ESSENTIAL MINERAL AND HAEMATO-BIOCHEMICAL STATUS OF ILL-THRIFT BUFFALO CALVES IN THE FLUORIDE ENDEMIC SOUTH-WEST PUNJAB, INDIA

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ABSTRACT

The present study aimed to evaluate essential mineral and haemato-biochemical status of the ill-thrift buffalo calves (n=21) in comparison to the healthy control (n=63) in the fluoride endemic south-west Punjab, India. Blood samples of the calves were analysed for plasma fluoride, calcium, phosphorus, magnesium, copper, molybdenum, zinc, iron, manganese, arsenic, ceruloplasmin and alkaline phosphatase activity, urea nitrogen, creatinine, glucose, cholesterol, triglycerides, serum total proteins and albumin, and Hb, PCV, TEC. Mean fluoride concentration in the ill-thrift calves (0.20±0.07 μgml^{-1}) was higher than the physiological limit of 0.10 μgml^{-1} and also was significantly ($P<0.05$) higher than that in the healthy control (0.07±0.01 μgml^{-1}). There was significant reduction ($P<0.05$) in plasma Pi, Zn, ceruloplasmin activity, Hb and TEC concentrations in the ill-thrift calves in comparison to the healthy control. The alkaline phosphatase activity was significantly ($P<0.05$) higher in the ill-thrift calves. It is concluded that higher plasma fluoride concentrations reflected moderately high fluoride intake which resulted in impaired metabolism of essential minerals Cu, Zn and P, and depressed haematopoitic activity that might caused ill-thrift in the buffalo calves from the fluoride endemic region of south-west Punjab in India.

Keywords: *Bubalus bubalis*, buffalo, Ill-thrift, buffalo calves, fluoride, minerals, haemato-biochemical

INTRODUCTION

Chronic fluoride intoxication or fluorosis, a serious health hazard for humans and animals, is endemic in many parts of the world including India (Khandare *et al*., 2005). In India, the disease occurs primarily due to intake of fluoride through drinking water. Ruminants are more susceptible to the disease due to higher water intakes with longer food and water retention in the gastrointestinal tract. Following absorption, about 99% fluoride gets deposited in skeletal tissues and produce osteofluorotic lesions characterised by overt dental and bone lesions, lameness, deformed hooves, anaemia, loss of body condition and ill-thrift (Radostits *et al*., 2007). Fluoride being a strong electro-negative ion interacts with many cations like calcium (Ca), phosphorus (P), magnesium (Mg), copper (Cu) and zinc (Zn) and adversely affects mineral metabolism. The interaction of fluoride

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with other minerals possibly plays important role in pathogenesis of chronic fluoride intoxication. The south-west region of Punjab comprising of Mukatsar, Bathinda, Mansa, Fazilka and parts of Ferozepur districts is a fluoride endemic area. The soils of the area are predominantly calcareous aridisols developed under hot and arid to semi-arid climatic conditions. High concentrations of fluoride had been detected in the groundwater of this region. Aulakh *et al.* (2009) had reported that the fluoride concentrations in 66% groundwater samples from this region were more than the safe limit of 1.0 ppm. Chronic exposure of fluoride is considered as one of the major factors for poor health and performance of livestock in this region. Exposure of fluoride to young calves is a serious concern because it may result in increased susceptibility to various infections, ill-thrift, high mortality and delayed puberty. Keeping this in view, the present study conducted in the fluoride endemic south-west Punjab aimed to assess mineral and haemato-biochemical status of buffalo calves manifesting ill-thrift in comparison to the healthy controls.

MATERIALS AND METHODS

A random survey was conducted in few villages (n=24) of the fluoride endemic south-west Punjab, India. The buffalo calves of 6 months to 1 year of age manifesting ill-thrift (n=21) were selected and for comparative analysis healthy calves (n=63) of identical age were taken as control. Blood samples of the calves were collected from jugular vein in heparin for plasma separation, without anticoagulant for serum harvesting and in EDTA for haemogram analysis. Fecal samples were collected and examined for parasitic ova/cyst by standard methods.

