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# EFFECT OF AGE, SEX, PREGNANCY AND LACTATION ON SERUM MINERAL PROFILE OF BANNI BUFFALO (*BUBALUS BUBALIS*)

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## *ABSTRACT:*

Present study reports the reference values of some important macro minerals viz. calcium (Ca), magnesium (Mg), phosphorus (P), sodium (Na), potassium (K), chloride (Cl) and micro minerals viz. copper (Cu), iron (Fe), zinc (Zn), cobalt (Co), manganese (Mn) in Banni buffalo (*Bubalus bubalis*). Further, it describes their variation due to age, sex and different physiological stages viz. pregnancy, dry and lactation. It was observed that the levels of Ca, Mg, P, K and Cl did not vary significantly between male and female calves unlike sodium. The Ca and Cl were apparently lower in lactating buffaloes than the dry ones. The variations observed pertaining to all the macro minerals between lactating and dry buffaloes were non-significant. The values of the micro minerals were within the physiological range of healthy buffalo. The Cu and Zn concentrations were found to be the lowest in pregnant buffaloes.

Similarly, lower value of Mn and Co was recorded in calves irrespective of sex. Conversely, Fe concentration was recorded to be the lowest during pregnancy. Nonetheless, data generated during the study may be of use for physiological characterization of this unique buffalo breed of Gujarat. Further, it may assist in monitoring the health and prognosis as well as diagnosis of deficiency diseases.

***KEY WORD:*** *Banni buffalo, Mineral, Age, Sex, Pregnancy, Lactation.*

## *INTRODUCTION:*

Minerals are biological facilitators. Both macro and micro (trace) minerals are absolutely essential for maintenance of normal growth and productivity in animals (Yatoo *et al*., 2013). Macro minerals *viz.* calcium, phosphorus, magnesium, sodium, potassium, chloride etc. are required for normal functioning of all biochemical process (Kumar *et al*.,2011). They act as catalyst in both enzyme and hormone systems and thus play role in maintenance of metabolic health of the animals (Ceylan *et al*., 2008). Similarly, the micro minerals, though required in minute amounts, are equally important for optimum growth, production, reproduction and immune defense mechanism of animals (Andrieu, 2008; Hesari *et al*., 2012). Body micro mineral profile, immunity and reproductive functions are directly related, where copper, cobalt, zinc, iron and manganese are very crucial element. The most devastating economic results of mineral deficiencies are late puberty of heifers and low reproductive rates of cows associated with prolong calving intervals (Shahjalal *et al*., 2008).

It is unequivocal that several factors viz. age, sex and different physiological stages affect metabolism and thereby the blood biochemical profile of animals (Jain *et al*., 2009). However, no study has been carried out to understand the effect of these factors on mineral profile of Banni buffalo (*Bubalus bubalis*), an indigenous breed of Gujarat, India. Recently, breed registration committee, ICAR, New Delhi has recognized this "*sui-genesis*" buffalo germplasm as 11$^{th}$ buffalo breed of India because of its unique genetic makeup. Hence, there is an urgent need of some basic research on this breed so as to establish reference values for biochemical profile since it is pivotal for correct interpretation of laboratory data and form the very basis for diagnosis, treatment and prognosis of diseases (Tebogo *et al*., 2014).

In view of the above, present study was carried out to determine the baseline values for some important macro and micro minerals of Banni buffalo as well as to investigate their alteration due to age, sex and physiological stages viz. pregnancy, dry and lactation.

## MATERIAL AND METHODS:

Forty two clinically healthy buffaloes from Cattle Breeding Farm, Bhuj were randomly selected and categorized into seven groups (n=6): group-I (male calves ≤1 year), group- II (bulls >1 year), group-III (female calves ≤1 year), group-IV (pregnant lactating buffaloes), group-V (non-pregnant lactating buffaloes), group-VI (pregnant dry buffaloes) and group-VII (non-pregnant dry buffaloes). All the buffaloes of the experimental groups were reared under standard feeding and husbandry conditions. The health of the selected animals was regularly examined based on behavior, rectal temperature, pulse rate, respiratory rate and fecal consistency.

