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Effect of molybdenum induced hypocuprosis on copper and molybdenum levels of plasma, hair and liver in buffalo calves

C SINGH[1], S S RANDHAWA[2] and SWARAN SINGH[3]

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 001 India

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ABSTRACT

The objective of this study was to determine the clinical effects, changes in concentrations of Cu and Mo in plasma, hair, liver and red cell parameters of buffalo calves fed on high Mo diet. Male buffalo calves (12), 1–1.5 year-old were assigned to either control (4) or deficient group (8) on the basis of their body weight. Control group was regularly supplemented with 30 mg of elemental copper and calves of deficient group were fed fresh green fodder sprayed with 0.04% sodium molybdate solution to provide 30 ppm of molybdenum for 180 days. Samples of blood, hair, and liver tissue were collected at regular intervals. Hemoglobin and PCV were determined in whole blood. Concentrations of copper and molybdenum were measured in plasma, hair and liver tissue. Fodder samples were analysed for proximate principles, cell wall constituents, Ca, P, Mg, Cu, Mo and Zn. Salient clinical signs were intermittent diarrhea, varying degree of skin and hair depigmentation. Bilateral swelling of hock joints and transitory lameness occured in one calf each. Daily weight gain was lower in molybdenum fed calves as compared to control however, the weight loss occurred only during 45–135 days. Plasma copper was unaffected. Copper concentration of hair and liver declined in 180 days. There was no change in plasma molybdenum concentration. Hair molybdenum content increased after 180 days of molybdenum feeding. Liver molybdenum concentration was significantly high throughout the experiment as compared to control group. Haemoglobin and PCV were not affected by excess molybdenum feeding. Hair copper concentration of 6 ppm or below may be taken as indicator of molybdenum induced copper deficiency in buffaloes.

Keywords: Buffalo, Copper, Hair, Liver biopsy, Molybdenum

Copper in animal body system participates in cellular respiration, bone formation, proper cardiac function, myelination of spinal cord, connective tissue development and skin, hair and wool pigmentation through physiologically important metalloenzymes, viz. cytochrome oxidase, lysyl oxidase, superoxide dismutase, dopamine ß-hydoxylase and tyrosinase. Natural copper (Cu) deficiency is recognized as one of the major deficiencies in cattle and sheep. It is caused by low levels of Cu or high levels of its antagonists primarily molybdenum(Mo),and/or sulphur (S) in diet, of which Mo is the most important under natural feeding conditions. Molybdenum induced copper deficiency causes clinical syndromes, viz. chronic diarrohea, unthriftiness in calves, falling disease in cattle, osteoporosis, epiphyseal widening in growing cattle, anemia in severe deficiency (Underwood and Suttle 1999) and vitiligo in buffaloes (Randhawa *et al*. 2009)

Many studies exist on experimental Mo induced Cu deficiency in cattle (Phillippo *et al*. 1987, Bremner *et al*. 1987, Xin *et al*. 1991 Gengelbach *et al*. 1994). There are however, few experimental studies on Mo induced Cu deficiency in buffalo calves (Randhawa 1993, Soodan and Randhawa 1998). There is evidence of differences in tolerance among different ruminant species viz. cattle, sheep, deer, goat (Mason *et al.* 1984, Zervas *et al*. 1990). A comparative experimental study in cow and buffalo calves had shown that clinical effects and tolerance to dietary Mo excess differs between cattle and buffaloes (Randhawa 1993). In most of the earlier experiments, Cu deficiency in cattle was induced by adding Mo @ 5–10 ppm (DM) in diet. In the present work, higher level of dietary Mo (30ppm wet basis) was used. Since, a few clinical or experimental work exist in scientific literature on Mo induced Cu deficiency in buffaloes, the present study was to determine the clinical effects, changes in concentrations of Cu and Mo in plasma, hair, liver and red cell parameters of buffalo calves fed on high Mo diet.

MATERIALS AND METHODS

Animals and feeding: Male, local bred buffalo calves (12), 1–1.5 years of age were housed in groups of 4 on concrete floor. Seasonal green fodder was offered @ 250g/kg body wt. once daily to each calf. All the calves were dewormed, 2 months prior to the experiment, with a single

Present address: [1]Professor (singhcharanjit11@gmail.com), [2]Director of Research (sarnarinder@gmail.com), [3]Professor (drswaran68@gmail.com).

dose of albendazole. The calves were assigned to either healthy control group (4) or deficient group (8) randomly on the basis of their body weight. Control calves were regularly supplemented with 30 mg of elemental Cu as copper sulphate throughout the experiment. Calves of deficient group were offered fresh green fodder sprayed with 0.04% sodium molybdate solution uniformly, so as to achieve a dietary level of 30 ppm of Mo for 180 days.