Concentrations of plasma copper (Cu), molybdenum (Mo), zinc (Zn), iron (Fe) and manganese (Mn) were analysed by atomic absorption spectrophotometer (AAS, PerkinElmer A Analyst 700, USA). For this, the plasma samples were wet digested as per Kolmer *et al.* (1951). For digestion, 3 ml of plasma and equal volume of concentrated nitric acid (HNO_3 98% GR, Merck Specialties Pvt. Ltd., Mumbai) were mixed in the digestion flask, kept overnight at room temperature followed by digestion on low heat (70 to 80°C) using digestion bench until volume of the mixture reduced to 1 ml. To this, 3 ml of double acid mixture of concentrated HNO_3 and perchloric acid ($HClO_4$ 70% GR, Merck Specialties Pvt. Ltd., Mumbai) in 3:1 ratio was added and low heat digestion continued until the digested sample became watery clear and emitted white fumes. Final volume of the digestate was made up to 10 ml with double distilled water. Concentrations of arsenic (As) in the digested plasma were estimated on graphite furnace AAS using matrix modifiers pladinum (1%, Merck KGA, Germany) and magnesium nitrate (1%, Sigma Aldrich, USA) to control chemical interferences. Concentrations of calcium (Ca) and magnesium (Mg) in plasma were estimated on AAS without digestion of the samples as described by PerkinElmer (2000). For this, the plasma samples were diluted to 1:100 with 0.1% (w/v) lanthanum chloride to control strong phosphate interference. Plasma fluoride was analysed by digital ion analyser (Orion 4 star bench top pH ISE meter, Thermo Scientific, Singapore) and fluoride electrodes (Thermo Scientific, USA). For the fluoride estimation, the plasma samples were diluted 1:1 with total ionic strength adjustment buffer II (TISAB II). Plasma inorganic phosphorous (Pi) was measured by method of Taussky and Shorr, (1953).

Activity of ceruloplasmin (Cp) in plasma was analysed by the method of Houchin (1958). Total proteins and albumin in serum and plasma urea nitrogen, creatinine, glucose, cholesterol and triglycerides were analysed on Microlab 300 (Merck, Netherlands) using diagnostic kits (Merck Specialties Pvt. Ltd., Goa). Plasma alkaline phosphatase activity (ALP) was estimated on System Vitros DT6011 Chemistry (Ortho-Clinical diagnostics, Johnson and Johnson, USA). Red cell parameters (Hb, PCV, TEC) were estimated by Advia 2120 Haematology System (Siemens Medical Solutions Diagnostics, USA). Mean values for different parameters were calculated and compared between the ill-thrift and control group by student's t-test using Statistical Package for Social Sciences (SPSS) for Window version 11.0.1 SPSS Inc. USA computer software program.

RESULTS AND DISCUSSION

Fluoride concentrations in the ill-thrift calves were significantly ($P<0.05$) higher than that in the healthy control (Table 1). In the ill-thrift calves, the fluoride concentrations were higher than the physiological upper limit of 0.1 μgml^{-1}, but the concentrations were only moderately high that reflected moderately high fluoride intake in the calves. Mild dental lesions characterized by yellow discolouration of incisors were observed in few (n=9) of the ill-thrift calves. Plasma fluoride concentrations reflect status of current fluoride intake in cattle (Grace *et al*., 2003b). The teeth of calves are sensitive to small changes in the plasma fluoride concentrations and the severity of lesions depends upon concentration of plasma fluoride. Severe dental lesions appeared at the concentrations of 0.5 μgml^{-1} or higher; the lesions tended to be less severe between 0.2 and 0.5 μgml^{-1}, while very few adverse effects were apparent at the concentrations less than 0.2 μgml^{-1} (Suttle *et al*., 1972). Once fluoride is absorbed from the gut and reaches to the haemopoeitic organs, it affects haemopoiesis and blood picture (Swarup and Singh, 1989), and absorption, excretion, distribution and retention of several minerals (Vashishth *et al*., 1997). It also affects activities of several enzymes (Singh and Swarup, 1999). Clinical signs of fluorosis may not appear for weeks to months in animals ingesting moderate amount of fluoride (Underwood and Suttle, 1999).

