Effect of Molybdenum Induced Copper Deficiency on Peripheral Blood Cells and Bone Marrow in Buffalo Calves

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ABSTRACT : Copper deficiency was induced in eight male buffalo calves by adding molybdenum (30 ppm wet basis) to their diet. Copper status was monitored from the liver copper concentration and a level below 30 ppm (DM basis) was considered as deficient. Haemoglobin, haematocrit, total and differential leucocyte numbers were determined. The functions of peripheral neutrophils were assessed by *in vitro* phagocytosis and killing of *Staphylococcus aureus*. The effect of molybdenum induced copper deficiency on bone marrow was monitored. The mean total leucocyte count was unaffected whereas a significant fall in neutrophil count coincided with the fall in hepatic copper level to 23.9±2.69 ppm. Reduced blood neutrophil numbers was not accompanied by any change in the proportion of different neutrophil precursor cells in bone marrow. It was hypothesised that buffalo calves were more tolerant to dietary molybdenum excess than cattle. It was concluded that neutropenia in molybdenum induced copper deficiency occurred without any effect on their synthesis and maturation process. Bone marrow studies in healthy calves revealed higher percentage of neutrophilic myelocytes and metamyelocytes as compared to cattle. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 4 : 509-515)***

Key Words : Buffalo, Bone Marrow, Copper Deficiency, Molybdenum, Neutropenia

INTRODUCTION

Neutrophils, macrophages and monocytes play a central role in immune responses against microbial pathogens. Studies suggest that alteration in the functions of neutrophils (Boyne and Arthur, 1981, 1986; Jones and Suttle, 1981; Babu and Failla, 1990b), macrophages (Babu and Failla, 1990a) and lymphocytes (Bala et al., 1991; Cerone et al., 1998) occurred in copper deficiency. *In vitro* studies have shown that the bactericidal activity of neutrophils and macrophages is decreased in cattle (Jones and Suttle, 1981; Boyne and Arthur, 1986; Xin et al., 1991) and rat (Babu and Failla, 1990a,b). However, in most of earlier studies the phagocytic and killing activity of neutrophils has been determined for fungal elements (Boyne and Arthur, 1986; Jones and Suttle, 1981). There are only few reports (Xin et al., 1991; Stabel et al., 1993; Gengelbach et al., 1997) of investigations into the bacterial phagocytosis and killing in copper deficient bovines and these too have provided contrasting results.

A well defined neutropenia occurs in copper deficient humans (Becton et al., 1986; Phillip et al., 1990; Percival, 1995) and this has only occasionally been looked for in ruminants (Arthington et al., 1996a,b; Gengelbach et al., 1997; Cerone et al., 1998). But, the results differ. For example, increased numbers of monocytes were observed by Cerone et al. (1998) whereas Arthington et al. (1996a,b) reported an increase in neutrophil count. Such studies are still meagre in water buffaloes. Though, copper deficiency results in anaemia (Radostits et al., 1994) the effect on erythopoiesis in bone marrow is believed not to have been investigated in ruminants.

The present study was therefore designed to evaluate the effect of molybdenum (Mo) induced copper (Cu) deficiency on total and differential leucocyte count, synthesis of leucocytes in bone marrow and function of blood neutrophils in buffalo calves.

MATERIALS AND METHODS

Animals, diets and experimental induction of copper deficiency

Twelve male Murrah buffalo calves (*Bubalus bubalis*), aged 1-1.5 years, were maintained on *ad lib* feeding of a basal diet of green succulent fodder. Samples of green fodder were collected twice a month, mixed and analysed for proximate principles and cell wall constituents (table 1) (AOAC, 1970; Van Soest and Wine, 1967). Eight of these calves (Treatment group) were depleted of Cu by adding sodium molybdate uniformly to the diet, as a spray for 180 days, to provide 30 ppm of Mo in fresh green fodder. Taking into account the dry matter of the green fodder the Cu:Mo ratio of the ration approximated 0.087. Four calves receiving only the basal diet and additional Cu (40 mg), twice a week, served as controls (Control group). The calves in each group were housed separately in pens with concrete floor. Water was offered three times a day. The mineral composition of fodder was determined by Inductively Coupled Argon Plasma Emission Spectroscopy

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Table 1. Nutrient composition of basal diet of mixed green fodder (DM basis)

Proximate principles and cell wall constituents		Minerals	
Dry matter	24.8%	Na	0.13%
Crude protein	11.1%	K	2.8%
Crude fibre	23.2%	Ca	0.63%
NDF	67.7%	P	0.33%
ADF	50.4%	Cu	10.5 ppm
Ether extract	1.7%	Mo	0.95 ppm
NFE	56.8%		

(ICP-AES) (Model Labtum-8440 Australia) by the technique of Jones (1977).

