic trial was conducted at the end of study. The study revealed that dietary fluoride caused significant decline ($P < 0.05$) in serum Ca and Zn on day 90 but serum ALP activity increased significantly ($P < 0.05$). However, serum Fe and Cu remained unaffected. The data suggest that 60 ppm fluoride has an adverse effect on serum minerals and ALP activity in buffalo calves.

Key words: Alkaline phosphatase activity, fluoride, minerals, buffalo

Domestic animals are prone to fluorosis due to the ingestion of high fluoride containing water, pasture, grass, mineral supplements and fodder grown near industrial polluted areas (Wheeler and Fell, 1983). Several researchers have reported osteodental fluorosis in buffaloes and cattle (Shupe, 1980, Choubisa, 1999). In addition to pathological changes in bone and teeth, high fluoride level also interfere the biochemical constituents in serum, some of which may be of diagnostic significance. Fluorosis also interferes with metabolism of Ca and P in the body (Underwood, 1977). Fluorine is a cumulative poison and reports (Krook and Maylin, 1979, Crissman *et al.*, 1980) indicate that even the recommendation of safe level of dietary fluoride does not protect the animal against severe chronic fluoride poisoning. Adequate information is available on clinical aspects of fluorosis. However, information is scarce regarding the subclinical changes at higher levels of fluoride fed for a short period resulting in absence of clinical manifestation in buffaloes. The present communication describes the influence of high fluoride intake on serum minerals and ALP activity in buffalo calves.

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MATERIALS AND METHODS

Eight, male Murrah buffalo calves of about 6-8 months age were randomly divided into two groups of 4 each on the basis of their body weight (92-115Kg). Both the groups were fed a normal farm ration consisting of concentrate mixture and roughage (chopped green jowar and wheat bhusa in 1:1 ratio) to meet their nutrient requirements as per ICAR (1985) recommendations. Clean tap water was provided *ad libitum*. All the animals were examined regularly for their health throughout the experimental period.

Concentrate mixture (CM) in both the groups was common and comprised of maize (25 parts), barley (10 parts), deoiled mustard cake (15 parts), wheat bran (15 parts), mineral mixture (2 parts) and common salt (1 part) having 20% crude protein and 70% total digestible nutrients. NaF (chemical grade) was added in the concentrate mixture of group II, in required quantity (132.60 mg) so as to make F concentration of 60 ppm on

Table 1
Proximate and mineral composition (DM basis) of experimental concentrate mixture and roughages

Attributes	Concentrate mixture	Dry roughage (Wheat bhoosa)	Green roughage (Jowar)
Dry matter (%)	093.00	094.00	025.00
Crude protein (%)	020.02	003.01	008.10
Crude fiber (%)	007.31	034.20	036.00
Eather extract (%)	007.52	001.95	002.01
Total ash (%)	011.30	011.20	010.00
NFE (%)	053.86	045.75	047.78
Calcium (%)	000.74	000.37	000.43
Phosphorous (%)	000.69	000.11	000.22
Iron (ppm)	410.00	650.00	432.00
Zinc (ppm)	104.00	040.50	057.00
Copper (ppm)	020.00	007.00	011.00
Fluoride (ppm)	060.00	004.00	006.00

DM basis where it was not added in diet of group I which acted as control. Mineral mixture used in the study was prepared in the laboratory as per BIS specification (2002).

Samples of feed and fodder were analyzed for their proximate nutrients (Table 1) as per (AOAC, 1984). Blood samples of the experimental calves were taken at zero day and subsequently at monthly intervals upto 3 months. The serum was analysed for calcium, phosphorus, iron, zinc, and copper by atomic absorption spectrophotometer (AAS, 1976) and ALP activity with Chemistry Analyser using Kit (ERBA Trans-asia). The data was analysed by Completely Randomized Design (ANOVA) as per Snedecor and Cochran (1968).

RESULTS AND DISCUSSION

Examination of experimental buffalo calves did not reveal any sign of fluoride toxicity like discoloration or mottling of teeth or exostoses of long bones. Serum Ca, P, Fe, Zn, Cu and ALP activity in both the experimental groups have been presented in Table 2. There was a significant decline ($P< 0.05$) in serum Ca level in group II as compared to group I on day 90 which was probably because fluoride causes decrease in absorption of dietary calcium and also enhances excretion of calcium in urine (Ramberg *et al.*, 1971, Chang *et al.*, 1977). The findings are in agreement with those of Hillman *et al.* (1979) and Singh and Swarup (1999) who have also reported hypocalcaemia in fluoride affected cattle and buffaloes. However, Wheeler *et al.* (1985) and Vashishth *et al.* (1998) did not report decline in serum calcium.

