



ISSN (E): 2277- 7695  
ISSN (P): 2349-8242  
NAAS Rating: 5.23  
TPI 2021; SP-10(12): 249-252  
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Received: 28-10-2021  
Accepted: 30-11-2021

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## A study on haemato-biochemical alterations in *Murrah* buffaloes affected with primary hypocuprosis

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### Abstract

A study was conducted in various parts of Haryana state including Hisar, Fatehabad, Rohtak and Sonipat districts to assess the alterations in various haemato-biochemical parameters in *Murrah* buffaloes affected with primary hypocuprosis. Findings of the study revealed significantly lower mean values of Hb, PCV and TEC in affected animals. Mean values of serum ceruloplasmin, copper, cobalt, zinc, manganese and hair copper concentrations were found significantly lower in hypocuprosis affected buffaloes as compared to healthy control group.

**Keywords:** *Murrah* buffalo, primary hypocuprosis, haematology, biochemistry

### Introduction

Copper and other various micronutrients act as component or co-factor of many enzyme systems and plays important role in growth and development of animal body. Their deficiency may disrupt the normal metabolism and therefore has received greater attention in modern intensive animal husbandry practices. Hypocuprosis, both primary and secondary, exerts a wide range of pathological effects in animals which are manifested as depressed growth, severe prolonged diarrhoea, bone disorders, anaemia, nervous disorders, cardiovascular diseases, temporary infertility, achromotrichia, leucoderma and reduced milk yield (McDowell, 1992) [17]. Therefore, a study was conducted in various parts of Haryana state to investigate the haemato-biochemical changes in *Murrah* buffaloes affected with primary hypocuprosis.

### Materials and Methods

Blood, serum and hair samples of a total of 108 *Murrah* buffaloes were collected randomly from various parts of Haryana state including Hisar, Fatehabad, Rohtak and Sonipat districts. Out of these, 80 buffaloes were kept in village conditions and 28 belonged to organised dairy farms. Among these buffaloes, 63 were heifers in which 42 were pregnant and 21 were non-pregnant. Out of these, 45 were adult buffaloes in which 26 were pregnant and 19 were non-pregnant. In the present study, out of a total of 108 buffaloes, overall 49.07 per cent (53/108) animals were found suffering from hypocuprosis. In addition, blood and serum samples of 10 apparently healthy buffaloes were also collected for comparison and statistical analysis of haemato-biochemical findings.

Haematological examination of collected blood samples for haemoglobin (Hb) and packed cell volume (PCV) were carried out by standard acid hematin method and micro haematocrit technique, respectively. Total erythrocytic count (TEC), total leucocytic count (TLC) and differential leucocytic count (DLC) were estimated as per the procedure described by Jain (1986) [8]. Serum ceruloplasmin levels were determined as per the procedure described by Bauer *et al.* (1974) [2]. For determination of mineral levels, collected serum samples were first digested as per the method described by Kolmer *et al.* (1951) [9]. Digested serum samples were further used for estimation of serum copper, cobalt, zinc, manganese, iron and molybdenum concentration as per the method described by Gitelman *et al.* (1973) [7] with the help of double beam atomic absorption spectrophotometer (AAS) using air-acetylene flameatomic absorption spectrophotometer. Hair samples were dusted off for extraneous contamination, cut into 1 cm length and washed with detergent, acetone and double glass distilled water 3-4 times and transferred to oven for drying at 70 °C for 24 hour. These hair samples were further digested (Kolmer *et al.*, 1951) [9] and used for estimation of hair copper concentration by employing

double beam atomic absorption spectrophotometer (AAS) using air-acetylene flame atomic absorption spectrophotometer (Gitelman *et al.*, 1973) [7]. Data collected were analysed as per standard statistical method as described by Snecdecor and Cochran (1967) [25]. For statistical comparison and analysis of haemato-biochemical findings, data of 10 buffaloes from each group i.e. affected with hypocuprosis and apparently healthy animals were used.

