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A study on macro minerals estimation in Mithun species in Nagaland state of India

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Abstract
Mithun (*Bos frontalis*) is a unique ruminant found mainly in subtropical rainforest of North-Eastern Hill Region (NEHR) of India. This unique livestock species is also found in Bhutan, Myanmar, Bangladesh, China and Malaysia and considered to be a descendent of wild gaur. Presently there is very little work done regarding the mineral deficiency status of Mithun, Therefore, a study on the mineral status of feeds, fodders and animals is worthwhile in identifying deficient minerals and advocating suitable corrective measures for good health, optimum production and reproductive performance. In this study the serum macro-mineral namely Calcium, Phosphorus, Sodium, Potassium and Magnesium 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 macro minerals among the four groups of animals examined.

Keywords: Mithun -Nagaland -macro minerals -estimation

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, hormonal and serum enzyme level (Sharma *et al*., 2006) [20, 24]. Mineral deficiency varies with the trace-minerals, the degree and duration of the dietary deficiencies, age, sex, species of the animal involved (Sharma *et al*., 2005) [25]. Clinical signs linked to trace elements are well described in cattle (Graham, 1991) [5]. Dairy cattle are more prone to mineral deficiency due to their increased requirement for lactation (McDowell *et al*., 1993) [11]. NEHR of India is one of the mega biodiversity centres of the world (Annual Report, MoEF 2001) [14], harbours a large variety of Mithun-eating tree leaves, herbs, shrubs and grasses. The nutrient requirements of this semi-wild prized animal is mostly fulfilled by browsing on these fodder resources of forest origin. The deficiency of minerals is an area problem (McDowell *et al*., 1985) [10]. Soil mineral status keeps on changing due to pressure on land for maximum crop production, fertilizer application and natural calamities, thus altering the mineral contents of feeds and fodders and hence their supply to the animals

Materials and Methods
Blood samples from semi intensive Mithun animals were collected from two districts of Nagaland i.e., Dimapur and Phek district. A total of 101 blood samples were collected irrespective of age, sex and stage of lactation during this study.

Grouping of the animals
Before collecting the blood samples from the animals, the following details were recorded i.e. species, age, sex and strain of the Mithun. Blood samples were collected from all the different age groups of the mithun. A performa /clinical case record sheet was prepared to generate the information regarding age, physiological state, production levels, plane of nutrition, and health status of the animals under study Animals were divided into four groups depending on the clinical status of the animals i.e., apparently healthy groups, Low productive potential group, overgrowth or cracks in hooves and horn 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: Serum sample was digested as per the procedure described by Kolmer *et al.* (1951) [7]. To 3 ml of serum, an equal amount of concentrated HNO_3 was mixed in the digestion tube. The samples was kept overnight at room temperature followed by digestion on low heat (70-80 °C) using digestion bench, until the volume of the sample was reduced to about 1 ml. To this, 3 ml of double acid mixture (3 part concentrated HNO_3 and 1 part 70% $HClO_4$) was added and low heat digestion continued until the digested samples became watery clear and emitted white fumes. As per need, the addition of 3 ml double acid mixture followed by low heat digestion was repeated couple of times. Further heating was continued to reduce the volume to approximately 0.5 ml. Final volume of the filtrate was made up to 10 ml with triple distilled deionised 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 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-value recorded in Calcium, Phosphorous, Sodium, Potassium and Magnesium were 2.92, 1.64, 0.44, 7.79 and 4.00 respectively as given below in Table no 2.

Table 2: Serum macro-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
Ca(mg/dl)	9.7-12.4	10.12 ± 0.12^{ab}	9.89 ± 0.28^{a}	9.49 ± 0.18^{ac}	9.56 ± 0.22^{ac}	2.92*
P(mg/dl)	5.6-6.5	7.67±0.35	8.78±0.79	7.38±0.35	8.83±0.83	1.64^{NS}
Na (ppm)	3036 - 3496	3123.26±29.74	3053.45±94.05	3075.00±71.18	3134.37±13.45	0.44^{NS}
K(ppm)	145.5-228.54	384.91 ± 35.71^{a}	212.70 ± 11.08^{b}	193.24 ± 10.34^{b}	201.59 ± 22.82^{b}	7.79**
Mg(mg/dl)	1.8-3.81	2.45 ± 0.11^{ab}	3.43 ± 0.28^{ac}	2.86 ± 0.22^{a}	2.85 ± 0.37^{a}	4.00**

* significant at 5 % ($p \leq 0.05$) ** significant at 1 % ($p \leq 0.01$) NS: Non Significant

Values having different superscripts within the same column have significant difference

Serum Calcium

The overall mean value of serum calcium (mg/dl) in Group I, Group-II, Group-III and Group-IV were 10.12±0.12, 9.89±0.28, 9.49±0.18 and 9.56±0.22 in mithuns respectively (Fig no 1). Mean Ca values of the different groups were within the normal range however animals belonging to group I had higher value and there was significant difference (*P*<-0.01) with animals of group III and Group IV.