About 10 ml of blood was collected aseptically from each animal of all the experimental groups by jugular vein puncture into serum clot activator tubes (Greiner Bio-One GmbH, Austria) to separate out the serum. Subsequently, the clear serum samples were collected in sterilized vials and stored in refrigerated condition till analyzed.

The macro minerals viz. calcium (Ca), magnesium (Mg), inorganic phosphorus (Pi), sodium (Na), potassium (K), chloride (Cl) were estimated employing ready to use kit (Ecoline, Merck specialities Private Ltd., India) in Clinical Analyzer-635 (Systronics India Ltd., India). The micro minerals viz. copper (Cu), iron (Fe), manganese (Mn), zinc (Zn) and cobalt (Co) were estimated employing atomic absorption spectrophotometer (Model AAS-4141, ECIL-INDIA).

All the results were expressed as mean ± standard deviation (SD) and were statistically analyzed using two-way ANOVA as per method of Snedecor and Cochran (1994). $p < 0.05$ were considered to be statistically significant.

## RESULTS AND DISCUSSION:

The mean ± SE values of the estimated macro and micro mineral concentrations of the different experimental groups of Banni buffaloes have been presented in table-1 and table-2, respectively.

Table-1 reveals that level of inorganic phosphorus was significantly ($p < 0.05$) higher in male calves than that of bulls, which corroborates the report of Doornenbal *et al*. (1988) in beef cattle. The greater amount of phosphorus in male calves may be attributed to higher level of growth hormone in growing animals that increases renal reabsorption of phosphate (Roslol and Capen, 1997). The non-significant difference of phosphorus in lactating and dry buffaloes was in agreement with the study of Hagawane *et al*. (2009) in buffaloes and Filipejova and Kovacik (2009) in cattle. The apparently higher level of phosphorus in non-pregnant buffaloes than pregnant buffaloes was in consistence with the findings of Rao *et al*. (1999) in ongole cows.

Conversely, the concentrations of calcium, magnesium, sodium and potassium varied non significantly between male calves and bulls, which are comparable with the observations of Mikniene *et al*., (2014). Bhattacharyya *et al*., (1994) in non-descript goat, reported that the higher level of serum Ca, Pi and Mg found in pubertal stage might be either due to increased mobilization of minerals from bone to cope with high demand for growth at this stage or due to increased rate of resorption from the gastrointestinal tract. The non-significant variation in calcium level between male and female calves observed in this study was in accordance with the study of Padodara *et al*., (2012) in Jaffarabadi buffaloes. However, the recorded values of calcium in both male and female calves were comparatively lower than the other groups. Similarly, the lower calcium level in pregnant lactating group as compared to non pregnant buffaloes could be due to utilization of calcium for mineralization of fetal skeleton (Jacob *et al*., 2002). It may also be attributed to the hike of calcitonin hormone during pregnancy (Kovacs, 2001). Nonetheless, the higher calcium level in dry buffaloes than the lactating groups was in line with the study of Paul *et al*., (2011) in Surti buffalo. Previous report suggested that calcium requirements for milk production have a significant effect on maternal mineral and skeletal homeostasis during lactation (Lisegang *et al*., 2005). The lower calcium level in lactating group is due to excessive drainage of blood calcium into milk and insufficient adjustment by the parathormone through mobilization of bone calcium (Paul *et al*., 2011). The magnesium level was non-significantly higher in bulls and adult females than in the male and female calves, which corroborates the report of Padodara *et al*., (2012). The findings of higher magnesium level in pregnant buffaloes than non pregnant buffaloes may be due to its requirement for formation of bone in growing fetus. It was reported that magnesium, though in smaller amount than calcium, is also one of the important constituent incorporated in the bone (Zofkova and Kancheva, 1995).

