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Bovine fluorosis and its effects on essential minerals, haemogram and biochemical status in the fluoride endemic South-West Punjab of India

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ABSTRACT

The present study was undertaken to assess prevalence of fluorosis and its effects on essential mineral and haemato-biochemical status in dairy animals from the fluoride endemic Mansa and Fazilka districts of the South-West Punjab, India. A base line survey was carried out in which blocks of the selected districts were taken as a stratum and from each stratum, the buffaloes and cattle were selected randomly. Blood samples of the selected animals were analysed for plasma Ca, Pi, Mg, Cu, Mo, Zn, Fe, Mn, alkaline phosphatase, Hb, PCV and TEC. The fluoride contents of 35.6 and 9.9% drinking water samples from Mansa (1.46±0.17 ppm, n=112) and Fazilka (1.18±0.36 ppm, n=91) district, respectively, were higher than the permissible limit of 1.5 ppm. The prevalence of fluorosis (plasma F > 0.10 µg/ml) in Mansa district was 95.3% in buffaloes (0.26±0.01 µg/ml, n=261) and 100.0% in cattle (0.31±0.02 µg/ml, n=35); while in Fazilka district, the prevalence was 92.0% in buffaloes (0.17±0.01 µg/ml, n=112) and 95.7% in cattle (0.21±0.01 µg/ml, n=118). The fluorotic buffaloes and cattle had significantly lower plasma Pi, Zn, Mn, PCV, TEC, and significantly higher plasma Mo and alkaline phosphatase activity. Plasma Ca, Mg, Cu, Fe and Mn, and Hb concentrations did not vary between the fluorotic and normal animals. It can be conclude that fluorosis is widely prevalent, and is causing essential mineral imbalances and lowering of haemogram in the dairy animals in the Mansa and Fazilka districts of the South-West Punjab of India.

Key words: Bovine, Biochemical, Fluorosis, Haemogram, Minerals

Fluorosis is a serious health hazard for humans and animals, endemic in many parts of the world including India (Khandare *et al.* 2005). Ruminants are reported to be more susceptible to the disease which could be due to higher water intake with longer food and water retention in the gastrointestinal tract (Swarup and Dwivedi 2002). Fluoride being a strong electro-negative ion interacts with cations like calcium (Ca), phosphorus (P), magnesium (Mg), copper (Cu) and zinc (Zn) and adversely affects mineral metabolism (Suttle 2010). Prolonged fluoride exposure may cause reduced erythropoietic activity as a result of damage to bone marrow (Wheeler and Fell 1983) leading to anaemia in animals. The South-West Punjab comprising of Mansa, Fazilka, Ferozepur, Bathinda and Mukatsar districts is a semi-arid fluoride endemic region characterised by high environmental temperature, low rainfall, shorter fodder growing period length, and high concentrations of fluoride in groundwater (Aulakh *et al.* 2009). The present study was undertaken to assess prevalence of bovine fluorosis and its impact on mineral and haemato-biochemical status in relation to drinking water fluoride contents in the Mansa and Fazilka districts of the South-West Punjab of India.

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MATERIALS AND METHODS

A survey was conducted in the Mansa and Fazilka districts of the South-West Punjab by using stratified random sampling technique. Each block of the Mansa (Bhikhi, Mansa, Budlada, Jhunir and Sardulgarh) and the Fazilka (Abohar, Fazilka, Khuian Sarwar and Jalalabad) district was taken as a stratum. Dairy buffaloes and cattle from the each stratum were selected randomly. Blood samples were collected from jugular vein in the heparinised mineral free glass vials (dipped overnight in 30.0% nitric acid and then washed with double glass distilled water). Plasma was separated immediately by centrifugation at 3,000 rpm and stored at –20°C in deep freeze till further analysis. For haemogram analysis, about 2 ml blood was collected in plastic vials containing disodium ethylene diamine tetra acetate. The drinking water samples were collected in glass vials, filtered through Whatman filter paper No. 1 and stored in refrigerator at 4°C till further analysis.