Concentration of copper in the liver and hair

A liver biopsy was obtained by the method described by Holtenius (1961). The upper third of area over 11th intercostal space was prepared for aseptic technique. Following local analgesia with 2% lignocaine HCl, a Silverman's Liver biopsy needle (14 gauge and 2.5 inch long) was inserted along the anterior border of 12th rib with its stylet *in situ*. Approximately 100-150 mg of liver tissue was obtained from each calf. Each biopsy sample was washed with double glass distilled water to remove blood, and dried at 90-100°C overnight. The dried liver tissue was wet digested in one cycle of distilled nitric acid and one cycle of hydrogen peroxide (30%) (Jones, 1977). Hair samples (1-2 gm.) were obtained from chest area prior to the experiment. Thereafter freshly grown hair were cut on day 60, 120 and 180. After washing the hair with detergent, samples were rinsed in deionised water and double distilled water and dried in oven. Hair samples were digested in one cycle each of nitric acid, perchloric and hydrogen peroxide. The digests were diluted with distilled water and the Cu concentration was measured by ICP-AES.

Whole blood constituents

Venous blood samples were collected from 8 treatment and 4 control group calves after 0, 60, 120 and 180 days of Mo feeding. Haemoglobin (cyanmethaemoglobin method) and packed cell volume (PCV) (microhaematocrit method) were determined as per Jain (1986). The total and differential leucocyte counts were determined by counting 200 cells from Giemsa stained smears.

Phagocytic and bactericidal functions

Blood for separation of neutrophils was obtained from 4 Treatment and 4 control group calves. *In Vitro* phagocytosis and bacterial killing capacity of peripheral neutrophils were compared between control and deficient groups.

Separation of neutrophils : A modification of the method of Carlson and Kaneko (1973) was used. Blood samples were centrifuged at 500 g for 20 min and the plasma, buffy coat and a small upper part of the red pellet was discarded. The red cells were lysed by adding isotonic ammonium chloride solution (0.83%) at 37°C, and after separation the white cell pellet was suspended in cold PBS (4-5°C, pH 7.2). The viability of the neutrophils was determined by the method of trypan blue dye exclusion (Jain and Jasper, 1967) and was always above 98%. The cell count of neutrophil suspension was made on a haemocytometer and the suspension was adjusted with PBS to the final working concentration of 5×10^6/ml for phagocytic and bactericidal tests and twice of this (1.0×10^7) for the NitroBlueTetrazolium (NBT) Reduction test. To ensure that neutrophils formed the predominant cell type, only leucocyte with lobed nuclei were counted at a magnification of 400.

Phagocytic index and bactericidal assay : The technique described by Yeo et al. (1993) was used with modifications. A pure culture of *Staphylococcus aureus* ATCC 6538P (Institute of Microbial Technology, Chandigarh, India) was grown overnight on nutrient agar plate. The bacterial suspension, washed three times in PBS, was counted on Petroff-Hauser bacterial counter (Hausser and Son, Philadelphia, USA) and the working concentration was adjusted to 5×10^8 organisms/ml with Hanks Balanced Salt Solution (HBSS). Stoppered plastic tubes, each containing 400 µl of working bacterial suspension and 200 µl of fresh non-haemolysed homologus serum, were incubated in a shaking water bath at 37°C for 30 min. to allow bacterial opsonisation. The working neutrophil suspension (400 µl) was added to each of the tubes and they were further incubated in shaking water bath at 37°C for 30 min. to allow phagocytosis to occur. Extracellular bacteria were removed by differential centrifugation (400 g for 1 min.) and the cell button was suspended in 1.0ml HBSS. An aliquot (100 µl) (To) of the reaction mixture was removed and diluted in sterile distilled water to get 1:10, 1:100 and 1:1,000 dilutions. Another 100 µl sample was taken to prepare smears, which were stained with Giemsa to determine the percentage of neutrophils phagocytosing bacteria and the number of bacteria in phagocytosing neutrophil. The remaining 800 µl of the reaction mixture was further incubated for 2 h and 100 µl (T_2) of the reaction mixture was again diluted as for T0. Aliquots of 100 µl from each of the three dilutions of T0 and T_2 were separately spread over nutrient agar plates. The inoculated plates were incubated for 36-48 h. and the number of colonies were counted on each plate to determine the number of colony forming units (CFU). The percentage bacterial survival was derived by the formula.

$$\text{Bacterial survival (\%)} = \frac{\text{Bacterial CFU of } T_2}{\text{Bacterial CFU of } T_0} \times 100$$

Nitroblue tetrazolium reduction test : As the oxidative metabolism of neutrophils forms the important microbicidal mechanism (Badwey and Karnovsky, 1980) and copper dependent superoxide dismutase forms an integral part of this pathway (Tizzard, 1996) this was evaluated by the NBT reduction test.

