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A study on micro minerals estimation in Mithun (*Bos frontalis*) in Nagaland, India

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Abstract
The North-Eastern Hill Region (NEHR) of India is home to the Mithun (*Bos frontalis*), which is primarily found in subtropical rainforest. Mithun can also be found in Myanmar, Bangladesh, Bhutan, China, and Malaysia and is said to be descended from wild gaur. There is currently very little research being conducted on Mithun's mineral deficient condition. Therefore, a study on the mineral status of mithun is valuable in identifying deficient minerals and also advocating suitable mineral supplementation in the diet for good health, optimum production and enhance the reproductive performance. In this study the serum micro-mineral namely Iron, copper, cobalt, manganese and zinc and its biochemical concentrations in Mithuns were estimated by digestive procedure of mineral estimation by dividing the animals into four groups based on the clinical status of the animals, the study revealed no significant deficiency of micro minerals among the four groups of animals examined.

Keywords: Nagaland, Mithun (*Bos frontalis*), micro mineral, deficiency

Introduction
Mineral nutrients are very important for several metabolic functions, and their deficiencies alter the activity of certain enzymes and the functions of specific organs, thus impairing the metabolic pathways as well as vitamins, hormones, and serum enzyme levels (Sharma *et al*., 2006) [1]. Mineral deficiency varies with the trace minerals, the degree and duration of the dietary deficiencies, age, sex, and species of the animal involved (Sharma *et al*., 2005) [2]. Clinical signs linked to trace elements are well described in cattle (Graham, 1991) [3]. Dairy cattle are more prone to mineral deficiency due to their increased requirement for lactation (McDowell *et al*., 1993) [4]. The NEHR of India is one of the world's major biodiversity hotspots, home to a diverse range of mithun-eating tree leaves, herbs, shrubs, and grasses world (Annual Report, MoEF 2001) [5]. The nutrient requirements of this semi-wild, coveted animal are primarily met by nibbling on these forested food resources. Mineral deficiency is a local issue (McDowell *et al*., 1985) [6]. Soil mineral status changes as a result of land pressure for maximum crop output, fertiliser application, and natural calamities, modifying the mineral content of feeds and fodders and hence their availability to animals.

Materials and Methods
Before collecting the blood samples from the animals, the following details were recorded: species, age, sex, and strain of the mithun. Blood samples from Mithuns were collected from Nagaland. A total of 101 blood samples were collected irrespective of age, sex and stage of lactation during this study.

Grouping of the animals
Before obtaining blood samples from the animals, the following information was gathered: The species, strain, age, and gender of mithun. Blood samples were taken from Mithuns of all ages. A clinical case record sheet was prepared to generate information regarding the age, physiological state, production levels, plan of nutrition, and health status of the animals under study. The mithuns were divide d into four groups depending on their clinical status, i.e., apparently healthy groups, low productive potential groups, overgrowth or cracks in hooves and horns groups, and unthriftiness in young mithun groups (Table 1).

Table 1: Grouping of Mithuns based on health status

Sl. No.	No. of animals	Group	Health status
1	52	Group-I	Apparently healthy
2	15	Group-II	Low productive potential
3	21	Group-III	Overgrowth or cracks in hooves and horns
4	13	Group-IV	Unthriftiness in young mithuns

Digestion procedure for mineral estimation

Digestion of serum samples: The serum samples were digested as per the procedure described by Kolmer *et al.* (1951) [7]. In the digestion tube, 3 ml of serum were mixed with an equal amount of concentrated HNO_3. The samples were kept overnight at room temperature, followed by digestion on low heat (70-80 °C) using a digestion bench, until the volume of the sample was reduced to about 1 ml. To this digested mixture, 3 ml of a double acid mixture (3 parts concentrated HNO3 and 1 part 70% HClO4) was added, and low-heat digestion was continued until the digested samples became watery and clear and emitted white fumes. As per the need, the addition of a 3 ml double acid mixture followed by low-heat digestion was repeated a couple of times. Further heating was continued to reduce the volume to approximately 0.5 ml. The final volume of the filtrate was made up to 10 ml with triple-distilled deionized water after luke-warming the solution.

While digesting the serum samples, simultaneous digestion of the reagent blank was undertaken, and the final volume was similarly made up to 10 ml to have a blank.

Statistical analysis

The statistical analysis was carried out using SPSS statistical analysis software (SPSS Version 11.5 USA) as per the standard procedure (Snedecor and Cochran, 1989).

Results

The F-values recorded in iron, copper, cobalt, manganese, and zinc were 0.11, 2.75, 0.23, 0.43, and 1.39, respectively, as given below in Table 2.