The plasma Pi and Zn concentrations were significantly ($P<0.05$) lower in the ill-thrift calves in comparison to the healthy control. Although, plasma Cu concentrations did not differ between the healthy control and ill-thrift calves, but the activity of plasma ceruloplasmin; a Cu containing enzyme considered as a sensitive indicator of the Cu status in ruminants (Kincaid, 1999) was significantly ($P<0.05$) lower in the ill-thrift calves that suggested impairment of Cu metabolism in them. Fluoride forms complexes with Ca, P and Mg in the gastrointestinal tract and thereby reduces their absorption. It was due to this, Jagadish *et al.* (1998) had reported lower serum Ca concentrations in fluorotic cattle and buffaloes. Decline in serum Ca in fluorosis might be due to decrease in absorption as well as enhanced excretion of Ca via urine (Bharti *et al*., 2007). Fluoride supplementation (60 ppm) as sodium fluoride in buffalo calves had resulted in significant decline in serum Ca and Zn, increase in serum ALP, and no effect on serum Fe and Cu concentrations (Bharti *et al*., 2008). Depression in serum Zn on fluoride exposure might be due to poor absorption of Zn from the intestine and higher excretion in the urine. Ranjan *et al.* (2008) had reported lower plasma Ca, Cu,

Zn and Mn, and higher plasma Co concentrations in the fluorotic cattle. Although, fluoride has a strong antagonism with Ca and Mg, the fluoride exposure in the ill-thrift calves was probably not high enough to disturb the strong homeostatic regulation of Ca and Mg in the body. The lower plasma Pi concentrations in the ill-thrift calves might be due to its reduced absorption in the gastrointestinal tract as fluoride forms complexes with P and reduces its absorption (WHO, 2002). Conversely, McLaughlin *et al.* (2001) had reported an increase in the plasma Pi along with decline in Ca concentrations in the fluorotic cattle, buffaloes and goats, which might be due to compensatory rise in plasma Pi concentrations secondary to plasma Ca depression in the fluorotic animals. Plasma Fe concentrations did not differ between the ill-thrift and healthy control calves, which was in agreement with Vashishth *et al.* (1998); Bharti *et al.* (2008).

Lower concentrations and impaired metabolism of essential minerals (Cu, Pi and Zn) due to high fluoride exposure might be responsible for the ill-thrift in calves in the fluoride endemic south-west Punjab, India. The role of P, Zn and Cu deficiency in causing loss of appetite, decreased growth, unthrifty appearance (rough skin and unkempt hair coat) and loss of weight in calves had been well documented (Little, 1980; McDowell, 2003). Copper deficiency had been associated with general weakness, stunted growth, infertility, parakeratosis and achromotrichia in crossbred dairy cattle of all ages (Damir *et al*., 1988). It had been observed that the inadequate Cu status was not associated with adult disorders, but it was an important risk factor for poor calf health and performance (Enjalbert *et al*., 2006).

Analysis of haemogram revealed that the Hb and TEC concentrations in the ill-thrift calves were significantly ($P<0.05$) lower than that in the healthy control (Table 2). Reduced erythropoietic activity as a result of damage to bone marrow resulting from prolonged exposure to high fluoride (Wheeler and Fell, 1983) could be the reason of the lowering of PCV and TEC values in the ill-thrift calves. Impaired P and Cu metabolism could be another factor that caused lowering of Hb and TEC in the ill-thrift calves as both the elements are essential for normal erythropoiesis (McDowell, 2003).

The ALP activity in the ill-thrift calves was significantly ($P<0.05$) higher than that in the healthy control. It might be due to chronic fluoride exposure resulting in the stimulation of osteoblastic activity (Arya *et al*., 1990). A close correlation had been reported between fluoride ingestion and blood levels of fluoride, ALP and osseous abnormalities in cow (Swarup *et al*., 2001). Increased serum ALP activity can occur in the fluoride exposed buffaloes without evidence of clinical disease (Madan *et al*., 2009). The plasma urea nitrogen, creatinine, glucose, cholesterol, triglycerides, serum total proteins and albumin concentrations were comparable in the ill-thrift and healthy control calves. It is concluded that moderately high plasma fluoride concentrations reflected moderately high fluoride intake which resulted in lower concentrations and impaired metabolism of essential minerals Cu, Zn and P, and depressed haematopoitic activity and that might caused ill-thrift in the buffalo calves from the fluoride endemic south-west Punjab, India.