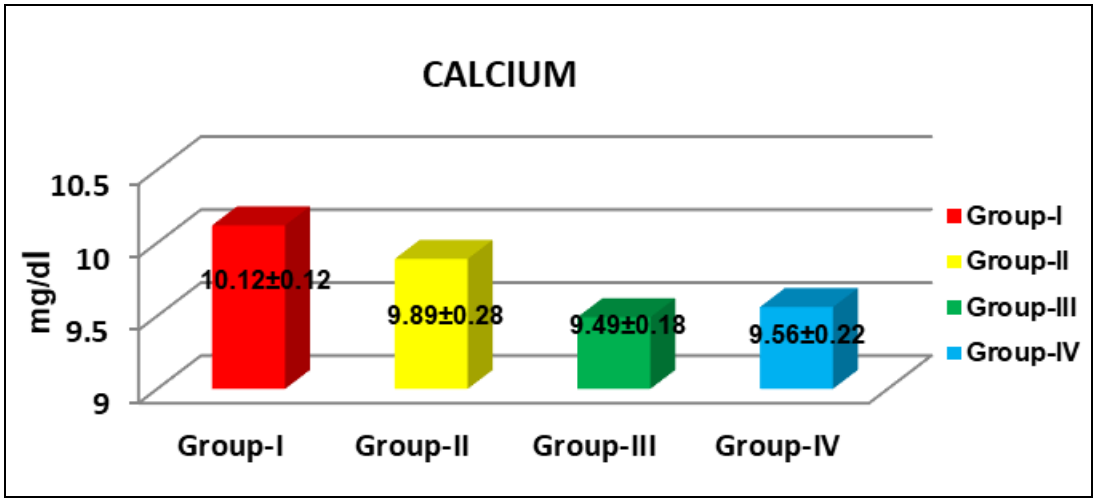

Fig 1: Graphical representation of serum calcium levels in mithuns grouped clinically.

Serum Phosphorus

The overall mean value of serum phosphorus (mg/dl) in Group I, Group- II, Group- III and Group- IV were 7.67±0.35, 8.78±0.79, 7.38±0.35 and 8.83±0.83 in mithuns (Fig no 2). The mean value of serum P in all the groups was non-significantly higher than that of the normal values.

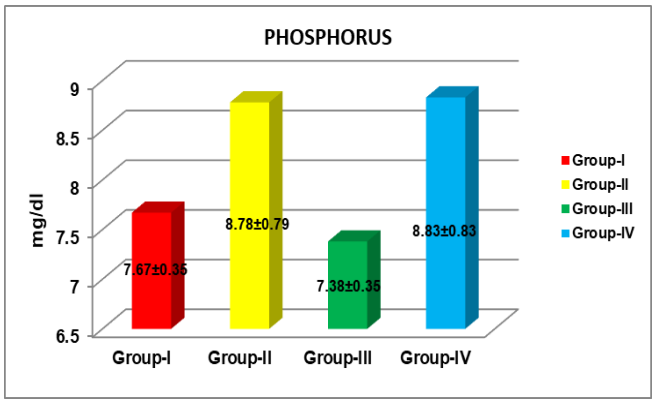

Fig 2: Graphical representation of serum phosphorus levels in mithuns grouped clinically.

Serum Sodium

The overall mean value of serum sodium (ppm) in Group-I, Group-II, Group-III and Group-IV were 3123.26±29.74, 3053.45±94.05, 3075.00±71.18 and 3134.37±13.45 (Fig no 3), which was within the normal range i.e 3036-3496 ppm (132-152 mEq/L) as per Radostits *et al.* (2007) [15]. The mean value of all the different groups was non-significant between the groups

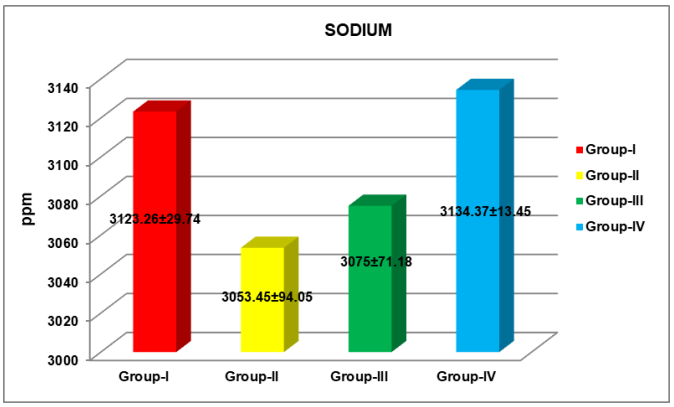

Fig 3: Graphical representation of serum sodium levels in mithuns grouped clinically.