The test was done by the method of Bhuyan (1992) but modified for quantitative assay described by Roth et al (1982). Briefly, 5×10^6 cells in 500 µl, 400 µl of NBT dye solution (3 mg/ml in PBS) and 150 µl of endotoxin (LPS, Sigma Chemicals, St. Louis, USA) activated plasma were mixed in a test tube and incubated in a shaking water bath. Simultaneously, a negative control was run. The reaction was terminated before incubation in the control and after incubation in the treatment tube by adding 40 µl of formol saline (40% formaldehyde diluted to 10 times with normal saline)(Gordon et al., 1975) was also run. The formazen dye formed within neutrophils was then extracted with 5 ml of pyridine. The optical density (OD) of the control and treatment mixtures was determined at a wavelength of 580 µm after centrifugation. The results were expressed as the OD/5.0×10^6 neutrophils/30 min/5.0 ml of pyridine.

Bone marrow studies

Bone marrow aspirate was obtained from four control and six treatment group calves. Aspirate (0.1-0.2 ml) was collected aseptically from dorsal ends of 10-11th rib using Jamshidi needle (Baxter Health Care Corporation, Valenicia, USA) following local anaesthesia on days 0, 60, 120 and 180 of the experiment. Haematoxylin and Eosin stained bone marrow smears were used to categorise bone marrow cells and to workout the myeloid:erythroid (M:E) ratio (Jain, 1986).

Statistical analysis

Since initial values of bone marrow cells differed, these were analysed by analysis of covariance (Das and Giri, 1986). The other results were analysed by Student's 't' test using the CPCS-I statistical package on a personal computer. All values were expressed as means±SEM.

RESULTS

The mean liver Cu content had declined to 23.9±2.69 ppm (DM basis) in treatment group after 180 days of Mo feeding (table 2). The mean Cu level in the hair had also declined significantly by 120 days (table 2). Black colour of the skin and hair of the deficient calves had changed to rusty by 120 days. The average weight gain in treatment group was 0.107 kg/day and was lower than that of 0.127 kg/day achieved in control group buffalo calves. The clinical signs were most intense in the calf having the lowest liver Cu content (15 ppm).

The mean TLC showed a declining trend whereas the circulating neutrophils decreased gradually in calves of group II (table 3). A significant ($p<0.05$) fall in neutrophil numbers coincided with decline in liver Cu concentration to below the critical level. The decrease in neutrophil : lymphocyte (N:L) ratio also became significant with the development of Cu deficiency (table 3). Haemoglobin and haematocrit values were unaffected. No significant difference was observed in myeloid:erythroid ratio as well as in the percentage of myeloblasts, promyelocytes, metamyelocytes, band cells and mature neutrophils in bone marrow. Bone marrow results also revealed that erythropoiesis was also not affected.

Neither the number of *S. aureus* in each engulfing neutrophil nor the percentage of neutrophils that phagocytosed *S. aureus* was affected by Cu status (data not given). There was also no treatment difference in NBT reduction test values. However, when temporal changes in bacterial survival rate in neutrophils were observed in individual calves, the most deficient calf consistently supported high intracellular survivability of *S.aureus* throughout the experiment.

Since no data is believed to exist on normal myelogram of buffaloes, the cellular composition of bone marrow aspirate determined prior to the initiation of the experiment in 10 healthy buffalo calves (4 control and 6 treatment group) is being reported (table 5). The mean M:E ratio was 0.71 and ranged between 0.13-1.29 and the percentage of rubriblasts, prorubricytes and metarubricytes were 1.58± 0.55, 7.02±1.57, 26.1±2.03 and 25.7±4.21, respectively. The average percentage of myelocytes, metamyelocytes, band cells and mature neutrophils were 6.83±0.88, 8.43±0.81, 5.74±1.79 and 10.35±1.39, respectively.