Plasma and water fluoride concentrations were analysed by using digital ion analyser (Orion 4 star bench top pH ISE meter, Thermo Scientific, Singapore) and fluoride

electrodes (Thermo Scientific, USA) using total ionic strength adjustment buffer II. For the estimation of plasma Cu, molybdenum (Mo), zinc (Zn), iron (Fe) and manganese (Mn) concentrations, the samples were wet digested as per Kolmer *et al.* (1951). For this, 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 (70–80°C) using digestion bench until volume of the mixture was 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 was continued until the digested sample became clear and emitted white fumes. Final volume was made up to 10 ml with double distilled water. The digested samples were analysed for minerals on the atomic absorption spectrophotometer (AAS, PerkinElmer Analyst 700, USA). For the estimation of plasma Ca and Mg concentrations, the plasma samples were diluted to 1:100 with 0.1% (w/v) lanthanum chloride to control strong phosphate interference and then analysed with the help of AAS. Plasma inorganic phosphorus (Pi) concentrations were estimated by the method of Taussky and Shorr (1953). The Hb, PCV and TEC concentrations were estimated on the Advia 2120 haematology system (Siemens Medical Solutions Diagnostics, USA). Activity of plasma alkaline phosphatase (ALP) was estimated on System Vitros DT6011 Chemistry Analyser (Ortho-Clinical Diagnostics, Johnson and Johnson, USA) using DT slides. The data were analysed by using Statistical Package for Social Sciences (SPSS for Window version 11.0.1°, SPSS Inc. USA) computer software program. The mean values were calculated and compared by using student's t-test (Singh *et al.* 1998).

RESULTS AND DISCUSSION

Fluoride concentration (ppm) in water samples: High fluoride concentrations (>1.5 ppm; WHO 1984) were detected in the drinking water samples from Bhikhi, Mansa, Budlada and Jhunir blocks of Mansa district, and Abohar, Khuian Sarwar and Jalalabad blocks of Fazilka district. Overall 35.6 and 9.9% water samples from Mansa and Fazilka district respectively had high fluoride concentrations (Table 1). Similarly, Aulakh *et al.* (2009) reported high fluoride concentrations in the groundwater samples from Bathinda district of the South-West Punjab. Significant ($P<0.05$) inter block variations were observed in the water fluoride concentrations; groundwater from Bhikhi and Mansa blocks in the Mansa district and Abohar and Jalalabad blocks in the Fazilka district had considerably higher fluoride contents as compared to the other blocks of the respective districts. The fluoride contents of all the water samples from the Sardulgarh and Fazilka blocks were within the permissible limit of 1.5 ppm. The endemic fluorosis in India is associated primarily with the high fluoride contents in the drinking water (Singh and Yadava 2003). Choubisa (1999) reported that osteo-dental fluorosis in cattle was associated with drinking water levels of 1.5 to 4.0 ppm. However, Radostitis *et al.* (2007) documented comparatively higher fluoride levels of 5.0 ppm, 10.0 ppm and 30.0 ppm in the drinking water of cattle showing minor tooth lesions, excessive tooth wear and serious systemic effects, respectively.

Plasma fluoride: The mean plasma fluoride concentrations in buffaloes and cattle from Mansa and Fazilka districts were higher than the critical limit of 0.1 µg/ml (Table 2). The concentrations were high in all the blocks of Mansa as well as Fazilka district. The prevalence of fluorosis (plasma fluoride > 0.1 µg/ml) in buffaloes and cattle was 95.3 and 100.0% in Mansa, and 92.0 and 95.7% in Fazilka district, respectively. However, the fluorosis was mild in severity as the plasma fluoride concentrations were considerably lower than 1.0 µg/ml value suggestive of clinical fluorosis. Plasma fluoride concentrations reflect short-term changes in fluoride intake with levels less than 0.1 µg/ml in normal animals while more than 1.0 µg/ml indicates a high fluoride uptake (Suttle *et al.* 1972).

