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Research Article

CERTAIN MICROMINERAL CONCENTRATIONS DURING DIFFERENT STAGES OF GROWTH IN FEMALE PANDHARPURI BUFFALOES

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Abstract: The present study was conducted on 24 Pandharpuri female buffaloes maintained at College farm of KNP College of Veterinary Science, Shirwal. The Pandharpuri buffaloes were categorized into different groups according to age comprising 6 (six) animals in each group *Viz.* Group I: Buffalo calves (up to 6 months), Group II: Young growing buffalo calves (6 months to 12 months), Group III: Buffalo heifers (12 months to 30 months) and Group IV: Adult Buffalos (above 30 months). Blood samples were collected and analyzed for micro minerals by using AAS (Cu, Fe, Zn), ICP-AES (Mo). The present data reveals that, there was non significant increasing trend in concentration of serum copper and significant apparently increasing trend in concentration of serum iron among different age groups. There was no significance difference in concentration of serum zinc and serum molybdenum among different age groups with no consistent trend.

Keywords: *Copper, Iron, Zinc, Molybdenum, Buffalo, Puberty, Heifers, Adult*

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Introduction

The buffalo milk is having high consumer demand due to high fat percentage. A 54% of total buffalo (*Bubalus bubalis*) population of the whole world is in India. The Pandharpuri buffaloes are found in Western Maharashtra and some parts of Karnataka. The Pandharpuri buffalo has the lactation length of 350 days with 1500 kg milk production [1]. Growth embraces a series of changes in size and structure by which an individual of any species develops from fertilized egg to maturity [2]. True growth involves an increase in the structural tissue such as muscle, bone and also in the organs [3]. The biochemical changes taking place during different stages of growth have been attributed to the changes in the activities of certain minerals. Growth of animals occurs due to mineral deposition in body. Copper plays important role as a structural component in macromolecules acting as coordination centre. It is also a common redox cofactor for a number of oxidase and monooxygenases that are essential for life. Impaired immunity, neurological function, oxidant defence, and depigmentation resulting from Cu deprivation can be linked to specific enzymes such as Cu-Zn superoxide dismutase which catalyse the superoxide anion [4]. Molybdenum acts as a cofactor for the enzymes like xanthine dehydrogenase/oxidase, aldehyde oxidase and sulphite oxidase [5], which are having important role to control conditions like ischemia, reperfusion or which help in reoxygenation of an injured tissue [5]. High level of molybdenum interfere with Serum copper and vice-versa, and disturb the body functions like growth, fertilization and immunity. Zn is essential for the function of more than 200 Zn-containing enzymes which are found in all major metabolic pathways involved in carbohydrates, lipid, protein and nucleic acid metabolism, zinc plays an important role in stabilization of biomembrane. Its deficiency may cause oxidative stress [4]. Iron is a vital constituent in the proteins structures that is involved in the transportation and consumption of oxygen. Furthermore, many other trace minerals, several enzymes also contain iron or activated by iron [6]. The signs of iron deficiency in animals may include: anemia, anorexia, reduced growth rate, or increased rate of weight loss, reduced immune response, laziness, Pale mucous membranes, Atrophy of the papillae of the tongue [7]. A large number of factors such as species, breed, sex, age, malnutrition, illness, reproductive status and physiological variations can affect the serum minerals values [8]. There is scanty information about the micromineral status in Pandharpuri buffaloes and thus the present investigation is undertaken to study the serum micro minerals *viz*. Fe, Cu, Zn and Mo in female buffalo calves during growth and puberty.

Materials and Methods

The present study was conducted on 24 Pandharpuri female buffaloes maintained at College farm of KNP College of Veterinary Science, Shirwal. The Pandharpuri buffaloes were categorized into four groups according to age comprising 6 (six) animals in each group *Viz.* Group I: Buffalo calves (up to 6 months), Group II: Young growing buffalo calves (6 months to 12 months), Group III: Buffalo heifers (12 months to 30 months) and Group IV: Adult Buffalos (above 30 months). Blood samples from experimental animals were collected and the separated serum samples were analyzed for Copper, Iron and Zinc. by Atomic Absorption Spectrophotometer (Elico, SL-194) in KNP College of Veterinary Science, Shirwal and for Molybdenum by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP- AES) at Sophisticated Analytical Instrument Facility (SAIF) of Indian Institute of Technology (IIT), Powai, Mumbai-400076.

Digestion procedure of serum samples

One ml serum sample was taken in 25 ml volumetric flask, to which 5 ml of di-acid mixture was added. The di-acid mixture contained 70% perchloric acid (one part) and concentrated nitric acid (four parts). The contents of volumetric flask were boiled gently for 30-45 minutes to oxidize all the easily oxidizable material. The solution was boiled until it became nearly colourless.