Table 2. Effect of dietary molybdenum feeding on liver and hair copper status (ppm : DM basis) of buffalo calves

Tissue	Group	Days of experiment				
		0	60	90	120	180
Liver Cu	Con.	228.8±78.4	-	299.0±37.1	-	230.2±11.5
	Treat.	249.2±49.0	-	204.4±78.4	-	23.9±2.69*
Hair Cu	Con.	9.66±0.52	8.47± 0.74	-	12.2±1.65	8.04±0.18
	Treat.	8.82±0.82	9.34±0.80	-	7.20±0.62*	4.75±0.25*

- Not sampled, * Significant differences at $p<0.05$ between the groups, Con.: control group, Treat.: treatment group.

Table 3. Effect of molybdenum induced copper deficiency on haematological parameters

Variable	Group	Days			
		0	60	120	180
Hb (g/l)	Con.	97.5±6.2	75.5±4.4	89.0±3.8	77.0±2.9
	Treat.	99.9±2.5	84.7±2.7	80.3±2.7	73.0±3.2
PCV(l/l)	Con.	0.36±0.02	0.26±0.01	0.28±0.01	0.28±0.01
	Treat.	0.35±0.01	0.29±0.01	0.27±0.01	0.26±0.01
TLC ($\times 10^9$)	Con.	13.3±2.93	15.2±2.30	10.7±1.85	13.1±2.20
	Treat.	13.8±2.02	10.5±1.02	12.6±1.54	11.3±1.27
Lymph. ($\times 10^9$)	Con.	9.74±0.45	11.1±0.89	7.85±0.43	10.0±0.13
	Treat.	9.38±0.42	7.60±0.35	9.50±0.26	9.18±0.17
Neut. ($\times 10^9$)	Con.	3.09±0.49	3.08±0.78	2.65±0.36	2.98±0.06[a]
	Treat.	3.91±0.42	2.25±0.26	3.06±0.30	2.01±0.15[b]
Mono. ($\times 10^9$)	Con.	0.40±0.05	0.11±0.07	0.13±0.08	0.00±0.00
	Treat.	0.27±0.08	0.34±0.12	0.14±0.04	0.06±0.03
Eos. ($\times 10^9$)	Con.	0.07±0.04	0.07±0.07	0.03±0.03	0.13±0.09
	Treat.	0.28±0.08	0.30±0.19	0.02±0.02	0.11±0.03
Neut:Lymph. Ratio	Con.	0.32±0.06	0.36±0.09	0.35±0.06	0.30±0.01[a]
	Treat.	0.43±0.07	0.30±0.04	0.32±0.04	0.22±0.02[b]

Means of a variable with superscripts differ at $p<0.05$ between the groups. Con.: control group, Treat.: treatment group.

Table 4. Effect of molybdenum induced copper deficiency on bone marrow cells

Variable* (%)	Group	Days			
		0	60	120	180
Prorubricytes	Con.	3.96±1.11	5.54±0.59	5.98±1.40	5.61±1.04
	Treat.	9.04±2.21	5.51±0.50	10.3±1.56	4.55±1.12
Rubricytes	Con.	22.5±1.16	23.5±1.44	35.9±2.48	32.3±4.62
	Treat.	27.8±3.18	33.6±0.53	32.4±2.51	39.0±4.54
Metarubricytes	Con.	32.9±8.15	34.0±7.82	22.4±1.53	21.7±2.28
	Treat.	20.2±3.65	25.3±1.67	16.3±2.33	19.6±2.81
Myeloblasts & Promyelocytes	Con.	1.54±0.10	1.35±0.42	0.98±0.39	2.92±0.82
	Treat.	6.44±1.16	2.57±0.59	2.89±0.74	1.58±0.67
Metamyelocytes	Con.	6.44±1.49	9.75±1.28	8.19±1.00	11.6±1.64
	Treat.	9.15±2.17	5.32±1.37	6.54±1.05	10.4±2.36
Band cells	Con.	11.4±1.57	11.5±1.71	7.73±1.66	7.83±0.62
	Treat.	4.40±2.66	8.29±1.27	6.88±0.93	8.41±2.07
Neutrophils	Con.	8.75±1.69	6.49±1.78	7.70±2.43	5.15±1.05
	Treat.	12.9±2.74	6.77±1.19	9.52±1.77	6.87±1.20
M:E ratio	Con.	0.70±0.21	0.68±0.21	0.54±0.84	0.67±0.08
	Treat.	0.73±0.12	0.55±0.04	0.66±0.10	0.60±0.11

* Percentage of bone marrow cells. Con.: control group, Treat.: treatment group.