Table 2: Serum micro-mineral and biochemical concentrations in Mithun (*Bos frontalis*) according to their clinical grouping (Mean± SE)

Serum Minerals	Normal value (Cattle)	Group-I (Apparently healthy) N=52	Group-II (Low productive potential) N=15	Group-III (Overgrowth or cracks in hooves and horns) N=21	Group-IV (Unthriftiness in young mithuns) N=13	F-Value
Fe (ppm)	5.51	15.06±2.94	12.90±0.87	13.25±0.83	13.67±2.31	0.11^{NS}
Cu (ppm)	0.60-1.50	1.30±0.21ac	0.66±0.08bc	0.66±0.07b	0.54±0.17b	2.75*
Co (ppm)	0.05-0.07	0.01±0.00	0.017±0.00	0.01±0.00	0.01±0.00	0.23^{NS}
Mn(ppm)	0.20-0.22	0.17±0.02	0.18±0.01	0.20±0.01	0.16±0.02	0.43^{NS}
Zn (ppm)	0.80-1.19	2.46±0.20	1.93±0.10	1.92±0.12	2.30±0.36	1.39^{NS}

NS- Non-significant

* Significant at 5% ($p \leq 0.05$)

Serum iron

The mean values of serum iron (ppm) in mithuns of different clinical conditions are given in Table 2.

The overall mean values of serum iron (ppm) in Group-I, Group-II, Group- III and Group-IV were 15.06±2.94, 12.90±0.87, 13.25±0.83 and 13.67±2.31 (Fig.1). Mean serum Iron values of all the groups showed non-significant ($p<0.05$) higher values of serum iron values and there was no significant difference among the different groups.

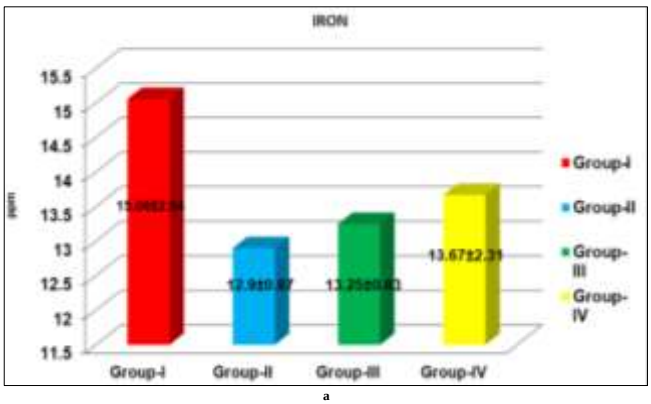

Fig 1: Graphical representation of serum iron levels in mithuns grouped clinically

Serum copper

The overall mean values of serum copper (ppm) in Group-I, Group-II, Group- III and Group-IV were 1.30±0.21, 0.66±0.08, 0.66±0.07 and 0.54±0.17 (Fig 2). Normal Cu level is 1.04± 0.06 ppm in mithun as cited by Rajkhowa *et al.* (2003) [8]. Mean serum copper values of Group I showed a significant difference ($p<0.05$) with that of Group-III and Group-IV respectively. Mean serum copper concentration observed in all the groups were within the lower normal range.

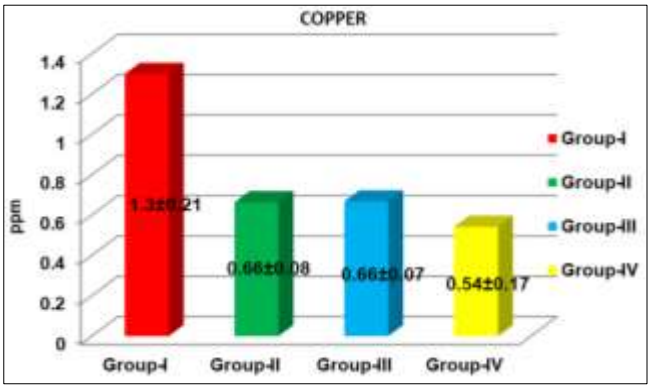

Fig 2: Graphical representation of serum copper levels in mithuns grouped clinically

Serum cobalt

Mean ±S.E (ppm) values of serum cobalt of mithun in Group-I, Group-II, Group-III and Group-IV were 0.01±0.00, 0.017±0.00, 0.01±0.01 and 0.01±0.00 (Fig. 3), respectively. There was no significant ($p<0.05$) difference in the mean serum cobalt values of different groups. The mean values of serum cobalt of the different groups were lower than that of the normal.

