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## A study on prevalence and clinical observations of hypocupraemia in *Murrah* buffaloes in Haryana state

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### Abstract

The objective of the present study was to assess the prevalence of copper deficiency in *Murrah* buffaloes from four districts of Haryana state *viz.* Hisar, Fatehabad, Rohtak and Sonapat. The study was undertaken in 108 heifer and adult buffaloes kept under village conditions and organized dairy farms. Analysis of serum samples of these animals revealed overall copper deficiency in buffaloes was 49.07 per cent, out of which 52.38 per cent heifers and 44.44 per cent were adults. The mean serum copper concentration of buffaloes kept under village conditions and organized dairy farms was found  $0.625 \pm 0.043$  ppm and  $0.675 \pm 0.039$  ppm, respectively. Similarly, low levels of ceruloplasmin was observed in animals reared under village conditions ( $13.919 \pm 0.732$  mg%) than the animals kept at organized dairy farms ( $16.741 \pm 1.251$  mg%). Serum ceruloplasmin level was observed lower in pregnant buffaloes ( $13.555 \pm 0.784$  mg%) than the non-pregnant animals ( $15.325 \pm 1.071$  mg%).

**Keywords:** buffalo, copper deficiency, prevalence, clinical observations

### Introduction

Various micronutrients including Copper (Cu) plays important role in growth and development of animal body system as they act as component or co-factor of many enzymes. Deficiency of these nutrients may disrupt the normal metabolism and therefore, has received greater attention in modern intensive animal husbandry practices. Micronutrients *viz.* copper (Cu), cobalt (Co) and zinc (Zn) play a vital role in livestock health and production. Their deficiency results in impairment of function or induces structural and physiological abnormalities (Underwood, 1979) [29]. 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, achromatrichia, leucoderma and reduced milk yield (McDowell, 1992) [13]. Copper also played important role in normal functioning of the immune system in ruminants. It alters the cell-mediated immunity i.e. increasing the mast cell production, and has major protective role in infectious diseases (Stable *et al.*, 1993) [28]. Many previous studies conducted on mineral status of buffaloes in Haryana state (Mandal *et al.*, 1996; Yadav *et al.*, 1998; Mann, 2000 and Sharma *et al.*, 2003) [11, 31, 10, 25] revealed that over 50 per cent animals show copper level below normal values. Copper responsive diseases have also been recognized in dairy cows and buffaloes in Punjab (Randhawa, 1993) [21]. The deficiency of copper, cobalt, selenium and zinc is usually manifested as an “ill-thrift” condition in the absence of specific clinical signs, so it requires bio-chemical appraisal of serum for the diagnosis. Therefore, a study was conducted to investigate the prevalence of copper deficiency in *Murrah* buffaloes in various parts of Haryana state so that suitable therapy can be advocated in the affected areas for optimal animal health and increased production.

### Materials and Methods

Serum samples of a total of 108 *Murrah* buffaloes were collected randomly from various parts of Haryana state including Hisar, Fatehabad, Rohtak and Sonapat 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.

For estimation of serum copper levels, collected serum samples were first digested as per the method described by Kolmer *et al.* (1951) [7]. Digested serum samples were used for estimation of serum copper concentration as per the method described by Gitelman *et al.* (1973) [5] using atomic absorption spectrophotometer.

Serum ceruloplasmin levels was estimated according to the procedure described by Bauer *et al.* (1974) [1]. Data collected were analysed as per standard statistical method as described by Snedecor and Cochran (1967) [26].

## Results and Discussion

### Prevalence

In the present study, out of a total of 108 buffaloes, overall 49.07 per cent animals were found deficient in serum copper levels. The highest prevalence (52.17%) of hypocupremia was found in buffaloes belonged to Fatehabad district followed by animals belonged to Hisar (51.28%), Rohtak (45.83%) and Sonipat (45.45%) districts (Table 1). Findings of the present investigation were in accordance with many previous studies conducted by Yadav *et al.* (1998) [31], Maan (2000) [10] and Sharma *et al.* (2003) [25] who also reported copper deficiency in buffaloes belonged to various parts of Haryana state with prevalence ranging from 46.26 to 68.05 per cent. Hypocupremia in the present study was found more prevalent in buffaloes reared under village conditions (55.00%) than the buffaloes kept at organized dairy farms (32.14%) (Table 2). This might be due to no feeding or less feeding of balanced ration at villages in comparison to organized farms where feeding of balanced ration along with feed supplement is commonly practiced (Maan, 2000) [10]. These findings were in agreement with Randhawa and Randhawa (2002) [20] but in contrary to Mata (1990) [12], who recorded higher average serum copper and ceruloplasmin levels in buffaloes