Positive but moderate degree correlation was observed between the water fluoride contents and plasma fluoride concentration in buffaloes ($r^2 = 0.52$, $P<0.01$) and cattle ($r^2 = 0.56$, $P<0.01$) from the Mansa district. Closely similar effects were observed in buffaloes ($r^2 = 0.40$, $P<0.01$) and

Table 1. Fluoride contents (ppm) of drinking water for livestock

District	Block	n	Mean±SE (Range)	F > 1.5 ppm (%)	F > 5.0 ppm (%)
Mansa	Bhikhi	28	1.52 ± 0.11^{ab} (0.70-3.50)	50.0	0
	Mansa	21	2.32 ± 0.42^{a} (0.30-7.00)	68.4	14.3
	Budlada	22	1.11 ± 0.22^{bc} (0.30-2.90)	23.1	0
	Jhunir	20	1.72 ± 0.95^{ab} (0.30-12.00)	16.7	5.0
	Sardulgarh	21	0.52 ± 0.09^{c} (0.20-1.10)	0	0
	Overall	112	1.46±0.17 (0.20-12.00)	35.6	3.6
Fazilka	Abohar	21	2.01 ± 0.67^{a} (0.20-9.30)	14.3	4.7
	Fazilka	22	0.72 ± 0.07^{b} (0.40-1.10)	0	0
	Khuian Sarwar	24	0.69 ± 0.07^{b} (0.30-1.90)	4.2	0
	Jalalabad	24	1.17 ± 0.19^{ab} (0.40-4.30)	16.7	0
	Overall	91	1.18±0.36 (0.20-9.30)	9.9	1.2

Within a district, figures bearing at least one common superscript do not differ significantly ($P<0.05$).

cattle (r^2 = 0.53, P<0.01) from the Fazilka district. Thus, the animals consuming drinking water with higher fluoride contents had higher plasma fluoride concentrations. Gupta *et al.* (2015) observed that with an increase in fluoride concentration in drinking water there was an increase in concentration of fluoride in cow and buffaloes. Fluorosis is endemic in parts of Punjab, Haryana, Rajasthan, Bihar, Madhya Pradesh, Odisha and Andhra Pradesh, having high fluoride contents in underground water (Randhawa and Singh 2010). In soil, fluoride is present as calcium fluoride and most plants have limited capacity to absorb soil fluoride. Unless, there is contamination of forages or pasture with industrial fumes or dust, or irrigation with high fluoride water, there is little risk of fluoride ingestion through forages. Ingestion of fluoride rich soil can be an important cause on the overgrazed pasture. Grains and their by-products are usually low in fluoride, but rock phosphate based salts mainly di-calcium phosphate can be the principle source of fluoride for animals (Suttle 2010). Patra *et al.* (2000) reported that consumption of fodder and water contaminated by the fumes and dusts emitted from superphosphate fertiliser plants resulted in the development of chronic fluorotic lesions in cattle and buffalo. Particularly in India, fluorosis is primarily geogenic in origin caused by high fluoride in the groundwater.

The plasma fluoride concentrations of cattle were significantly (P<0.05) higher as compared to the buffaloes in both the districts. Plasma fluoride content reflects the current rate of fluoride intake (Radostitis *et al.* 2007). The higher plasma fluoride contents in cattle suggest higher fluoride intake by them. Interspecies variations had been reported in the susceptibility of fluorosis. Franke (1989) reported that cattle were most sensitive to fluorosis, followed by sheep, horses, pigs, rabbits, rats, guinea pigs, and poultry. Contrary to the present findings, Sharma *et al.* (1995) reported that buffaloes were more susceptible to the fluorosis as compared to the cattle.

Effects of fluorosis on essential mineral status: Plasma Ca and Mg concentrations did not vary between the fluorotic and non-fluorotic animals. Fluoride has a strong antagonism with Ca and Mg (Maiti and Das 2004, Ranjan *et al.* 2008), but fluoride exposure to the dairy animals in the present study was not high enough to disturb the strong homeostatic regulation of plasma Ca. Plasma Pi concentrations were significantly (P<0.01) lower in the fluorotic buffaloes and cattle as compared to their non-fluorotic counterparts. In the gastrointestinal tract, fluoride forms complexes with phosphorus and reduces its absorption (WHO 2002). On the contrary, McLaughlin *et al.* (2001) reported an increase in plasma Pi 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 Zn and Mn concentrations were significantly lower, whereas, plasma Mo concentrations were significantly higher in the fluorotic buffaloes and cattle. Lower Zn and Mn concentrations might be due to poor absorption of these elements from the gut and their higher excretion in the urine because of the strong binding of electro-negative anion fluoride with the electro-positive cations Zn and Mn. Similar to the present findings, Singh and Swarup (1999) and Ranjan *et al.* (2008) had observed significantly lower serum Zn and Mn concentrations in the fluorotic cattle. Plasma Cu and Fe concentrations did not differ between the non-fluorotic and the fluorotic buffaloes and cattle. Similarly, Vashishth *et al.* (1998) and Bharti *et al.* (2008) reported no change in serum Fe concentrations of lambs and buffalo calves fed higher levels of fluoride. However, Ranjan *et al.* (2008) reported significantly lower serum Cu concentrations in the fluorotic cattle.