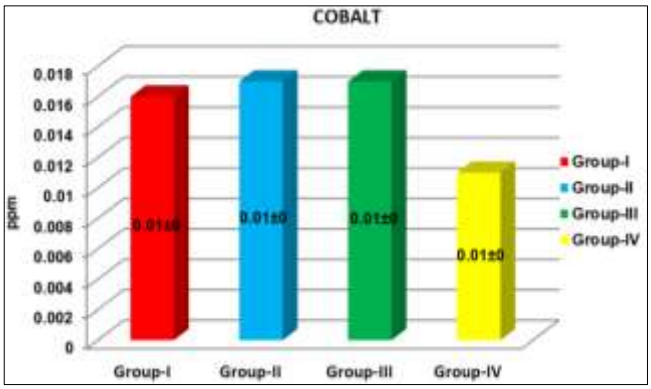

Fig 3: Graphical representation of serum cobalt levels in mithuns grouped clinically.

Serum manganese

The overall mean values of serum manganese (ppm) in Group-I, Group-II, Group- III and Group-IV were 0.17±0.02, 0.18±0.01, 0.20±0.01 and 0.16±0.02 (Fig. 4) The mean values of serum manganese were not found to be significantly (p<0.05) different. The mean values of serum manganese of all the groups were lower than that of the normal value.

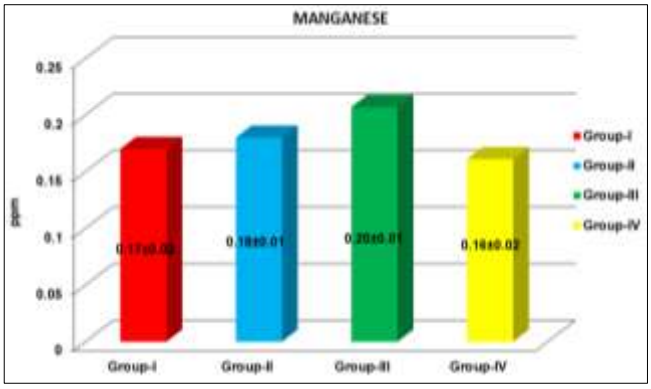

Fig 4: Graphical representation of serum manganese levels in mithuns grouped clinically.

Serum zinc

The overall mean values of serum zinc (ppm) in Group-I, Group-II, Group- III and Group-IV were 2.46±0.20, 1.93±0.10, 1.92±0.12 and 2.30±0.36. The mean values of serum zinc were not found to be significantly (p<0.05) different. The mean values of serum zinc were found to be comparatively higher than that of the normal.

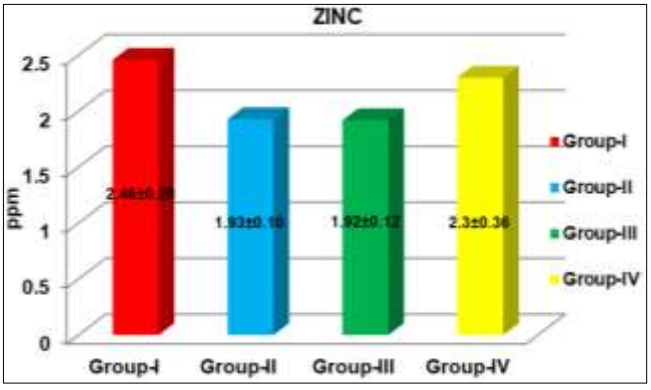

Fig 5: Graphical representation of serum zinc levels in mithuns grouped clinically.

Discussion

The overall mean of serum iron in all the clinical groups, i.e., Group I, Group II, Group III, and Group IV, was comparatively higher than that of the normal values. Normal values of serum Fe are 5.51 0.29 ppm in mithun (Rajkhowa *et al*. 2003) [8] and 57-162 µg/dl in cattle as per Radostits *et al*. (2007) [9]. The overall average values of serum iron in Mithun were in the range of 12.27±0.59 -25.47±13.73 ppm as against the critical limit of 0.89 ppm as suggested by McDowell (1987) [10].

Higher values of serum Fe has also been reported by various workers in different parts of India (Bhat *et al*., 2011; Sharma *et al*., 2005; Verma *et al*., 2008) [11-13]. The majority of tropical soils are acidic, resulting in forage levels of iron in excess of requirements (Jena *et al*., 2011; Kaneko 1980) [14, 15] revealed that elevated iron could be due to refractory anaemia, hemolytic iron overload, or liver disease. It could also be due to extra iron supplements in the diet through mineral supplements, as 89.10% of the animals are supplemented with mineral mixtures.

