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Blood biochemical profile in sub-oestrus buffaloes

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Abstract
Normal reproduction is a primary and inevitable basis for sound animal husbandry enterprise and is one of the most important considerations determining the profitability. However, buffalo is considered as the animal of poor reproductive efficiency due to several features including delayed puberty and sexual maturity, sub-oestrus, anoestrus, true anoestrus and seasonality in breeding. Ovarian disorders are the most important causes of infertility characterized by cessation of sexual cycles and psychic manifestation of oestrus in dairy animals. Anoestrus is a functional disorder of ovaries causing lowered fertility in dairy animals especially in buffaloes. It is responsible for tremendous economic losses to the farmers by decreasing calf crop and milk production. True anoestrus (smooth ovaries) and sub-oestrus (functional ovaries) are the most common forms of postpartum anoestrus remain undiagnosed in field conditions and are responsible for prolonged calving interval and culling at breeding age in these animals. The intensity of oestrus signs in buffaloes is generally weak and also mounting activity is not pronounced as in cattle. Summer anoestrus is an important condition contributing to infertility in buffaloes. Heat stress causes increased cortisol secretion which blocks oestradiol induced sexual behavior. Deficiency of trace minerals especially calcium, phosphorus and magnesium also influence the ability of animals to utilize other micro-minerals. The influence of these minerals on certain enzyme system may affect reproductive efficiency. In the present study blood biochemical profile in sub-oestrus and true anoestrus buffalo (n=12 each) were compared on day 0, 7, 14, 21 and 28 irrespective of stage of estrus cycle. The mean serum manganese and zinc was recorded significantly higher in cyclic as compared to non-cyclic buffaloes on different days of cycle. However, mean serum copper was higher on day14 and 28 in cyclic as compared to non-cyclic buffaloes. The mean serum calcium, phosphorus, their ratio and cobalt was recorded non significantly higher in sub-oestrus buffaloes as compared to non-cyclic buffaloes.

Keywords: Sub-oestrus buffalo, blood biochemical, serum calcium, phosphorus, cobalt, zinc, manganese

Introduction
Lack of minerals especially calcium and phosphorus upsets the proper functioning of the genital organs (Acharya, 1960) [1]. Dhoble and Gupta, (1986) reported that minerals like calcium, phosphorus and magnesium also influence the ability of animals to utilize other micro-minerals. The influence of these minerals on certain enzyme system may affect reproductive efficiency, which might be reflected in lower blood level of them. The involvement of phosphorus in phospholipids and cAMP synthesis may be a key factor to its effect on reproduction. A close correlation between the reproductive hormones and inorganic phosphorus exist and marginal phosphorus deficiency may lead to aneostrus condition (Hurley and Doane, 1989) [10]. Calcium and phosphorus play an intermediate role in the action of hormones and enzymes at sub-cellular levels in an integrated fashion in the initiation of estrus in animals (Ali *et al*. 1991) [3].

Material and Methods
Postpartum dairy buffaloes maintained at Livestock farm of Veterinary College and organized buffalo herds of Jabalpur (M.P.) were used for this study. Selection of sub-estrus buffalo was made by history of anoestrus and gynaecological examination of genitalia twice at 10 days interval. Sub-oestrus buffaloes ware further confirmed by ex-foliative vaginal cytology according to Tongu *et al.* (2015) and change in serum progesterone concentration at an interval of 10 days.

Blood biochemical profile in sub-oestrus buffaloes was studied. For this selected 10 buffaloes each of sub-oestrus and true anoestrus were subjected to blood collection on day 0, 7, 14, 21 and 28. Serum calcium and phosphorus were estimated using commercial kits whereas copper, cobalt, zinc and manganese were estimated by Atomic Absorption Spectrophotometer (AAS).

Animals: Postpartum dairy buffaloes maintained at Livestock farm of Veterinary College, Adhartal and organized private dairy farms situated at Katiya Ghat and Pariyat area of Jabalpur (M.P.) were used for this study.