Effects of fluorosis on haemato-biochemical status: The PCV and TEC concentrations in the fluorotic buffaloes and

Table 2. Plasma fluoride concentrations in buffaloes and cattle (Mean±SE, range)

District		Plasma fluoride (µg/ml)					
	Blocks →	Bhikhi (n=76)	Mansa (n=45)	Budlada (n=52)	Jhunir (n=47)	Sardulgarh (n=41)	Overall (n=261)
Mansa	Buffalo	0.22±0.01^{a} (0.05-0.97)	0.29±0.02^{bc} (0.07-0.94)	0.21±0.04^{a} (0.05-0.48)	0.34±0.03^{c} (0.08-0.82)	0.24±0.02^{ab} (0.12-0.82)	0.26±0.01^{A} (0.05-0.97)
	Cattle (n=35)			-			0.31±0.02^{B} (0.16-0.66)
Fazilka	Blocks →	Abohar	Fazilka	Khuian Sarwar		Jalalabad	
	Buffalo	n=20	n=25	n=31		n=36	n=112
		0.20±0.04^{a}	0.14±0.03^{bc}	0.19±0.06^{a}		0.16±0.03^{ac}	0.17±0.01^{A}
		(0.09-0.83)	(0.01-0.34)	(0.09-0.34)		(0.08-0.37)	(0.01-0.83)
	Cattle	n=47	n=26	n=27		n=18	n=118
		0.20±0.02^{a}	0.25±0.02^{b}	0.19±0.03^{a}		0.18±0.04^{a}	0.21±0.01^{B}
		(0.08-0.58)	(0.13-0.59)	(0.10-0.57)		(0.10-0.32)	
(0.08-0.59)							

Superscripts in small letters compare blocks in a row; figures bearing at least one common superscript do not differ significantly (P<0.05). Superscripts in capital letters compare buffaloes with cattle within a district; figures bearing different superscripts differ significantly (P<0.05).

Table 3. Mineral status of non-fluorotic and fluorotic animals (Mean±SE)