Table 1. Plasma mineral concentrations and ceruloplasmin activity in the ill-thrift and healthy calves (Mean ± SE).

Parameter	Healthy control (n=63)	Ill-thrift (n=21)
Fluoride (μgml^{-1})	0.07±0.01	0.20±0.07*
Ca ($mgdl^{-1}$)	10.90±0.24	10.55±0.46
Pi ($mgdl^{-1}$)	6.29±0.17	5.77±0.26*
Mg ($mgdl^{-1}$)	2.73±0.11	2.98±0.19
Cu (μgml^{-1})	0.71±0.01	0.69±0.03
Mo ($ngml^{-1}$)	79.88±15.78	89.67±28.92
Zn (μgml^{-1})	1.33±0.01	1.05±0.02*
Fe (μgml^{-1})	3.67±0.28	3.86±0.58
Mn ($ngml^{-1}$)	69.55±7.90	65.32±12.39
As ($ngml^{-1}$)	17.35±3.65	23.13±9.89
Ceruloplasmin ($mgdl^{-1}$)	10.58±0.62	8.95±0.65*

*Significant at (P<0.05).

Table 2. Haemogram in the ill-thrift and healthy calves (Mean ± SE).

Parameter	Healthy control (n=63)	Ill-thrift (n=21)
Hb (gdl^{-1})	9.72±0.21	9.01±0.33*
PCV (%)	31.03±0.75	28.74±1.26
TEC ($x10^6 \mu l^{-1}$)	7.79±0.23	6.88±0.29*

*Significant at (P<0.05).

Table 3. Biochemical profile in the ill-thrift and healthy calves (Mean ± SE).

Parameter	Healthy control (n=63)	Ill-thrift (n=21)
Total proteins (gdl^{-1})	7.08±0.14	7.00±0.27
Albumin (gdl^{-1})	2.97±0.10	2.87±0.17
Urea nitrogen ($mgdl^{-1}$)	11.06±0.51	11.38±1.47
Creatinine ($mgdl^{-1}$)	1.34±0.04	1.29±0.08
Glucose ($mgdl^{-1}$)	78.78±2.17	72.94±3.73
Cholesterol ($mgdl^{-1}$)	123.59±4.77	119.45±7.62
Triglycerides ($mgdl^{-1}$)	13.97±1.47	13.90±3.05
Alkaline phosphatase (uL^{-1})	128.94±17.25	202.79±30.50*

*Significant at (P<0.05).

REFERENCES

Arya, O., F. Wittwer, A. Villa and C. Ducom. 1990. Bovine fluorosis following volcanic activity in the southern Andes. *Vet. Rec.*, **126**: 641-642.

Aulakh, M.S., M.P. Khurana and D. Singh. 2009. Water pollution related to agriculture, industrial, and urban activities and its effect on the food chain: Case studies from Punjab. *J. New Seeds*, **10**: 112-137.

Bharti, V.K., M. Gupta and D. Lall. 2008. Ameliorative effects of boron on serum profile in buffalo (*Bubalus bubalis*) fed high fluoride ration. *Trop. Anim. Health Prod*., **40**: 111-116.

Bharti, V.K., M. Gupta, D. Lall, T.C. Balamurugan and S. Imam. 2007. Effect of boron on urinary and faecal excretion of minerals in buffalo calves fed high fluoride ration. *Anim. Nutr. Feed Tech*., **7**: 125-130.

Damir, H.A., M.E.S. Barri, S.M. El-Hassan, M.H. Tageldin, A.A. Wahbi and O.F. Idris. 1988. Clinical copper and zinc deficiencies in cattle of western Sudan. *Trop. Anim. Health Prod*., **20**: 52-56.

Enjalbert, F., P. Lebreton and O. Salat. 2006. Effects of copper, zinc and selenium status on performance and health in commercial dairy and beef herds: Retrospective study. *J. Anim. Physiol. Anim. Nutr*., **90**: 459-466.

Grace, N.D., P. Loganathan, M.J. Hedley and G.C. Wallace. 2003b. Ingestion of soil fluorine: its impact on the fluorine metabolism of dairy cows. *New Zeal. J. Agr. Res*., **48**: 23-27.