maintained at villages than the buffaloes at organized farms. In the present study, prevalence of copper deficiency was found more in heifers (52.38%) than in adult buffaloes (44.44%) (Table 1). These findings were in agreement with Randhawa (1999) [19] and Pavlata *et al.* (2005) [17]. This might be due to increased requirement of growing animals, apart from that in general practice farmers provided feed supplement to the adult animals only for more production, so adults meet their requirement from these feed supplements like cotton seed cake, grains, mineral mixture and green fodder which are rich source of copper (Maan, 2000) [10], while no feed supplement was provided to heifers. Only wheat straw with less quantity of green fodder is given to heifers which are poor source of copper, containing upto 5 ppm copper only (Maan, 2000 and Garg *et al.*, 2005) [10, 4]. Higher prevalence of copper deficiency was found in pregnant buffaloes (55.88%) as compared to non-pregnant animals (37.50%). The average value of serum Cu (ppm) was found to be significantly lower in pregnant buffaloes ( $0.57 \pm 0.36$ ) than the non-pregnant buffaloes ( $0.753 \pm 0.62$ ) (Table 3). Similar trend was found in ceruloplasmin levels in these buffaloes. These findings of present study were in agreement with Xiao-Yun *et al.* (2006) [30] and Kumar *et al.* (2007a) [8]. Miller *et al.* (1972) [14] attributed such variations might be due to deposition of copper in liver of developing foetus throughout the gestation period, which acts as a reservoir of copper after the birth.

**Table 1:** Prevalence of copper deficiency in buffaloes in various parts of Haryana

District	Heifer			Adult			Over all deficiency
	Pregnant	Non-Pregnant	Total	Pregnant	Non-Pregnant	Total	
Hisar	12/18 (66.66)	2/6 (33.33)	14/24 (58.33)	3/7 (42.85)	3/8 (37.50)	6/15 (40.00)	20/39 (51.28)
Fatehabad	5/10 (50.00)	¼ (25.00)	6/14 (42.85)	5/7 (71.42)	½ (50.00)	6/9 (66.66)	12/23 (52.17)
Sonipat	4/6 (66.66)	3/6 (50.00)	7/12 (58.33)	2/6 (33.33)	¼ (25.00)	3/10 (30.00)	10/22 (45.45)
Rohtak	4/8 (50.00)	2/5 (40.00)	6/13 (46.15)	3/6 (50.00)	2/5 (40.00)	5/11 (45.45)	11/24 (45.83)
Total	25/42 (59.52)	8/21 (38.09)	33/63 (52.38)	13/26 (50.00)	7/19 (36.84)	20/45 (44.44)	53/108 (49.07)

Figures in parenthesis indicate per cent

**Table 2:** Prevalence of copper deficiency in buffaloes kept under village conditions and organised dairy farms

Group	Pregnant	Non- Pregnant	Over all deficiency
Village conditions	33/49 (67.34)	11/31 (35.48)	44/80 (55.00)
Organised dairy farm	5/19 (26.31)	4/9 (44.44)	9/28 (32.14)
Total	38/68 (55.88)	15/40 (37.50)	53/108 (49.07)

Figures in parenthesis indicate per cent

**Table 3:** Average serum copper and ceruloplasmin values in pregnant and non-pregnant buffaloes in Haryana

Variable	Pregnant (n = 68)	Non-Pregnant (n = 40)	Z <sub>cal</sub>
Cu (ppm)	$0.571 \pm 0.361$	$0.753 \pm 0.062$	Z <sub>cal</sub> = 2.729*
Ceruloplasmin (mg%)	$13.555 \pm 0.784$	$15.325 \pm 1.071$	Z <sub>cal</sub> = 1.349

\*Z<sub>cal</sub> at 5% level of significance is 1.96

**Table 4:** Average serum copper and ceruloplasmin valves in buffaloes kept at village conditions and organised dairy farms