Materials: Calcium with Aresenazo III (1, 8-Dihydroxy-36-disulpho-2, 7-naphthalene-bis (AZO)-dibenenearsonic acid), at neutral pH yields a blue colour complex. The intensity of the colour formed is proportional to the calcium concentration in the sample.

Serum phosphorous estimation
Quantitative determination of phosphorus in serum was done using commercial kit of Lab Care Diagnostic.

Principle of test: Inorganic phosphate reacts in acid environment with molybdic acid to form an unreduced phosphor molybdic acid complex, which absorb light at 340nm.The absorbance is directly proportional to phosphorus concentration in the sample.

Statistical analysis
The mean value of Ca, P, Zn, Mn, Cu and Co were analyzed by two way analysis of variance (ANOVA) with standard statistical software (SPSS Version 16.0).

Experimental design: For the study of blood biochemical profile in 10 buffaloes each of sub-oestrus and true anoestrus were subjected to blood collection at weekly interval for five days i.e. on day 0, 7, 14, 21 and 28. Serum was separated and stored at -20^{o}. Serum zinc, copper, cobalt and manganese were estimated by Atomic Absorption Spectrophotometer, whereas calcium and phosphorus were estimated by colorimetric method using commercial kits.

Results and Discussion

Mean serum calcium profile (mg/dl) in anoestrus and sub-oestrus buffaloes

The mean serum calcium in the present study was recorded 9.29±0.69 to 10.0±0.78mg/dl in non-cyclic and 9.76±0.55 to 11.37±0.79mg/dl in cyclic buffaloes. The analysis of result revealed non significantly higher concentration of calcium in sub-oestrus/cyclic buffaloes as compared to non-cyclic/anoestrus buffaloes. The results of present study were in accordance to the findings of Khasatiya *et al*. (2005). However, Kumar *et al.* (2016) [15] reported similar serum calcium concentration in cyclic but lower in non-cyclic buffaloes. The higher serum calcium in the present study was recorded as compared to the findings of Jayachandran *et al.* (2013) [11] in both the cyclic and non-cyclic buffalos, Kumar *et al.* (2015a) [14] in non-cyclic buffalo and Buhecha *et al.* (2016) [5] in cyclic buffaloes, respectively. The comparatively low level of calcium in non-cyclic buffaloes may be the cause of infertility as optimum level of calcium is required for normal reproductive cycle. It has sensitizing action on reproductive organs through various hormone, further, substantial the finding of different calcium level in anoestrus buffaloes (Kumar *et al*., 1992) [16].

Mean serum phosphorus profile (mg/dl) in anoestrus and sub-oestrus buffaloes

The mean serum phosphorus in the present study was recorded in 7.74±0.18 to 8.26±0.42 mg/dl in non-cyclic and 7.91±0.45 to 8.57±0.60 mg/dl in cyclic buffaloes. The analysis of result, revealed non significantly higher concentration of phosphorus in sub-oestrus buffaloes as compared to non-cyclic/true anoestrus buffaloes. The serum phosphorus concentration in the present study in both the cyclic and non-cyclic animal was higher than the report of Ali and Shukla (2012) [4], Jayachandran *et al.* (2013) [11], Khastiya *et al*. (2015) and Buhecha *et al*. (2016) [5]. The comparatively low level of phosphorus in non-cyclic buffaloes may be the cause of infertility as optimum level of phosphorus is required for normal reproductive cyclic. The involvement of phosphorus in phosphor/ lipid and cAMP synthesis may be a key factor to its effect on reproduction. A close correlation between the reproductive hormone and inorganic phosphorus exist and marginal phosphorus deficiency may lead to anoestrus condition (Hurly and Doane 1989) [10]. However, Newar *et al.* (1999) [19] did not find any difference in the phosphorus level between cyclic and anoestrus buffaloes.