Serum Potassium:

The overall mean value of serum K (ppm) in Group-I, Group-II, Group-III and Group-IV were 384.91±35.71, 212.70±11.08, 193.24±10.34 and 201.59±22.82, respectively (Fig no 4). The normal range is 3.9-5.8mEq/L (145.8-228.54 ppm) as per Radostits *et al.* (2007) [15]. The K value in Group-I is higher than the normal and the value from Group-II differ significantly (P<0.01)

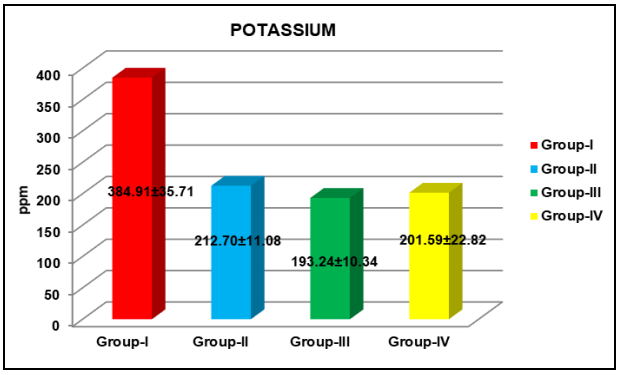

Fig 4: Graphical representation of serum potassium levels in mithuns grouped clinically.

Serum Magnesium

The overall mean value of serum Mg (mg/dl) in Group-I, Group-II, Group-III and Group-IV were 2.45±0.11, 3.43±0.28, 2.86±0.22 and 2.85±0.37, respectively as shown in Fig no 5, which were within the normal range i.e. 1.8-2-3 mg/dl (0.74-1.10 mmol/L) as per Radostits *et al.* (2007) [15] and 3.81 ± 0.12 in mithun (Rajkhowa *et al.*, 2003). The Mg values between Group-I and Group-II differ significantly (P<0.05).

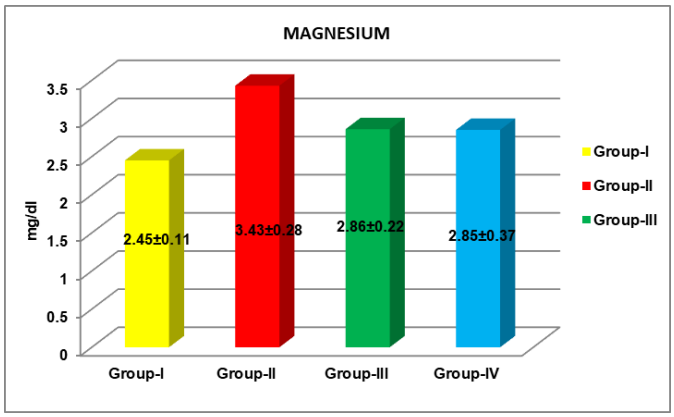

Fig 5: Graphical representation of serum magnesium levels in mithuns grouped clinically

Discussion

In mithuns grouped clinically, the mean serum calcium values were found to be within the normal range. As per Radostits *et al* (2007) [15] the normal range of Ca is 9.7-12.4 mg/dl (2.43-3.10 mmol/l and 97-124 ppm) in cattle. And in mithuns grouped according to the type of housing, mean serum calcium values were within the normal range. Similar observations have been recorded by Das *et al.* (2007) [4], Singh *et al.* (2011) [26] and Sharma *et al.* (2011) [23].