Parameter	Buffaloes		Cattle	
(n=28)	Non-fluorotic (n=332)	Fluorotic (n=10)	Non-fluorotic (n=140)	Fluorotic
Ca (mg/dl)	10.19±0.47 (5.61-13.93)	10.45±0.12 (5.92-15.36)	10.66±0.61 (8.00-13.53)	9.88±0.19 (5.46-15.21)
Pi (mg/dl)	6.58±0.28 (4.28-8.96)	5.56±0.07** (2.05-8.06)	6.64±0.35 (3.79-7.67)	5.42±0.10** (2.67-8.36)
Mg (mg/dl)	2.74±0.20 (1.53-4.86)	2.86±0.05 (1.05-5.53)	2.77±0.27 (1.98-4.67)	2.49±0.08 (1.20-5.53)
Cu (µg/ml)	0.73±0.05 (0.41-1.32)	0.66±0.02 (0.11-1.58)	0.68±0.10 (0.27-1.22)	0.59±0.03 (0.10-1.37)
Mo (ng/ml)	35.59±7.54 (ND-200.90)	113.46±9.03** (ND-932.20)	25.13±2.92 (13.97-41.28)	102.19±12.73* (2.88-912.70)
Zn (µg/ml)	1.62±0.12 (0.79-2.78)	1.13±0.03** (0.20-3.38)	1.30±0.23 (0.69-3.18)	1.01±0.04* (0.16-3.11)
Fe (µg/ml)	3.86±0.40 (0.50-8.29)	3.64±0.15 (0.14-8.34)	3.94±1.19 (1.41-7.21)	3.19±0.22 (0.22-8.41)
Mn (ng/ml)	108.36±15.93 (ND-270.70)	60.64±2.73** (ND-252.00)	171.09±24.32 (73.43-278.40)	63.37±5.14** (ND-252.60)
F (µg/ml)	0.08±0.003 (0.01-0.10)	0.25±0.007** (0.11-0.97)	0.09±0.002 (0.08-0.10)	0.24±0.01** (0.11-0.66)
Hb (g/dl)	10.39±0.37 (7.10-13.60)	9.91±0.09 (6.00-14.50)	9.53±0.60 (8.70-10.70)	8.23±0.15 (3.90-11.90)
PCV (%)	32.66±1.16 (21.00-43.20)	30.46±0.29* (18.10-45.00)	33.40±2.64 (28.20-36.80)	26.07±0.45** (16.00-44.00)
TEC ($\times 10^6$/µl)	8.04±0.38 (4.57-12.11)	6.83±0.07** (4.15-11.68)	9.59±0.90 (7.96-11.05)	6.12±0.11** (4.10-10.86)
ALP (uL)	151.62±5.88 (7.00-620.00)	264.44±4.91** (31.00-547.00)	99.85±7.81 (17.00-498.00)	274.00±4.38** (132.00-519.00)

*Significant at P<0.05; ** significant at (P<0.01); ND, non-detectable.

cattle were significantly lower and Hb concentrations were non-significantly lower than their non-fluorotic counterparts (Table 3). Reduced erythropoietic activity as a result of damage to bone marrow resulting from prolonged exposure to high fluoride levels (Wheeler and Fell 1983) could be the reason for lowering of PCV and TEC values in the fluorotic animals. Similarly, Sharma *et al.* (1997) had reported significantly lower Hb, PCV and TEC concentration in the animals showing clinical fluorosis. The alkaline phosphatase activity in the fluorotic buffaloes and cattle were significantly higher than the non-fluorotic buffaloes and cattle, respectively. A close correlation had been observed between fluoride ingestion and blood levels of fluoride, alkaline phosphatase and osseous abnormalities in cow (Swarup *et al.* 2001). Increased serum alkaline phosphatase concentrations in fluorosis might be due to stimulation of osteoblastic activity by fluoride exposure (Arya *et al.* 1990).

It can be concluded that fluorosis of moderate severity caused by high fluoride contents in the drinking water is widely prevalent in buffaloes and cattle in the Mansa and Fazilka districts of the South-West Punjab. The fluorotic animals tend to have lower plasma Pi, Zn, Mn, haemogram and higher alkaline phosphatase activity. Various fluorosis mitigation strategies viz. supplementation of calcium, aluminum, selenium, boron, ascorbic acid and some herbal preparations had been tested in the past with limited success (Ranjan *et al.* 2009b). The "Nalgonda Technique" of water defluoridation as developed in India in 1975, involves the addition of alum and lime (Nawlakhe *et al.* 1975). It is a low cost, simple technique that can be used at domestic as well as village levels to provide defluoridated drinking water to livestock.

REFERENCES

Arya O, Wittwer F, Villa A and Ducom C. 1990. Bovine fluorosis following volcanic activity in the Southern Andes. *Veterinary Record* **126**: 641–42.

Aulakh M S, Khurana M P and Singh D. 2009. Water pollution related to agriculture, industrial, and urban activities and its effect on the food chain: Case studies from Punjab. *Journal of New Seeds* **10**: 112–37.

Bharti V K, Gupta M and Lall D. 2008. Ameliorative effects of boron on serum profile in buffalo (*Bubalus bubalis*) fed high fluoride ration. *Tropical Animal Health Production* **40**: 111–16.

Choubisa S L. 1999. Some observations on endemic fluorosis in domestic animals (India). *Veterinary Research Communications* **23**(7): 457–65.

Gupta P, Gupta N, Meena K, Moon N J, Kumar P and Kaur R. 2015. Concentration of fluoride in cow's and buffalo's milk

in relation to varying levels of fluoride concentration in drinking water of Mathura city in India– A pilot study. *Journal of Clinical Diagnosis and Research*. **9**(5): 5–7.