The mean copper value of Group-IV (unthrifty young mithuns) of the clinical group was lower than that of the lower limit of the normal value (0.06 ppm). Normal range of copper in cattle, i.e., 0.60-1.50 ppm (9.5-23.6 µmol/l) as per McDowell (1992) [16]. Copper insufficiency can be linked to excessive Fe levels in the soil, feed, and cattle of certain Indian regions. This is in corroboration with the findings by Campbell *et al.* (1974) [17], who observed that high levels of iron over an extended period of time have an influence on copper availability. Sharma and Joshi (2006) [1] and Tiwary *et al.* (2007) [18] also observed deficient serum Cu levels in animals from the Garhwal hilly region and Haridwar district (plain region) of Uttarakhand, respectively. Kumar *et al.* (2007) [19] also reported a copper deficiency of 30.63% in the buffaloes of eastern Uttar Pradesh. Das *et al.* (2011) [20], Humphries *et al*. (1983) [21], Kumagai *et al.* (1996) [22], Shukla *et al.* (2009) [23], Yadav and Khirkwar (1999) [24], Dutta *et al.* (2000) [25], and Sharma *et al.* (2003b) [26] have also reported similar findings. McDowell (1985) suggested that decreased gut absorption as well as increased excretion of copper in animals resulted in a lower concentration of this mineral in serum. A high level of molybdenum and iron in feed may also contribute to the secondary copper deficiency (Underwood, 1981) [28].

The overall mean of serum cobalt in all the clinical groups, i.e., Group I, Group II, Group III, and Group IV, was comparatively lower than that of the critical level (0.05–0.07 ppm). The overall average value of serum cobalt in the clinical group was in the range of 0.011–0.016 ppm. McDowell *et al.* (1975) [28] has reported the critical level of cobalt as 0.05–0.07 ppm. Cobalt deficiency is the most severe mineral limitation for grazing livestock in tropical countries (McDowell and Conrad, 1990) [29]. A high manganese level in the soil decreases the uptake of Co by the fodders and thereby the uptake of Co by the animals, ultimately leading to its deficiency in the blood profile. A deficiency of cobalt has been reported by various workers in different parts of India (Sharma *et al.*, 2006; Kumar *et al.*, 2007) [1, 19]. Tiwary *et al.* (2009) [18] and Kumarasen *et al.* (2010) [30] reported a non-detectable level of cobalt in serum.

The overall mean of serum manganese in all the clinical groups, i.e., Group-I, Group-II, Group-III, and Group-IV, was comparatively lower than that of the critical level (0.20 ppm).

As per Radostits *et al*. (2007) [9], the normal range of manganese is 18–19 µg/dl, 3.3–3.5 µmol/l or 0.20-0.22 ppm in cattle, respectively. Shukla *et al*. (2009) [23] also reported a manganese deficiency of 87.97% in cattle whose mean serum concentrations (0.18 ppm) were below those of the critical levels (<0.20 ppm). Kumarasen *et al.* (2010) [30] also reported manganese deficiency in cattle in the sub-tropical hill agrosystem with a mean value of 1.1 µg/dl. Baruah *et al.* (1999) [31] also reported manganese deficiency in prepubertal Jersey heifers in Assam.

The overall mean of serum zinc in all the clinical groups, i.e., Group I, Group II, Group III, and Group IV, was comparatively higher than that of the critical level. As per Radostits *et al*. (2007) [9], the normal range of zinc is 0.80–1.19 ppm (12.2–18.2 to 18.2µmol/l) in cattle. Higher levels of zinc in serum have been reported by Kumar *et al.* (2005) [32]. Tiwary *et al*. (2009) [33] also reported that the zinc contents were normal in the serum of cattle and buffaloes in Uttarakhand and were above the critical levels (0.80 ppm). Similar observations have been reported by Baruah *et al.* (1999) [31], Gadberry *et al.* (2003) [34], and Hedaoo *et al.* (2008) [35]. A high zinc level could be due to the fortification of concentrate mixtures with mineral mixtures that have trace minerals in adequate amounts to meet the animal's requirements.

Conclusion

The metabolic and deficiency diseases are quite common under Indian conditions, particularly in the tropical areas where a short rainy season of 3–4 months and a long dry season of 8–9 months are experienced, and they are mainly due to the non-availability of a balanced diet or deficiency of specific nutrients in the soil. The conditions of tropical areas also significantly affect the quantity and quality of forages, and the availability of minerals decreases with the maturity of the fodder.

The climate of Nagaland is determined by its terrain in general; it is hot to moderate subtropical in areas with elevations of 1000 to 1200 m. The climate, the climatic environment is warm subtropical in areas with elevations of 1200 m and above; the temperature ranges from 0 °C in winter to 40 °C in summer depending on the elevation; the average annual temperature ranges from 18 °C to 20 °C and 23 °C to 25 °C in higher and lower elevations, respectively; Soil mineral status changes as a result of pressure on land for maximum crop output, fertilizer application, and natural calamities, modifying the mineral contents of feeds and fodders and hence their supply to animals. In this study, it has been found that there is a deficiency of cobalt and copper in the collected blood samples.

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