Sodium level recorded in different experimental groups of the study was within the reference interval reported by Ellah *et al*., (2014a and 2014b). Higher sodium level was observed in male buffaloes than that of female buffaloes, which was in agreement with the study of Mikniene *et al*., (2014). Nonetheless, elevated sodium level in bulls than the male calves disagreed the findings of Mikniene *et al*., (2014). The findings of non-significant variation of Na among the groups of lactating and dry buffaloes was in tune with the study of Kulkarni *et al*., (1984), who observed almost similar levels of sodium between lactating and dry buffaloes. The potassium concentration also apparently varied between the experimental groups. Jabbar *et al*., (2012) reported that potassium concentration vary with age. It was the highest at post-pubertal stage; while, lower at prepubertal stage. Kumar *et al*. (1992) also observed that plasma potassium level was higher in mature group of buffalo heifers as compared to other developmental stages. In fact, an ionic balance exists between potassium, sodium and magnesium. However, potassium requirement appears to be increased for livestock during stress such as pregnancy (Osman and Al-Busadah 2003). This holds good in the current study also as non-significant elevation of potassium was observed in pregnant buffaloes than non pregnant buffaloes. However, bulls had numerically higher potassium level than the adult females, which is in tune with the report of Mikniene *et al.*, (2014). The chloride concentration did not exhibit any noticeable difference amongst the groups. However, the values pertaining to chloride was in agreement with the reference intervals determined by Ellah *et al.*, (2014a). Apparently higher chloride level in dry buffaloes as compared to lactating buffaloes was in agreement with the study of Kulkarni *et al.*, (1984). Jabbar *et al.*, (2012) observed that chloride concentration was not affected by age. Arosh *et al.*, (1998) reported that anoestrous cows had significantly low concentration of chloride. They stated that the mineral plays an intermediate role in action of hormones and enzymes at sub cellular levels. The minerals act in integrated fashion in the synthesis of reproductive hormones, with positive action of such hormones on reproductive organs and initiation of estrus in animals. In contrast, Michałek (2010) found no significant differences in pregnant and non pregnant goats, and remained within the reference values. They implicated changes to varied levels of aldosterone and progesterone and their mutual proportions differing between the experimental groups.

Mean ±SE of serum micro mineral concentration of different experimental groups of Banni buffaloes have been presented in Table-1. The recoded values of the micro minerals were found to be within the physiological range prescribed for healthy adult ruminants. The copper and zinc concentrations were found to be the lowest in the pregnant buffaloes, which may be attributed to higher progesterone level and/or to the increased fetal demands and utilization of maternal copper and zinc for development of fetal nervous system (Elnageeb and Abdelatif, 2010). These two minerals play significant role in regulating progesterone production by luteal cells via involvement of superoxide dismutase (Sales *et al.*, 2011). Further, as a component of antioxidant arsenals, they contribute in counteracting pregnancy induced oxidative stress (Pathan *et al.*, 2011). Copper is also involved in steroidogenic enzymes like cytochrome $P_{450}$, 17α-hydroxylase and cytochrome $P_{450}$ side-chain cleavage and lysyl oxidase (Kendall *et al.*, 2006). Zinc is important for reorganization of ovarian follicles which are the source of progesterone. This occurs through the involvement of metalloproteinase-2 (MMP-2) enzyme, which is a member of zinc endopeptidase family (Gottsch *et al.*, 2000). Further, it influences the secretion and function of male hormone testosterone through the enzyme that controls the arachidonic acid cascade (Ceylan *et al.*, 2008). Thus, zinc is vital for sexual development and spermatogenesis. Similarly, manganese is also required for skeletal growth and development. In the present study, lowest level of manganese and cobalt was recorded in calves irrespective of sex. This may be attributed to their utilization by the young growing calves for proper bone formation (Hansen *et al*., 2006, Nagabhusana *et al*., 2008). Involvement of manganese in the synthesis and production of oestrogen and progesterone may be due to the fact that it acts as a cofactor of the enzyme required for synthesis of cholesterol (Karkoodi *et al*., 2012). Correspondingly, the concentration of iron was apparently lower in the pregnant animals, which corroborates earlier report (Qian *et al*., 2001). This may be due to the utilization of iron to neutralize free radicals like peroxides, super oxides or hydroxyl ions produced during pregnancy (Yatoo *et al*., 2013). Iron is a key component of catalase and peroxidase, thereby plays significant role in combating oxidative stress (Harvey, 2000; Antonyuk *et al*., 2009). It is also necessary for ovarian activity (Qian *et al*., 2001). Role of iron in immunity is reported by Eisa and Elgebaly (2010).