Khandare A L, Harikumar R and Sivakumar B. 2005. Severe bone deformities in young children from vitamin D deficiency and fluorosis in Bihar-India. *Calcified Tissue International* **76**: 412–18.

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

Maiti S K and Das P K. 2004. Biochemical changes in endemic dental fluorosis in cattle. *Indian Journal of Animal Sciences* **74**: 169–71.

McLaughlin M J, Stevens D P, Keerthisinghe D G, Cayley J W D and Ridley A M. 2001. Contamination of soil with fluoride by long term application of super-phosphate to pastures and risk to grazing animals. *Australian Journal of Soil Research* **39**: 627–40.

Nawlakhe W G, Kulkarni D N, Pathak B N and Bulusu K R. 1975. Defluoridation of water by Nalgonda technique. *Indian Journal of Environmental Health* **17**: 26–34.

Patra R C, Dwivedi S K, Bhardwaj B and Swarup D. 2000. Industrial fluorosis in cattle and buffalo around Udaipur, India. *Science of The Total Environment* **253** (1–3): 145–50.

Radostits O M, Hinchcliff K W, Gay C C and Constable P D. 2007. *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats*. 10th edn. W B Sounders Harcourt Publishers Ltd, London.

Randhawa S S and Singh S T. 2010. Mineral Toxicities in Dairy Animals. Proceedings of the National Symposium and 28th Annual Convention of Indian Society for Veterinary Medicine. pp 14–22. February 17–19, 2010. Hyderabad.

Ranjan R, Swarup D, Bhardwaj B and Patra C. 2008. Level of certain micro and macro minerals in blood of cattle from fluoride polluted localities of Udaipur, India. *Bulletin of Environmental Contamination and Toxicology* **81**: 503–07.

Ranjan R, Swarup D, Patra R C and Chandra V. 2009b. *Tamarindus indica* L. and *Moringa oleifera* M. extract administration ameliorates fluoride toxicity in rabbits. *Indian Journal of Experimental Biology* **47**: 900–905.

Sharma P S, Randhawa S S and Randhawa C S. 1997. Clinico-epidemiological features of bovine fluorosis in Punjab. *Indian Journal of Animal Sciences* **67**: 943–45.

Sharma S P, Randhawa S S and Brar R S. 1995. Geomedical studies on fluorosis in dairy animals in Punjab, India. *Annales de Zootechnie* **44**: 314–19.

Singh J L and Swarup D. 1999. Biochemical changes in serum and urine in bovine fluorosis. *Indian Journal of Animal Sciences* **69**: 776–78.

Singh S, Bansal M L, Singh T P and Kumar R. 1998. *Statistical Methods for Research Workers*. Kalyani Publishers, New Delhi.

Singh V P and Yadava R N. 2003. Ground Water Pollution. *Proceedings of the International Conference on Water and Environment.* December 2003, Bhopal, India.

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

Suttle N F. 2010. *Mineral Nutrition of Livestock*. 4th edn, pp 501–10. CABI Publishing, Oxfordshire, UK.

Swarup D and Dwivedi S K. 2002. *Environmental Pollution and Effects of Lead and Fluoride on Animal Health*. Indian Council of Agricultural Research, Pusa, New Delhi.

Swarup D, Dey S, Patra R C, Dwivedi S K and Ali S L. 2001. Clinico-epidemiological observation of industrial bovine fluorosis in India. *Indian Journal of Animal Sciences* **71**: 1111–15.

Tausky H H and Shorr E. 1953. A micro colorimetric method for determination of inorganic phosphorus. *Journal of Biological Chemistry* **202**: 675–85.

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

Wheeler S M and Fell L R. 1983. Fluorides in cattle nutrition: A review. *Nutrition Abstracts and Review* **53**: 741–69.

World Health Organisation. 1984. *Guidelines For Drinking Water Quality*. Volume 1, Recommendations. Geneva.

World Health Organisation. 2002. *Fluorides*: *Environmental Health Criteria*. pp 227. World Health Organisation, Geneva.