Sampling: Venous blood samples were collected by jugular venipuncture in mineral free heparinised vials for mineral estimation and in EDTA for hematology. Blood sampling for hematology were done twice prior to experimental induction. Hematology was performed on 0, 30, 60, 120 and 180 days of the experiment. Hair samples, 1g approximately, were clipped from head and chest areas by a stainless steel scissors once before and at 60 days intervals. After introduction to experimental diet, only freshly grown hair were collected for mineral analysis. Liver biopsies were performed on 0, 90th and 180th day of the experiment. Liver biopsy was obtained by the procedure described by Holtenius (1961). Area over upper third of 11th intercostal space was prepared for aseptic technique. Silverman liver biopsy needle (14 gauge, 3.5 inch long) was inserted along anterior border of 12th rib with stylet *in situ* after local analgesia with 2% lignocaine. Approximately 100–200 mg of liver tissue was obtained from each calf. Tissue samples were washed with double distilled water to remove blood and dried at 100°C for overnight. Dried tissue was weighed and prepared for Cu and Mo determination. Samples of green fodder were collected twice a month during the experimentation period and all the samples were thoroughly mixed to get a representative sample for determination of proximate principles and mineral composition.

Clinical observations: All the buffalo calves were observed daily for general behaviour, coat colour, consistency of feces and gait abnormalities. Body weight of each calf was recorded before feeding and watering on 0, 45, 90, 135 and 180 days of the experiment.

Analysis: Hemoglobin (Hb) was determined by cyanmethemoglobin method. Packed cell volume (PCV) was determined by microhematocrit method. Blood plasma samples (2.5 ml each) were prepared for mineral analysis by digesting in one cycle of distilled concentrated nitric acid (15 ml) and one cycle of hydrogen peroxide (2 ml of 30%). Hair samples were washed with detergent followed by demineralised water repeatedly to remove dust and detergent. Thereafter, hair samples were washed 3–4 times with double distilled water and transferred to oven for drying at 90^{o} C for 24 h. One gram of hair and fodder samples were digested in one cycle of distilled nitric acid followed by 4 ml perchloric acid and 2 ml of hydrogen peroxide. Plasma, hair and feed digestates were diluted to 10 ml with double distilled water. Analysis for proximate principles and cell wall constituents of fodder samples were performed as per AOAC (1970) and VanSoest and Wine (1967). Concentrations of Ca, P, Mg, Cu, Mo, Zn in fodder and Cu, Mo in plasma, hair and liver tissue were determined as per Jones (1977). Nutrient composition of fodder sample was 11.2% crude protein, 23.2% crude fibre, 67.7% neutral detergent fibre, 50.4 acid digestible fibre, 1.70% ether extract, 56.8% nitrogen free extact, 0.63% Ca, 0.61% Mg, 0.33% P, 10.5 ppm Cu, 100 ppm Zn, 1.56 ppm Mo.

Statistical analysis: All values were expressed as mean±SE. The result were analyzed by student's t test using CPCS–1 statistical package on computer. Differences with $P<0.05$ were considered significant.

RESULTS AND DISCUSSION

Overt clinical signs: The buffalo calves allocated to Mo diet developed profuse diarrohea by third day and continued for a fortnight. To ascertain that diarrhoea was because of Mo excess, supplementation rate was reduced to half (15 ppm) for 3 days. The diarrhoea ceased in all the calves. A second bout of diarrhoea appeared at 89th day for few days. The diarrhoea was always self limiting. Onset of diarrhoea was ascribed to high Mo intake rather than a consequence of Cu deficiency as it appeared within a week of the start of Mo feeding and was reversed immediately by decreasing the level of Mo in fodder to half. Previous reports also showed that diarrhoea was a clinical sign in cow heifers ingesting Mo @ 100 ppm DM (Lesperance and Bohman 1963), but not when it was 5–10 ppm (DM) (Humphries *et al*. 1983). One animal developed episodic gait abnormalities intermittently, extending over 2 to 3 days. No visible abnormality in feet, subcutaneous tissue, muscles or joints associated with lameness or otherwise was evident clinically. One calf slowly developed swelling of subcutaneous tissue and bones of both hock joints. Clinical signs of intermittent lameness were similar to the observation by Lesperance and Bohman (1963) with a comparable dietary level. Change in coloration of skin and hair coat from black to brownish became evident in 7 of the 8 calves by about 120 days of experiment. Skin depigmentation was of highest degree in 1, moderate in 3 and mild in 4 calves. None of the control calves developed any change in skin and hair coat colour. Severity of the depigmentation and unthriftiness were directly related. Changes in skin and hair coat colouration were similar to previous studies (Lesperance and Bohman 1963, Humphries *et al.* 1983, Phillippo *et al*. 1987).