Houchin, O.B. 1958. A rapid colorimetric method for quantitative determination of copper oxidase activity (ceruloplasmin). *Clin. Chem*., **4**: 519-523.

Jagadish, S., L.K. Singh, A. Samad and P. Bhalerao. 1998. Bovine fluorosis I: Clinical pathology and investigations in to source of fluorine. *Indian Vet. J*., **75**: 335-337.

Khandare, A.L., R. Harikumar and B. Sivakumar. 2005. Severe bone deformities in young children from vitamin D deficiency and fluorosis in Bihar-India. *Calcif. Tissue Int.* **76**: 412-418.

Kincaid, R.L. 1999. Assessment of trace mineral status of ruminants: A review. *In Proceedings of the American Society of Animal Science*. Washington State University, Pullman, USA.

Kolmer, J.A., E.H. Spanbling and H.W. Robinson. 1951. *Approved Laboratory Techniques*. Appleton Century Crafts, New York, USA.

Little, D.A. 1980. Observations on the phosphorus requirement of cattle for growth. *Res. Vet. Sci*., **28**: 258-260.

Madan, J., J.P. Puri and J.K. Singh. 2009. Growth, feed conversion efficiency and blood profile of buffalo calves consuming high levels of fluoride. *Trop. Anim. Health Prod*., **41**: 295-298.

McDowell, L.R. 2003. *Minerals in Animal and Human Nutrition*, 2nd ed. Academic Press Inc., New York, USA.

McLaughlin, M.J., D.P. Stevens, D.G. Keerthisinghe, J.W.D. Cayley and A.M. Ridley. 2001. Contamination of soil with fluoride by long term application of super-phosphate to pastures and risk to grazing animals. *Aust. J. Soil Res*., **39**: 627-640.

PerkinElmer. 2000. *Analytical Methods for Atomic Absorption Spectrometry*. PerkinElmer Instruments LLC, Singapore.

Radostits, O.M., K.W. Hinchcliff, C.C. Gay and

P.D. Constable. 2007. *Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats*, 10th ed. W.B. Sounders Harcourt Publishers Ltd, London, England.

Ranjan, R., D. Swarup, B. Bhardwaj and C. Patra. 2008. Level of certain micro and macro minerals in blood of cattle from fluoride polluted localities of Udaipur, India. *Bull. Environ. Contam. Toxico.,* **81**: 503-507.

Singh, J.L. and D. Swarup. 1999. Biochemical changes in serum and urine in bovine fluorosis. *Indian J. Anim. Sci*., **69**: 776-778.

Suttle, J.W., J.R. Carlson and E.C. Faltin. 1972. Effects of alternating periods of high- and low-fluoride ingestion on dairy cattle. *J. Dairy Sci*., **55**: 790-804.

Swarup, D. and Y.P. Singh. 1989. Bovine fluorosis in a brick kiln congested zone. *Indian J. Vet. Med*., **9**: 12-14.

Swarup, D., S. Dey, R.C. Patra, S.K. Dwivedi and S.L. Ali. 2001. Clinico-epidemiological observation of industrial bovine fluorosis in India. *Indian J. Anim. Sci*., **71**: 1111-1115.

Tausky, H.H. and E. Shorr. 1953. A micro colorimetric method for determination of inorganic phosphorus. *J. Biol. Chem*., **202**: 675-685.

Underwood, E.J. and N.F. Suttle. 1999. *The Mineral Nutrition of Livestock*, 3rd ed. CABI Publishing, Oxon, UK.

Vashishth, S.N., V. Kapoor, D. Lall and R. Kumar. 1998. Mineral status and serum alkaline phosphatase activity in lambs fed diets supplemented with fluorine and boron. *Indian Vet. J*., **75**: 17-21.

Vashishth, S.N., V. Kapoor, P.S. Yadav and A.B. Mandal. 1997. Effect of supplemental boron on nutrient utilization, mineral status and blood biochemical constituents in lambs fed high fluoride diet. *Fluoride*, **30**: 165-172.

Wheeler, S.M. and L.R. Fell. 1983. Fluorides in cattle nutrition: A review. *Nutr. Abstr. Rev*., **53**: 741-769.

World Health Organisation. 2002. *Fluorides*: *Environmental health criteria*. World Health Organisation, Geneva, Switzerland. 227p.