Table-1 *Mean ± S.E. values of serum micro minerals (ppm) in different age groups in Pandharpuri buffaloes*

Group No	Groups	Concentration (ppm)			
		Copper	Iron	Zinc	Molybdenum
I	Buffalo calves (up to 6 months)	1.92 ± 0.30	1.19^{c} ± 0.08	1.20 ± 0.16	0.50 ± 0.03
II	Young growing buffalo calves (6 months to 12 months)	2.21 ± 0.28	1.14^{c} ± 0.15	1.15 ± 0.15	0.55 ± 0.04
III	Buffalo heifers (12 months to 36 months)	2.33 ± 0.25	1.81^{b} ± 0.05	1.43 ± 0.14	0.42 ± 0.02
IV	Adult Buffalos (above 36 months)	2.46 ± 0.31	2.45^{a} ± 0.15	1.23 ± 0.10	0.46 ± 0.03
	Average	2.23 ± 0.15	1.65 ± 0.12	1.25 ± 0.07	0.48 ± 0.02

The precaution was taken while heating the solution not allowing it to go to dryness. The solution was cooled to which some distilled water was added. The solution was then diluted to 25 ml with deionized water. The prepared aliquot was used for the analysis of the minerals under study. The available data was subjected to statistical analysis, by using Web Agricultural Statistical Package of ICAR (www.icargoa.res.in/wasp/tt22.php) to compare the results by applying Completely Randomized Design (CRD).

Result and Discussion

The results and average values of influence of age on concentrations of serum microminerals are presented in [Tables-1].

Serum copper (Cu)

The mean ± SE values of serum copper (Cu) from calves to adult Group of Pandharpuri buffaloes are presented in [Table-1]. The average value of all age groups was comparable (± 0.50) to the findings reported by [9] and [10] in buffalo. However, in buffaloes reported lower values in buffaloes than the present findings [11-15]. Statistical analysis of present data reveals that there was no significant difference in the values of serum copper among the four different Groups. But indicated that there was an increase in serum copper levels in different age groups with advancement of age and which was in total agreement with [16]. This increasing trend in copper concentration may be associated with developing endocrine glands and its functioning during puberty [17] and may be due to high oestrogenic activity during follicular phase at puberty [18]. Secondly, in most species, the newborn is characterized by liver copper concentrations that are markedly higher than those found in adults. However, the copper in the most other tissues tends to be higher in the adult than in new born [19]. Almost similar increasing trend with the values of serum copper in crossbreed female calves and heifers was reported from a group of 1 to 3 months of age to a group of 22 to 24 months of age by by [20] and [21] in calves, heifers and adult local nondescript and crossbred (NXJ) cattle. Similar significant increase of copper in crossbreed adult from calf in two agro climatic zones of west Bengal were reported by [22]. [23] reported increasing trend in the values of copper from day 1 to 6 months of age in cow calves. The gradual increase in plasma copper from 0.69 to 0.97 µg/ml with the advancement of age in calves, heifers and adults were also reported by [24]. Similar findings were also reported by [9] in cow calves of 2.3 to 3 months of age to 22 to 26 months of age and by [25] in buffalo calves, heifers and adults. However, [26] in buffalo and [27] in cattle reported non consistent trend in the values of copper in calves, heifers and adult groups with the highest values in heifers. [28], [29], [30] in buffalo and [31] in cattle, reported higher values in heifers than adults. However, [32] in buffalo reported reverse results. [33] in water buffalo and Charolasia cattle reported higher values at 32 to 34 weeks of age as compared to 36 to 8 weeks of age. [34] reported decreasing trend in the values of copper from buffalo calves to adults through heifers. [35] stated that levels of copper were significantly related with age and puberty.[36] reported lowest value of plasma Cu in calves (3 months to 1 year old), intermediate in heifers and highest in adult cows. According to results of present study, Pandharpuri buffaloes had more serum copper than the reference values of cow [37]. The differences between buffalo and other ruminants in the serum concentration of microelements may be regarded as physiological peculiarities due to their adaptation to environmental conditions.

Serum Iron(Fe)

The mean ± SE values of serum iron (Fe) from calves to adult Group of Pandharpuri buffaloes presented in [Table-1]. The average value of all age Groups was comparable (± 0.50) to the findings reported by [21] and [9], in cattle and [14] and [15]) in buffalo. However, [38] in buffalo reported lower and [30] in buffaloes reported higher values than the findings of present study. Statistical analysis of present data reveals that there was a significant difference ($P<0.05$) in the values of serum iron among the four different Groups. These values show an apparently increasing trend from calves (Group I and II) to adult (Group IV) through Group III. This apparent increase in the serum iron with the advancement of age might be associated with correlated increase in body weight, since, [39] opined that the total haemoglobin in mammals is directly proportional to body weight. However, [20] reported highest concentration of serum iron in calves than adults and significantly lower iron concentration also observed in adult by [16] as compared to young stock of dairy cattle. Almost similar increasing trend with significant difference in the values of serum iron in crossbreed female calves and heifers was reported from a group of 1 to 3 months of age to a group of 22 to 24 months of age by [21] and by [40] in crossbreed calves from 15 to 180 days of age. [36] reported lowest value of plasma iron in calves (3 months to 1 year old), intermediate in heifers and highest in adult cows. This increase in iron concentration from calves to heifers in present study was correlated with maturity and similar results were also obtained by [41] and [42]. Lower values of Fe than present study in late pubertal heifers were observed by [43]. [44] also reported lower values of serum Fe in calves than Cows. Variation in the blood or serum iron in different age groups as reported by different workers might be due to variation in age group/groups and in climatic conditions.