Average body weight of the control animals increased from 92.7±12.7 to 118.3±18.9 kg and that of Mo supplemented group registered an increase from 138.9±12.7 to 158.5±23.2 kg. Mean weight gain was lower in Mo supplemented calves (0.107 kg/d) compared to the control (0.127 kg/d). Weight loss was not consistent in Mo fed group. Molybdenum fed calves grew at a faster rate over first 45 days than the healthy controls. The body growth was affected from day 45 to 135. The copper depleted calves lost body weight and the control calves maintained growth but at a slower rate. Mean weight gain rates became identical over the last 45 days. It could, therefore, be inferred that body growth was not consistently affected by Mo induced

Cu deficiency. Individual differences in weight changes were also observed. Two animals did not lose weight throughout the period of the experimentation, although the growth rates slowed down from 45–135 days. One calf had a weight loss during 90–135 days, whereas, 3 calves suffered negative body growth after 90 days to the end of the trial and the final live weight of these 3 calves was less than the initial. The feed intake was not different between 2 groups. Unlike previous reports in cattle, body growth was not consistently affected, differences in weight gains leveled off during last 45 days which suggested physiological adjustment to excess Mo feeding in buffalo calves. Gradual weight loss had earlier been documented in experimental Cu deficiency of buffalo calves conditioned by Mo (Randhawa 1993, Soodan and Randhawa 1998) but with the difference that daily Mo intake was higher (5–15 mg/kg body wt.) in those experiments. In the present study, growth failure did not seem to be affected by Cu deficiency as weight gain was comparable in control and deficient calves during last 45 days when liver Cu concentration was significantly low in deficient group. In contrast, variable response to weight changes had been reported in cattle. Growth rate was unaffected when high growth rate ration was used (Kellaway *et al*. 1978, Xin *et al*. 1991) or the growth was reduced in later stages with the development of Cu deficiency (Humphries *et al*. 1983, Phillippo *et al*. 1987) but at a very lower rate of Mo intake compared to the present study.

Copper status: Pre-induction liver Cu concentration in the control and experimental groups were similar statistically (Table 1). Thereafter, liver Cu levels of control group were maintained on 90th and 180th day of the experiment. Mean Cu concentration in liver decreased gradually in Mo supplemented group. The result showed that major fall in liver Cu occurred after 90 days and Cu deficiency developed in 180 days. Mean liver Cu level in Mo fed buffalo calves declined to below 30 ppm, a threshold level suggested by Radostitis *et al*. (2000) but higher than the marginal band (6–20 ppm) for diagnosis of Cu deficiency in cattle suggested by Underwood and Suttle (1999) in cattle. Plasma Cu concentrations were not significantly affected even when liver and hair concentration had decreased and the fall in hair Cu content was evident by 120 days (Table 1) and the hair Cu of all the Mo supplemented calves was below 6 ppm after 180 days.