## Results and Discussion

As serum/plasma copper level alone did not always reflect the copper status of ruminants (Xin *et al.*, 1991) [28], so serum ceruloplasmin and hair copper concentrations were also estimated along with serum copper for the confirmation of hypocuprosis. Copper bound to albumin and amino acids is delivered from the mucosal cells to the liver, for the hepatic uptake and incorporation into ceruloplasmin a protein representing 90-95 per cent of the serum copper content (Cousins, 1985) [5]. In the present study, significantly lower ceruloplasmin level was observed in copper-deficient animals as compared to healthy controls. Serum ceruloplasmin indirectly reflects the status of copper because ceruloplasmin and serum/plasma copper levels have been shown to be highly positively correlated by large number of workers (Mata, 1990; Soodan, 1996; Laven *et al.*, 2007 and Pourjafar and Dehkordi, 2008) [16, 26, 13, 18] in buffaloes and cattle. Decision to confirm the diagnosis of the copper-deficiency by hair copper concentration was taken considering experimental evidences in buffaloes (Randhawa, 1993 and Soodan, 1996) [21, 26] and support to these observations by the other researchers (Radositits *et al.*, 2003) [19]. Over and above the convincing results were also obtained regarding relationship between hair and serum copper level in present study i.e. low hair copper concentration was observed in copper-deficient animals. Randhawa (1999) [20] also reported low hair copper concentration in hypocuprotic buffaloes having low plasma copper concentration. Similar findings were observed by Maan (2000) [14].

## Haematological parameters

The haematological parameters *viz.* Hb, PCV, TEC, TLC and DLC were estimated in both the groups of buffaloes and compared statistically (Table 1). In the present study, mean value of Hb (g/dl) in animals of group I (9.35±0.65) was found significantly lower as compared to group II (11.96±0.64). Significantly lower mean value of PCV (%) was observed in copper deficient animals (33.70±1.44) in comparison to that of healthy control group (40.40±1.42). Mean TEC (x10<sup>6</sup>/μl) value of group I (5.46±0.13) was also found significantly lower than group II (6.40±0.22). Whereas, no significant alterations were observed in the mean values of TLC (x10<sup>3</sup>/μl) and DLC (%) (Neutrophils, lymphocytes, monocytes, eosinophils and basophils) in hypocuprosis affected buffaloes in comparison with healthy control animals. In the present study, significantly lower mean values of Hb, PCV and TEC were observed in copper deficient buffaloes (Group I) in comparison to healthy control animals (Group II). These findings of present investigation were in accordance with many similar previous studies conducted by Sharma *et al.* (2003) [24], Sharma *et al.* (2005) [23], Xiao-Yun *et al.* (2006) [27], Sangwan *et al.* (2007) [22] and Kumar *et al.* (2007b) [11]. The decline in Hb, PCV and TEC levels in hypocuprosis affected buffaloes reflected development of anaemia tending towards hypochromic macrocytic. Similar

finding has also been reported by Cardoso *et al.* (2001) [3] and Kumar *et al.* (2007a) [10]. In the present study, anaemia was not as severe as in cattle, which corroborate with the findings of Randhawa (1993) [21], but in contrary to the findings of Sharma *et al.* (2003) [24] and Xiao-Yun *et al.* (2006) [27]. A mild but non-significant low level of mean values of TLC and lymphocytes were observed in the present study in copper deficient animals as compared to healthy control group. Almost similar findings had been reported earlier by Sharma *et al.* (2003) [24]. The non-significant decline in TLC and lymphocyte were suggestive to the rarefaction of the white pulp of spleen and decreased number of lymphoid follicles. Whereas no significant difference was observed in mean values of neutrophils, monocytes, eosinophils and basophils in hypocuprosis affected animals in comparison of healthy control group. Similar findings were observed by Soodan (1996) [26] in molybdenum-induced hypocuprosis in buffalo calves. Non-significantly higher levels of monocytes in copper deficient animals ascribed to increased sub-population of monocytes due to hypocupremia (Cerone *et al.*, 1998) [4].

**Table 1:** Haematological parameters (Mean ± SE) of copper deficient (Group I) and healthy control (Group II) buffaloes

Parameter	Group I (N=10)	Group II (N=10)
Hb (g/dl)	9.35 ± 0.65*	11.96 ± 0.64
PCV (%)	33.70 ± 1.44*	40.40 ± 1.42
TEC (x10 <sup>6</sup> /μl)	5.46 ± 0.13*	6.40 ± 0.22
TLC (x10 <sup>3</sup> /μl)	5.69 ± 0.15	6.34 ± 0.21
<b>DLC</b>		
Neutrophils (%)	31.40 ± 1.85	26.90 ± 1.42
Lymphocytes (%)	63.80 ± 1.78	69.80 ± 1.20
Monocytes (%)	1.60 ± 0.38	1.30 ± 0.45
Eosinophils (%)	1.25 ± 0.40	1.18 ± 0.53
Basophils (%)	0.68 ± 0.33	0.63 ± 0.22

**Group I:** Animals affected with hypocuprosis.

**Group II:** Healthy control animals.

Values with superscript \*\* of group I differ significantly in a row at 5% level of significance from healthy control group (Group II).