Serum zinc(Zn)

The mean ± SE values of serum zinc (Zn) from calves to adult Group of Pandharpuri buffaloes presented in [Table-1]. The average value of all age groups was comparable (± 0.50) to the findings reported by [35],[33], in buffaloes [9], [45] and [27] in cattle. However, the values reported by [20] and [40] in cattle were higher than the present study. Statistical analysis of present data reveals that there was no significant difference in the values of serum zinc among the four different groups with no specific trend. [35] stated that levels of zinc were significantly related with age and puberty; increase Zn concentration in heifers than calves was observed by [42]. Increasing trend with the values of serum zinc in crossbreed female calves and heifers was reported from a group of 1 to 3 months of age to a group of 22 to 24 months of age [21]. Values of serum zinc in heifers in the present study was higher than the two groups of calves and adults. Similar finding with higher values of zinc inheifers was also reported by [9] and hence was congruent with the present report. However, the findings of [26], [25] and [27] in calves, heifers and adults exhibits apparently decreasing trend, which did not corroborate with present findings. [29] in buffalo reported higher values of serum zinc in heifers than adults and thus were in agreement with the present study. Similarly, [33]) in Charolasia cattle reported higher values at 32 to 34 weeks of age as compared to 36 to 38 weeks of age. [24] also reported decrease in zinc concentration from heifers to adult. This change might be attributed to hormone dependent metabolic activity and also indicative of their nutrient outgo in milk.

Serum Molybdenum(Mo)

The mean ± SE values of Molybdenum (Mo) from calves to adult Group of Pandharpuri buffaloes are presented in [Table-1]. Among different age groups in Pandharpuri buffaloes the values of serum molybdenum (ppm) in calves of Group I and Group II of present study were comparable (± 0.10) to the value reported by

[46]in buffalo and hence was in agreement. However, the value reported by [47] was apparently lower than in the present study and hence not in agreement. Value of serum molybdenum (ppm) in heifers of Group III of present study could not be compared with the values reported by various workers in the literature since; no one has reported the values for heifers. Value of serum molybdenum (ppm) in adult of Group IV of present study was comparable (± 0.10) to the values reported by [25], [48] and [49] in buffaloes. However, the value of present study was lower than that reported by [50]. Statistical analysis of present data reveals that there was no significant difference in the values of serum molybdenum among the four different Groups with no consistent trend. No report was found regarding serum molybdenum status in growing buffaloes or cows in different age groups with which present results could be compared. It would be therefore worth to discuss the plethora of variation in serum molybdenum content in association with that of copper. In general, slightly higher values of serum Cu recorded in present research during different age groups may also be related with antagonism of Mo and Cu and 3-way interaction between copper, molybdenum and sulphur. Since, in present study the Molybdenum in these groups was comparatively lower in buffaloes than earlier reports in literature with the exception of [47] who reported the average serum Mo level as 0.37 ppm in female buffalo calves. As reported by Suttle (1991), this interaction can occur with concentration of molybdenum and sulphur that are naturally present in feed stuff, and is involved in the formation of thiomolybdates in the rumen [51]. Sulphides are produced by micro-organisms in the rumen via the reduction of sulphate and degradation of sulphur amino acids. These sulphides react with molybdate to form thiomolybdates, which bind with copper and form a highly insoluble complex that does not release copper, even under acidic conditions and renders it unavailable to the animal for utilisation resulting in Cu deficiency because of more Mo. Thus, comparatively low level of Mo in present study might have favored increased bioavailability of Cu which was responsible for higher Cu levels in present study.

Conclusion

The serum copper (Cu) values shows an increasing trend with no significant difference as the age advances from calves to adult. The values of serum iron (Fe) show apparently increasing trend with significant difference. The values of serum zinc (Zn) and serum Molybdenum (Mo) show no specific trend. In general, the differences between buffalo and other ruminants in the serum concentration of microelements may be regarded as physiological peculiarities due to their adaptation to environmental conditions.

Application of research: Study of pandharpuri buffaloes in different age groups which will be helpful in diagnosing the associated deficiencies in the field if any.

Research Category: Veterinary Science.

Abbreviations: Copper (Cu); Zinc (Zn); Iron(Fe) ; Molybdenum(MO); Atomic absorption spectrophotometer (AAS); Web Agricultural Statistical Package (WASP); Indian Council of Agricultural Research (ICAR); Standard error (S.E.); Completely randomized Design (CRD); parts per million (ppm)

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Study area / Sample Collection: Research Farm, KNP College of Veterinary Science, Shirwal

Animal name: *Bubalus bubalis*

Conflict of Interest: None declared

Ethical approval: Ethical approval taken from KNP College of Veterinary Science, Shirwal 412 801, Maharashtra Animal and Fishery Sciences University, Nagpur, 440001, India.
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