The overall mean of serum inorganic phosphorus in all the clinical groups i.e., Group-I, Group-II, Group-III and Group-IV were slightly higher than that of the normal value. Radostits *et al.* (2007) [15] had cited normal range of P in cattle as 5.6-6.5 mg/dl (1.08-2.76mmol/L and 56-65 ppm). Mean plasma Pi content of 4.80±0.99mg/dl (1.55±0.32mmol/l) in beef cattle from Southern Brazil was reported by Vella *et al.* (2003) [30]. A comparatively higher level of Pi in crossbred cows had been reported by several workers as 6.11±0.39mg/dl (Rao *et al.*, 1981) [18], 5.20±0.11mg/dl (Randhawa, 1993) [16], 5.26mg/dl (Randhawa *et al.*, 2007) [17], 5.80 mg/dl (Singh *et al.*, 2005) [27] and 4.71±0.44 mg/dl. Shukla *et al.* (2009) [19] reported that the blood serum P (5.29mg/dl) concentration were above the critical levels in cattle and buffaloes serum in Pittorgarh district of Uttarakhand. Whereas in the mithun which are grouped according to their housing, the Mean±SE values of serum Inorganic phosphorus in Group-I (Intensive) and Group-II(Semi-Intensive) were slightly higher than that of the normal values whereas the animals in Group-III(Free-range) were towards the lower values (4.98±0.439). Tiwary *et al.* (2010) [29] also reported mean Pi level of 3.79±0.21 mg/dl in dairy cattle and buffaloes which was below the critical level. Jena *et al.* (2011) [6] reported an average serum P concentration (3.57±0.11 mg/dl) of cows in NECPZ (North Eastern Coastal Plain Zone) which was below the critical level of 4.5mg/dl. Similar observations of Pi deficiency has been reported (Yadav *et al.* (2002) [31]; Mishra (2006) [13]. The differences in the dietary level and composition, breed, season and certain factors like sample preparation, degree of

haemolysis, temperature, duration of sample collection and serum preparation time might have been the reason for the different observations in the various studies (McDowell, 2003). The phosphorus deficiency is widely prevalent throughout the world as P itself is the limiting mineral in the soil (McDowell, 1992; Underwood and Suttle, 1999) [9, 27].

The overall mean of serum sodium in all the clinical groups i.e. Group-I, Group-II, Group-III and Group-IV and in all the Housing groups i.e. Group-I, Group-II and Group-III were within the normal range and there was no significant difference between the groups. The normal range i.e 3036-3496 ppm (132-152 mEq/L) as per Radostits *et al.* (2007) [15]. Similar observations have been reported by Sharma *et al*., (2002) [21]. Sharma *et al*. (2009) [22] also reported of adequate serum sodium (135.85 – 155.45 Meq/L) level in buffaloes of Central Uttar Pradesh.

The overall mean of serum potassium in Group-II, Group-III and Group-IV of the clinical group and in Group-I and Group-III of the housing groups were within the normal range (152-226 ppm).The normal range is 3.9-5.8 mEq/L (145.8-228.54 ppm) as per Radostits *et al. (*2007) [15]. Group-I of the clinical group and Group-II of the housing group were comparatively higher than that of the normal level. Sharma *et al*. (2002) [21] also reported of normal level of K in cattle. Kumarasen *et al.* (2010) [8] reported lower level of K (5.9 mg/dl) in the serum of cattle reared in the sub tropical hill agro systems.

The overall mean of serum Magnesium in all the clinical groups i.e. Group-I, Group-II, Group-III and Group-IV and in all the Housing groups i.e. Group-I, Group-II and Group-III were within the normal range. The normal range i.e. 1.8-2-3 mg/dl (0.74-1.10 mmol/L) as per Radostits *et al.* (2007) [15] and 3.81 ± 0.12 in Mithun. Tiwary *et al*., (2010) [29]; Baruah *et al*., (2000) [1] and Sharma & Joshi (2006) [20, 24] also reported of similar findings in cattle. There was significant difference between Group-I and Group-II of the clinical group in their Mg values whereas the mithuns reared intensively and semi-intensively also showed significant difference and this difference might be due to different feeding practices (Das *et al*., 2002) [3]. Calves maintained on a simulated milk diet for a longer period of time induce hypo-magnesaemic tetany (Blaxter *et al*., 1954) [2].

Conclusion

Under the Indian conditions, particularly in tropical areas where short rainy season of 3-4 months and a long dry season of 8-9 months is experienced, the metabolic and deficiency diseases are quite common and it is mainly due to non-availability of 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 availability of minerals decreases with the maturity of the fodder, The Climate of Nagaland in general is controlled by its Terrain features, it is hot to warm sub tropical in areas with elevation of 1000 to 1200 m, the climatic environment is warm sub temperate in areas with elevations of 1200 m and above, the temperature ranging 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 elevation respectively, Soil mineral status keeps on changing due to pressure on land for maximum crop production, fertilizer application and natural calamities, thus altering the mineral contents of feeds and fodders and hence their supply to the animals. This mega biodiversity centres of the world, harbours a large variety of Mithun-eating tree leaves, herbs, shrubs and grasses. The nutrient requirements of this semi-wild prized animal is mostly fulfilled by browsing on these fodder resources of forest origin, hence in this study no macro nutritional deficiencies was observed.

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