Variable	Village Conditions (n = 80)	Organised Dairy Farms (n = 28)	Z <sub>cal</sub>
Cu (ppm)	$0.625 \pm 0.043$	$0.675 \pm 0.039$	Z <sub>cal</sub> = 0.654*
Ceruloplasmin (mg%)	$13.919 \pm 0.732$	$16.741 \pm 1.251$	Z <sub>cal</sub> = 1.428

\*Z<sub>cal</sub> at 5% level of significance is 1.96

### Clinical observations

In the present study, out of 108 buffaloes a total of 53 were found hypocupraemic based on estimation of serum copper levels. Among these 53 hypocupraemic buffaloes, 28 animals were found to have observable single or multiple clinical findings (Table 5). Poor body condition was the observable

clinical finding found in most of the buffaloes (85.71%) suffering from hypocupraemia followed by achromotrichia (85.71%), leucoderma (71.42%), anaemia (67.85%), repeat breeding (57.14%), alopecia (50%), anoestrous (32.14%), mastitis (10.71%), diarrhoea (7.14%), hoof deformity (7.14%), sudden falling of animal (3.57%) and paralysis

(3.57%). Poor body condition could be attributed to the disturbances in metabolism and absorption from the alimentary tract as copper is required for these functions (Gallagher, 1957 and Mudgal *et al.*, 2007) [3, 15] or might be due to other micro-nutrient deficiencies. Achromatrichia and leucoderma could be attributed to the disturbances in conversion of tyrosine to melanin as this conversion is catalyzed by copper containing enzyme, tyrosinase (Holstein *et al.*, 1979) [6]. Similar findings in the hypocupraemic animals have been reported earlier by Soodan (1996) [27], Sharma *et al.* (2005) [24], Kumar *et al.* (2007a) [8], Kumar *et al.* (2007b) [9] and Sharma *et al.* (2008) [23]. This was further supported by the improvement in the darkness and shining of both skin and hairs, which was suggestive of increased tyrosinase activity in the copper supplemented animals either orally or parentally. Anoestrous and repeat breeding in hypocupraemic buffaloes might be due to the change in reproductive processes as copper play a vital role in maintaining the activities of follicle stimulating hormone,

leutenizing hormone and estrogen (Desai *et al.*, 1982) [2]. Similar findings were reported by Randhawa and Randhawa (2002) [20] and Kumar *et al.* (2007a) [8]. Copper has major protective role in infectious diseases (Stable *et al.*, 1993) [28] by increasing the mast cell production. Therefore, mastitis as observed in three copper deficient animals might be due to suppressed cell mediated immunity. Shaheen (2005) [22] also found low levels of serum copper and zinc in mastitic cases in comparison to healthy control. Diarrhoea and hoof deformity were recorded in 7.14 per cent copper deficient animals showing clinical signs. Diarrhoea was not so consistent sign in present study as reported in earlier studies (Soodan, 1996 and Radostits *et al.*, 2003) [27, 18]. Paralysis and sudden falling of animals in copper deficiency might be due to demyelination of nerves and anaemic anoxia (Radostitis *et al.*, 2003) [18], respectively. Muskens *et al.* (2006) [16] reported wallerian type of degeneration of myelinated nerve fibers in the lateral and ventral spinal cord tracts of copper deficient suckling calves associated with ataxia of hind limb.

**Table 5:** Frequency distribution of clinical signs in copper deficient buffaloes (n = 28)

Sr. No.	Clinical signs	No. of animals affected
1	Poor body condition	24 (85.71%)
2	Anaemia	19 (67.85%)
3	Depigmentation of hair (Achromotrichia)	24 (85.71%)
4	Depigmentation of skin (Leucoderma)	20 (71.42%)
5	Alopecia	14 (50.00%)
6	Diarrhoea	02 (7.14%)
7	Sudden falling of animal	01 (3.57%)
8	Paralysis	01 (3.57%)
9	Hoof deformity	02 (7.14%)
10	Anoestrous	09 (32.14%)
11	Repeat breeding	16 (57.14%)
12	Mastitis	03 (10.71%)

## Conclusions

In the present study, hypocupraemia was found in heifer and adult as well as in pregnant and non-pregnant *Murrah* buffaloes reared in village conditions and organized dairy farms from various parts of Haryana state with overall copper deficiency in 49.07 per cent animals. Important clinical observations of hypocupraemia revealed poor body condition, skin disorders, reproductive problems and digestive disturbances.

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