It may be concluded that age, sex and physiological stages alter the micro-mineral profile of Banni buffalo. Therefore, such variation must be taken into consideration before supplementation of mineral mixture to animals of different age groups and sex as well as during pregnancy, lactation etc. for proper maintenance of health, optimization of productive and reproductive activities.

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**Table 1: Macro mineral concentrations in Banni buffaloes at different age, sex and physiological status**

| Parameters | Male calves | Bulls | Female calves | Pregnant lactating buffaloes | Non pregnant lactating buffaloes | Pregnant dry buffaloes | Non pregnant dry buffaloes |
|---|---|---|---|---|---|---|---|
| Inorganic phosphorus (mg/dl) | 7.67±0.50[b] | 5.81±0.22[a] | 6.16±0.53[ab] | 6.31±0.36[b] | 6.49±0.24[b] | 6.40±0.26[b] | 6.94±0.32[b] |
| Calcium (mg/dl) | 8.23±0.66[ab] | 9.72±0.59[bc] | 8.11±0.36[a] | 8.54±0.60[abc] | 10.38±0.24[abc] | 10.50±0.19[abc] | 11.96±0.34[c] |
| Magnesium (mg/dl) | 2.95±0.32[ab] | 3.63±0.21[abc] | 2.77±0.30[a] | 4.09±0.19[c] | 3.81±0.37[bc] | 3.85±0.21[bc] | 3.38±0.31[abc] |
| Sodium (mEq/L) | 128.88± 3.59[b] | 149.01±7.13[b] | 115.16±7.01[a] | 122.52±9.61[a] | 120.56±3.33[a] | 126.90±12.49[a] | 122.48±6.27[a] |
| Potassium (mEq/L) | 2.77±0.23 [a] | 3.50±0.13 [a] | 2.74±0.22 [a] | 3.19±0.28 [a] | 2.88±0.37 [a] | 3.05±0.95 [a] | 2.71±0.60 [a] |
| Chloride (mEq/L) | 88.17±3.38 [a] | 91.74±3.52 [a] | 89.58±3.57 [a] | 83.49±4.29 [a] | 81.86±2.76 [a] | 85.26±5.44 [a] | 89.11±10.06 [a] |

Means with same superscript within a row do not differ significantly from each other.

**Table 2 : Concentration of micro minerals in different experimental groups of Banni buffaloes**

| *Parameter (ppm)* | *Male buffaloes Group I* | | *Female buffaloes Group II* | | | | |
|---|---|---|---|---|---|---|---|
| | **Male calves Group- I A** | **Bulls Group- I B** | **Female calves Group- II A** | **Pregnant lactating buffaloes Group- II $B_{1a}$** | **Non pregnant lactating buffaloes Group- II $B_{1b}$** | **Pregnant dry buffaloes Group- $IIB_{2a}$** | **Non pregnant dry buffaloes Group- II $B_{2b}$** |
| *Copper* | 0.69±0.07[bc] | 0.85±0.04[c] | 0.53±0.07[ab] | 0.40±0.1[a] | 0.54±0.04[ab] | 0.40±0.06[a] | 0.77±0.02[c] |
| *Iron* | 0.84±0.14[a] | 0.94±0.37[a] | 0.70±0.15[a] | 0.61±0.37[a] | 0.84±0.16[a] | 0.69±0.21[a] | 0.94±0.17[a] |
| *Zinc* | 0.60±0.02[d] | 0.49±0.03[bcd] | 0.55±0.03[cd] | 0.39±0.02[ab] | 0.41±0.02[ab] | 0.33±0.06[abc] | 0.41±0.05[a] |
| *Cobalt* | 0.41±0.04[a] | 0.72±0.05[b] | 0.45±0.06[a] | 0.65±0.01[b] | 0.50±0.02[a] | 0.66±0.01[b] | 0.52±0.03[a] |
| *Manganese* | 0.20±0.06 | 0.28±0.06 | 0.22±0.06 | 0.33±0.07 | 0.37±0.06 | 0.42±0.05 | 0.42±0.08 |

Means with same superscript within a row do not differ significantly from each other.