Significant increase ($P< 0.05$) in serum P level in group II as compared to group I at 0 day might be due to animal factor and health status of the calves before the start of the experiment. However, significantly higher ($P< 0.05$) serum P on day 90 as compared to day 0 in group II appears to be due to intake of 60 ppm fluoride in the diet. The serum P levels depend on Ca concentration in the serum. In present study, there was fall in Ca level in group II on day 90 probably because of inhibition of calcium metabolism by fluoride which might have led to increase in serum P level accordingly. Singh and Swarup (1999) and Wu Zongjun *et al.* (1995) also reported higher serum P level in fluorotic cattle and buffaloes.

The data related to serum Fe levels revealed non-significant changes at the two time intervals within the same group as well as between the treatments. It was concluded that fluoride (@ 60 ppm of DM) intake in the ration for 3 months did not affect serum Fe level in buffalo calves. Vashishth *et al.* (1998) also reported non-significant changes in serum Fe level in lambs fed higher level of dietary fluoride. However, Singh and Swarup (1999) revealed significantly

Table 2
Effect of high fluoride intake on serum mineral and alkaline phosphatase activity in buffalo calves

Attributes	Treatments group	Periods (days)	
		0	90
Calcium (mg/dl)	Group I	10.73 ± 0.31	$11.25^{A}\pm0.31$
	Group II	$10.74^{a}\pm0.69$	$8.16^{bB}\pm0.50$
Phosphorus (mg/dl)	Group I	$4.80^{A}\pm0.28$	$5.16^{A}\pm0.29$
	Group II	$5.28^{aB}\pm0.33$	$6.29^{bB}\pm0.12$
Iron (ppm)	Group I	0.977 ± 0.028	1.013 ± 0.03
	Group II	1.028 ± 0.024	0.951 ± 0.02
Zinc (ppm)	Group I	$2.65^{A}\pm0.19$	$2.62^{A}\pm0.18$
	Group II	$2.15^{B}\pm0.10$	$1.95^{B}\pm0.15$
Copper (ppm)	Group I	0.387 ± 0.028	0.427 ± 0.02
	Group II	0.425 ± 0.030	0.375 ± 0.03
Alkaline phosphatase(IU/L)	Group I	$76.34^{aA}\pm6.83$	$93.97^{bA}\pm4.39$
	Group II	$90.21^{aB}\pm3.40$	$141.03^{bB}\pm9.34$

Group I – Fed basal ration alone, Group II – Fed basal ration supplemented with 60 ppm fluoride.
Means bearing different superscripts in row (a,b,c) and column (ABC) for a parameter differ significantly ($P = 0.05$).

lower serum Fe level in fluorotic buffaloes.

The serum Zn level did not reveal any significant change at the two time intervals within the same groups. However, group II had significantly lower ($P \leq 0.05$) serum Zn level as compared to group I on day 90. It was probably due to poor absorption of Zn from the intestine and higher excretion in the urine due to fluoride supplementation in group II. Singh and Swarup (1999) also reported significant decline in serum Zn level in fluorotic cows but the fall was non significant in fluorotic buffaloes.

There was no significant difference in the level of serum Cu within the group (at 0 and 90 days). The difference between the groups due to different treatments was also non-significant.

The serum ALP (IU/L) activity on day 0 and day 90 was 76.34 ±6.83 and 93.97±4.39, respectively in group I, while it was 90.21± 3.40 and 141.03± 9.34, respectively in group II at these intervals. The enzyme assay in both the groups revealed significant increase in serum ALP level at different time intervals within the groups and between the groups. Alkaline phosphatase level has diagnostic importance and indicates the damage of bone and affection of developing skeleton tissue in animals suffering from fluorosis. A close correlation has been reported between the fluoride ingested and fluoride contents in the blood, osseous abnormalities and ALP activity in bone of cow (Underwood, 1977). Increased serum ALP level in the present study might be due to stimulation of osteoblastic activity by fluoride (Arya *et al.*, 1990) as also reflected by decrease in the level of calcium and increase in the levels of P. Similar findings of higher level of serum ALP in the case of fluorosis was observed by Singh and Swarup (1999), Mehedintu *et al.* (2000) and Muralidhara *et al.* (2000) in cattle. Thus, the short term feeding of high level of fluoride does not produce toxicity or changes in bone but affect some serum minerals namely calcium and phosphorus levels and ALP activity in blood.

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