The results also showed that individual variation was less with hair than in plasma and the fall in hair Cu levels appeared before the liver Cu concentration decreased to 23.9 ppm (DM). No change in plasma Cu concentration was identical to the results of Lesperance and Bohman (1963) and Xin *et al*. (1991) in cattle and Randhawa *et al*. (2003) in buffaloes. Results of earlier studies showed a decline in plasma Cu if dietary Cu level was below the dietary requirement (4.0 ppm) concurrently with high Mo (Kellaway *et al*. 1978, Humphries *et al*. 1983, Phillippo *et al*. 1987). On comparing the plasma and liver Cu levels in the experiments demonstrating concurrent fall in plasma and liver Cu revealed that liver Cu levels were below 20 ppm. Therefore, it may be suggested that plasma Cu did not fall when liver Cu levels were above 20 ppm. Other workers had reported higher plasma Cu concentrations in sheep (Bremner and Young 1978), Holstein steers (Xin *et al*. 1991) and buffalo calves (Randhawa 1993) following Mo feeding. A major fall in mean liver Cu levels occurred in Mo fed buffalo calves in the later half of the study and it fell below 30 ppm, a threshold level suggested by Radostitis *et al*. (2000) in cattle. Contrary to other studies (Kellaway *et al*. 1978, Humphries *et al*. 1983), the fall in liver Cu content was not remarkable during the first 90 days. Delayed depletion of hepatic Cu reserves was probably due to high dietary Cu levels as indirectly evidenced by a concomitant rise in hepatic Cu concentration in control group. The results showed that hair Cu level appeared to be affected earlier compared to liver in Mo induced hypocuprosis in buffalo calves.

Most remarkable finding on the relationship between

Table 1. Effect of high dietary molybdenum feeding on copper and molybdenum levels in plasma, liver and hair in buffalo calves

Sample	Group	Days				
		0	60	90	120	180
Plasma Cu	C (n=4)	11.8±1.18^{a}	14.4±1.15^{a}	NS	17.1±1.60^{a}	16.0±1.16^{a}
(µmol/l)	E (n=8)	14.4±1.44^{a}	17.6±1.19^{a}	NS	16.8±1.15^{a}	14.5±1.22
Liver Cu	C (n=4)	228.8±17.4^{a}	NS	299.0±37.0^{a}	NS	230.2±11.5^{a}
(ppm DM)	E (n=8)	249.2±49.0^{a}	NS	204.2±78.4^{a}	NS	23.9±2.68^{b}
Hair Cu	C (n=4)	9.66±0.52^{a}	8.47±0.74^{a}	NS	12.2±1.65^{a}	8.04±0.18^{a}
(ppm DM)	E (n=8)	8.62±0.86^{a}	9.34±0.80^{a}	NS	7.20±0.62^{b}	4.75±0.25^{b}
Plasma Mo	C n=4)	0.26±0.15^{a}	0.45±0.06^{a}	NS	0.32±0.25^{a}	0.48±0.11^{a}
(ppm)	E(n=8)	0.16±0.11^{a}	0.42±0.20^{a}	NS	0.94±0.48^{a}	2.27±0.47^{a}
Liver Mo	C (n=4)	4.10±1.43^{a}	NS	5.22±1.53^{a}	NS	5.68±0.42^{a}
(DM)	E (n=8)	8.24±0.76^{b}	NS	42.0±9.34^{b}	NS	43.8±6.57^{b}
Hair Mo	C (n=4)	10.1±1.67^{a}	10.7±2.13^{a}	NS	11.7±2.32^{a}	9.50±1.26^{a}
(DM)	E (n=8)	8.00±0.87^{a}	19.0±4.0^{a}	NS	35.6±8.64^{a}	30.0±3.80^{b}

DM, dry matter; NS, not sampled ; Means with different superscripts differ significantly at P<0.05; C, control group; E, experimental; n, number of calves.

liver and hair Cu concentration was that mean level of Cu in hair started declining at a mean liver Cu level above 23.9 ppm and plasma Cu concentration was within the normal range. This finding contrasted to those of Suttle and Angus (1975) and Kellaway *et al*. (1978) in cattle who had concluded that hair Cu levels changed little above this hepatic Cu concentration.

Molybdenum status: Though, no significant change occurred in plasma Mo concentration it showed a tendency to be higher in Mo fed calves (Table 1). At the time of allocation of treatment, plasma Mo concentration was 0.26 ppm in the control and the 0.16 ppm in the Mo group. The plasma Mo concentration of the control animals varied non-significantly to a maximum level of 0.48 ppm. Buffalo calves on Mo supplemented diet recorded a gradual but statistically non-significant increase from 0.47 to 2.27 ppm by the end of the trial.

Wide variation among individual calves was observed in plasma Mo concentration over first 120 days such that only a few individuals showed high plasma Mo level until 120 days. By 180th day, 7 of the 8 experimental calves had high plasma Mo concentration. Mean liver Mo concentration was remarkably high throughout this trial compared to non significant increase in plasma Mo concentration. Mean levels of Mo in liver of the control and deficient group prior to Mo feeding were 4.10±1.43 and 8.24±0.76 ppm, respectively, the latter being significantly higher. In control group, mean liver Mo concentration varied from 4.10 to 5.69 ppm and was always significantly lower than that of the molybdenotic calves.