DISCUSSION

Buffalo calves were considered deficient by 180 days of Mo feeding since liver Cu concentration decreased to below 30 ppm (Radostits et al., 1994). The clinical signs of hair depigmentation, reduced growth rate, and a fall in concentration of Cu in hair were similar to those reported in earlier studies in cattle and buffaloes (Kellaway et al., 1978; Randhawa, 1993; Soodan, 1996; Arthington, et al., 1997a). Unlike cattle, however, scouring was not observed in buffalo calves in spite of a very high Mo level in feed.

Copper deficiency in ruminants occurs when dietary Mo level exceeds 3 ppm or dietary Cu:Mo ratio falls below 2.0 (Radostits et al., 1994). However, in our experiment,

Table 5. The cellular composition (%) of the bone marrow of healthy buffalo calves (percentage of 200 cells) N=10

	Mean	Range		Mean	Range
M:E Ratio	0.71±0.10	0.31-1.29	Myelocyte	6.87±0.88	5.47-21.8
Rubriblast	1.58±0.55	0.00-3.57	Metamyelocyte	8.43±0.81	3.89-13.14
Prorubricyte	7.02±1.57	3.93-19.5	Band Cell	5.74±1.79	0.00-15.5
Rubricyte	26.1±2.03	19.3-41.2	Mature Neutrophil	10.35±1.39	2.98-16.3
Metarubricyte	25.7±4.21	11.5-46.3	Myelocyte Eosinophilic	0.97±0.56	0.00-2.20
Myeloblast	1.94±0.59	0.00-5.05	Band Eosinophilic	0.12±0.08	0.00-0.80
Promyelocyte	2.45±0.74	0.00-7.35	Mature Eosinophil	1.07±0.33	0.00-2.59

N=Number of buffalo calves.

hypocuprosis developed slowly in spite of a very high level of molybdenum in the basal diet. The liver Cu concentration declined from 249.2 to 23.9 ppm (DM basis) in 180 days when Mo feeding was done @ 30 ppm (wet basis). In contrast, concentration of Cu in liver declined from 192 to 32 ppm (DM basis) in 150 days in cattle when Mo in diet was only 10 ppm (DM basis) (Xin et al., 1991). Similarly a dietary Cu:Mo ratio of 0.8 reduced concentration of Cu in liver from 104 to 28.2 ppm (DM basis) within 8 weeks in cow calves (Humphries et al., 1983) as compared to the present study with very low Cu: Mo ratio (0.087). In another study in cow calves (Cerone et al., 1994), hypocupraemia (plasma copper level 5.9 μmol/L) occurred within 120 days when 30 ppm of Mo and 225 ppm of sulphate (DM basis; Cu : Mo of 0.2) was added to the diet five days per week. Our findings were supported by a comparative study in cow and buffalo calves (Randhawa, 1993), where the levels of Cu in liver decreased from 115 to 21.8 ppm (wet basis) within 35 days of Mo feeding @ 15 mg/kg b.wt. in cow calves whereas liver stores were depleted from 129.7 to 52.9 ppm (wet basis) in 52 days by feeding Mo to buffalo calves at a rate similar to cow calves. Also, the clinical signs were more intense in cow than buffalo calves. It may thus be hypothesised that buffaloes appear to be more tolerant to dietary Mo intake compared to cattle. Non observance of any differences in haemoglobin and PCV values in Cu deficient and control calves were similar to findings of other investigators (Soodan, 1996; Randhawa, 1993; Arthington et al., 1997a,b).

The number of circulating neutrophils as well as the neutrophil:lymphocyte ratio decreased ($p<0.05$) with fall in liver Cu concentration. Neutropenia (6-39%) with low or normal total leucocyte count ($1.3\text{-}13\times10^6$/ml) was also identified in humans, (Goyens et al., 1985; Sriram et al., 1986; Fujita et al., 1989; Phillip et al., 1990; Percival, 1995). However, earlier studies in buffalo calves have failed to reveal significant fall in neutrophil numbers (Randhawa, 1993; Soodan, 1996). The difference might be attributed to less severe Cu deficiency at the time of leucocyte count determination (liver Cu level 14.8 ppm wet basis) and/or dietary Mo supplementation was for a shorter period of time (75-90 days). Higuchi and colleagues (1988) also concluded from their comprehensive studies on interaction of Cu deficiency and circulating neutrophils in humans that neutropenia occurred in severe but not in mild or moderate Cu deficiency. Though, Cerone and others (1998) did not find significant neutropenia, the average total leucocyte, lymphocyte and neutrophil count tended to be lower in deficient cattle. In contrast, study of Arthington et al (1996a) showed that mean neutrophil count was higher in Cu deficient cattle. The increased neutrophil count in this study was, however, found to be associated with high plasma fibrinogen level (indicating a pathogenic challenge) at the time cell count determination in Mo treated than in control group. Also, in comparison to the present study neutrophil count was determined at one time and the mean liver Cu concentration was higher than ours (49±1.7 ppm vs 23.9±2.69 ppm) at the time of blood count.