## Biochemical parameters

The serum biochemical parameters including ceruloplasmin, copper, cobalt, zinc, manganese, iron and molybdenum were estimated in both the groups of buffaloes and compared statistically (Table 2). In the present study, mean value of serum ceruloplasmin (mg %) was found significantly lower in the hypocuprosis (Group I) affected animals (7.35±0.46) in comparison with healthy control group (16.29±1.24). Mean serum copper concentration (ppm) of group I (0.28±0.06) was significantly lower as compared to group II (0.66±0.03). Mean level of serum cobalt (ppm) in animals of group I (0.29±0.08) was significantly lower than group II (0.46±0.04). Significantly lower value of serum zinc (ppm) was observed in group I (1.58±0.56) than the group II (4.61±0.52 ppm). Mean manganese (ppm) level of groups I (0.22±0.05) was found significantly lower as compared to group II (0.43±0.04). Whereas, non-significant alterations were observed in the mean values of serum iron (ppm) and molybdenum (ppm) in hypocuprosis affected buffaloes in comparison with healthy control animals. Hair copper concentrations of all the animals were estimated in both the groups and compared statistically (Table 3). In the present study, mean value of hair copper level (ppm) of hypocuprosis affected buffaloes (4.54±0.69) was found significantly lower than healthy control group (9.15±0.83 ppm). In the present study, mean serum copper concentration of deficient buffaloes

found significantly lower than the healthy control, but mean copper concentration of healthy control (0.66±0.03 ppm) was also close to the critical level (0.60 ppm), indicating that there was less intake of dietary copper as most of the fodder in many districts of Haryana was found to be highly deficient in copper in many earlier studies (Maan, 2000 and Sharma *et al.*, 2005) [14, 23]. Similarly, Yadav and Khirwar (1999) [30] also reported copper deficiency in Jind district of Haryana and found positive correlation between copper content of soil, fodder and buffaloes in this area. Therefore, hypocuprosis in the present study was suggestive of decreased intake of dietary copper due to its low level in fodder which leads to primary copper deficiency. This was further supported by the normal level of serum molybdenum (major antagonist of copper) in copper deficient animals in the present study because high molybdenum level is the major cause of secondary copper-deficiency in many areas of adjoining state Punjab (Randhawa, 1999) [20]. Significantly lower values of serum cobalt, zinc and manganese in hypocuprosis affected buffaloes in the present study might be due to their primary deficiencies along with copper-deficiency as observed earlier in many studies (Mandal *et al.*, 1996; Yadav *et al.*, 1998; Baruah *et al.*, 2000; Maan, 2000; Sharma *et al.*, 2003; Garg *et al.*, 2005 and Lal *et al.*, 2007) [15, 29, 1, 14, 24, 6, 12]. Additionally, low levels of serum zinc in copper-deficient animals also ascribed to increased requirement of zinc in Cu-Zn superoxide dismutase at tissue level due to low level of copper (Radostits *et al.*, 2003) [19].

**Table 2:** Serum biochemical parameters (Mean ± SE) of copper deficient (Group I) and healthy control (Group II) buffaloes

Parameter	Group I (N=10)	Group II (N=10)
Serum Ceruloplasmin (mg %)	7.35 ± 0.46*	16.29 ± 1.24
Copper (ppm)	0.28 ± 0.06*	0.66 ± 0.03
Cobalt (ppm)	0.29 ± 0.08*	0.46 ± 0.04
Zinc (ppm)	1.58 ± 0.56*	4.61 ± 0.52
Manganese (ppm)	0.22 ± 0.05*	0.43 ± 0.04
Iron (ppm)	5.55 ± 0.47	5.30 ± 0.54
Molybdenum (ppm)	0.20 ± 0.02	0.18 ± 0.04

**Group I:** Animals affected with hypocuprosis.

**Group II:** Healthy control animals.

Values with superscript ‘\*’ of group I differ significantly in a row at 5% level of significance from healthy control group (Group II).

**Table 3:** Hair copper concentration (Mean ± SE) of hypocuprosis affected (Group I) and healthy control (Group II) buffaloes

Parameter	Group I (N=10)	Group II (N=10)
Hair copper concentration (ppm)	4.54 ± 0.69*	9.15 ± 0.83

**Group I:** Animals affected with hypocuprosis.

**Group II:** Healthy control animals.

Values with superscript ‘\*’ of group I differ significantly in a row at 5% level of significance from healthy control group (Group II).

## Conclusions

Findings of the present study revealed anaemia and significantly lower levels of serum ceruloplasmin, copper, cobalt, zinc, manganese and hair copper concentrations in *Murrah* buffaloes affected with primary hypocuprosis.

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