Hair Mo content increased gradually from 8.00±0.87 to 30.00±3.80 ppm in Mo supplemented group, whereas, the levels varied from 10.1±1.67 to 11.7±2.32 ppm in the control group. The difference in mean hair Mo between control and molybdenotic calves was significant on 180th day. The comparative data on plasma, hair and liver Mo concentrations from this study showed that liver appeared to be the storage organ in buffalo calves.

No change in plasma Mo concentration was difficult to explain as there was no decrease in feed intake. It may be merely hypothesized that Mo was being mobilized to body tissues as there was a marked elevation in liver Mo concentration. Other potential factors of rapid plasma Mo clearance might be due to deposition in bones (Lesperance and Bohman 1963, McDowell 2003), or kidney (Suttle, 1980) and/or by whole body retention (Grace and Suttle, 1979). The data from the present study, therefore, suggested that plasma Mo concentration in buffalo calves was poorly related with Mo intake up to a dietary level (30 ppm wet basis).

The increase in liver Mo concentration testified the findings of Lesperance and Bohman (1963), Xin *et al*. (1991) in cattle and that of Randhawa *et al*. (2003) in buffalo calves. The increment was, however, much more in the buffalo calves than the results of Lesperance and Bohman (1963, 3–5 ppm wet basis) Cook *et al*. (1966, 1.81–2.06 ppm wet basis) and Xin *et al*. (1991, 15.98 ppm DM basis) in cattle. It may be suggested from the present results that high dietary Mo feeding in buffaloes caused higher accumulation of Mo in liver. In other words, liver of buffaloes appeared to be mass storage organ for Mo compared to cattle.

Dietary Mo intake was also reflected in hair Mo level, though it was delayed compared to liver Mo concentration. Coat colour changes during Mo feeding in cattle with normal blood Cu and ceruloplasmin activity have also been recorded (Wang *et al*. 1988) Therefore, high liver and hair Mo content might be taken as an index of high Mo intake in buffalo calves.

Red cell parameters: Mean Hb and PCV values were unaffected by Mo feeding, however a consistent, gradual and comparable significant fall ($P<0.05$) occurred in red cell parameters in both groups of buffalo calves by 60 days of the experiment (Table 2). Thus red cell mass was unaffected by high Mo intake.

No significant effect on red cell mass was identical to the findings of Randhawa (1993) and Soodan and Randhawa (1998) in Mo induced hypocuprosis in buffalo calves. Previous reports in cattle showed that effect was variable. Hemoglobin and hematocrit was either unaffected in cow heifers (Humphries *et al.* 1983, Gangelbach *et al*. 1994) and Holstein steers (Xin *et al*. 1991) or low in Holstein heifers with similar level of dietary Mo (Phillippo *et al*. 1987). This finding was also confirmed by examination of bone marrow aspirate in healthy control and Mo fed calves which showed normal erythroid cellular profile (Randhawa *et al*. 2002). It may be proposed that buffaloes feeding on diets containing 10.5 ppm Cu suffers from molybdenosis induced hypocuprosis at a dietary level of 30 ppm of Mo wet basis in 180 days and hair Cu concentration of 6 ppm or below may be taken as an indicator of Cu deficiency in buffaloes.

Table 2. Influence of high dietary molybdenum feeding on red cell mass in buffalo calves

Variable	Group	Days				
		0	30	60	120	180
Hb (g/l)	C (n=4)	97.6±0.68 ^{a}	93.8±0.66 ^{a}	75.5±0.55 ^{b}	89.1±0.42^{b}	77.0±0.35^{b}
	E (n=8)	99.9±0.86 ^{a}	96.7±0.51^{a}	84.7±0.30^{b}	80.4±0.30^{b}	73.0±0.35^{b}
PCV (l/l)	C (n=4)	0.36±2.06^{a}	0.36±2.40^{a}	0.26±1.47^{b}	0.28±1.35^{b}	0.28±1.22
	E (n=8)	0.37±1.27^{a}	0.36±1.39^{a}	0.30±1.33^{b}	0.27±1.19^{b}	0.26±1.24 ^{b}

N, number of animals ; C, control group ; E, experimental group.

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