Five possible pathways which may cause neutropenia are: early death or destruction of the progenitor cells in bone marrow, impairment of the synthesis or a decrease in the rate of cellular maturation in the bone marrow, decreased rate of secretion of neutrophils from the bone marrow or a rapid clearance of neutrophils from the circulation. The results of bone marrow studies indicated that unlike human studies (Dunlap, 1974; Zidar et al., 1977; Hirase et al., 1992) synthesis and maturation of neutrophils was not affected by Cu deficiency in buffalo calves. Therefore, a rapid clearance of neutrophils from circulation or decreased secretion of neutrophils into circulation could be speculated as the possible causes of the fall observed in circulating neutrophils in our study. Reduced viability of blood neutrophils had also been observed in Mo and iron induced Cu deficiency in cattle (Boyne and Arthur, 1981).

The results of *in vitro* bacterial phagocyosis by the neutrophils in this study agreed well with the earlier findings in cattle (Jones and Suttle, 1981; Xin et al., 1991; Stable et al., 1993; Gangelbach et al., 1997). Boyne and Arthur (1986) recorded reduced phagocytosis of Candida *albicans* by neutrophils from cattle when mean liver Cu content was 6 ppm but not when the level was 20 ppm (Boyne and Arthur, 1981). Recently, Cerone et al. (1998) had also demonstrated that phagocytosis of sheep erythrocytes was poor in Cu deficient cattle. The studies

showing difference from the present study employed yeast cells or sheep erythrocytes as target particles, whereas we used *Staphylococcus aureus* in our experiment. Since the surface nature of the target particles affected their phagocytosis by the neutrophils (Tizzard, 1996), it is possible that phagocytic cells responded differently to bacteria as compared to yeast or sheep erythrocytes. It may also be that phagocytic activity was not reduced because of less severe deficiency achieved in this study as compared to that achieved (6 ppm) by Boyne and Arthur (1986).

The observance of reduced bactericidal activity in the most deficient calf was considered as an effect rather than an artifact of assay sensitivity. In spite of assay to assay fluctuations in bacterial survivability in neutrophils (possibly caused by variations in ratio of *S. aureus* to neutrophils in the incubation mixture) a consistent temporal trend emerged in the deficient calf. It was also observed that this calf exhibited relatively more intense signs of deficiency and had the lowest level of Cu in the liver (15 ppm) than the other deficient calves (range 18.2-28.3 ppm). Xin et al. (1991) had also reported a significant reduction in bacterial killing in Mo induced Cu deficiency, but only in the last month of the 8 months long experimental trial.

Oxidative metabolism of neutrophils assessed by NBT reduction test was, however, unaffected in Cu deficient group as well as in the calf manifesting lower neutrophilic bactericidal activity. The results of previous studies of Boyne and Arthur (1986) and Cerone et al. (1998) had revealed a decline in NBT reduction potential of neutrophils in Cu deficiency in cattle. The difference might be attributed to the fact that deficiency was more intense (liver Cu 6 ppm vs 23.9 ppm; plasma Cu 5.9 μmol/l vs 14.5 μmol/l). It may also be that the effects of deficiency induced in our study were so subtle that NBT reduction test was not sensitive enough to allow detection.

The mean as well as the range of M:E ratio in healthy buffalo calves was comparable to the values of 0.79±0.13 (Winquist, 1954), 0.71±0.29 (Wilde, 1964) and 0.61-0.97 (Schalm and Lasmanis, 1976) in adult cattle. The differential cell count, however, revealed that neutrophilic myelocytes and metamyelocytes were higher the present study than the percentage recorded by Wilde (1964) and Schalm and Lasmanis (1976).

IMPLICATIONS

We therefore suggest from our study that Mo induced Cu deficiency in buffalo calves reduces the number of peripheral neutrophils when hepatic Cu concentration falls to below 25 ppm (DM basis).

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