Serum calcium phosphorus ratio in anoestrus/sub-oestrus buffaloes

The mean serum calcium phosphorus ratio in the present study was found slight higher or similar in cyclic as compared to non-cyclic animal. In general the level of blood calcium alone might not affect the normal physiology of animal directly but alteration in calcium phosphorus ratio affect the ovarian activity through the blocking action of pituitary gland resulted prolongation of first post-partum oestrus and ovulation (calcium, phosphorus and magnesium) also influence the ability of animals to utilize other micro-minerals, influencing certain enzyme system and affect reproductive efficacy (Dhoble and Gupta, 1996) [6]. Ali *et al.* (1991) [3] reported that calcium and phosphorus play intermediate role in action of hormone and enzyme at sub cellular level in an integrated fashion for initiation of oestrus in animals. Kumar *et al.* (2016) [15] also reported significantly lower calcium phosphorus ratio in post-partum anoestrus than the normal cyclic buffaloes. The disturbed calcium phosphorus ratio in post-partum animal was also reported (Kumar and Saxena 2010 and Roginosu *et al*., 2011) [17, 21]. Calcium deficiency further causes the release of paratharmone which leads to more excretion of phosphorus leading to imbalance.

Serum copper profile

Table 1: Mean serum copper profile (mg/l) in anoestrus and sub-oestrus buffaloes

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
(Cyclic)	0.51±0.19	0.55±0.18	0.98 ^{A}±0.30	0.53±0.19	0.75 ^{A}±0.21
(Noncyclic)	0.42±0.04	0.36±0.03	0.39 ^{B}±0.08	0.43±0.11	0.55 ^{B}±0.11

Mean values with different superscripts (A&B) in column differs significantly (p<0.05).

The mean serum copper in the present study was recorded significantly higher concentration of copper in sub-oestrus/ cyclic buffaloes as compared to non-cyclic/true anoestrus

buffaloes (table 1). The serum copper level in the present study was in agreement with the study of Yadav *et al*. (2006) [24] and Akhtar *et al*. (2009) [2] who reported low copper level in anoestrus buffaloes as compared to cyclic buffalos. However, Jayachandran (2013) [11] and Paul *et al*. (2000) [20] could not find any significant difference in plasma copper concentration in cyclic and non-cyclic buffaloes. The association of copper with resumption of ovarian activity and fertility has been reported by Manickam *et al*. (1977) [18].

Serum Manganese profile

Table 2: Mean serum manganese profile (in mg/l) in anoestrus and sub-oestrus buffaloes

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
(Cyclic)	$0.80^{A}\pm0.41$	$0.59^{A}\pm0.14$	$0.60^{A}\pm0.20$	$0.59^{A}\pm0.18$	$0.67^{A}\pm0.13$
(Noncyclic)	$0.22^{B}\pm0.03$	$0.22^{B}\pm0.10$	$0.35^{B}\pm0.09$	$0.38^{B}\pm0.19$	$0.59^{B}\pm0.12$

Mean values with different superscripts (A &B) in column differs significantly ($p<0.05$).

The mean serum manganese in the present study was recorded significantly higher in sub-oestrus/cyclic buffaloes as compared to non-cyclic/true anoestrus buffaloes (Table 2). The result of serum manganese of the present study was in accordance to the finding at Kumar *et al*. (2016) [15] who reported manganese concentration significantly higher in normal cyclic as compared to post-partum anoestrus buffaloes. However, Khasatiya *et al*. (2015) [12] and Jayachandran *et al*., (2013) [11] could not find any significant difference in blood manganese level the anoestrus and regular cyclic buffaloes.

Serum zinc profile

Table 3: Mean serum zinc profile (in mg/l) in anoestrus and sub-oestrus buffaloes

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
(cyclic)	$1.79^{A}\pm0.48$	$1.18^{A}\pm0.31$	$1.37^{A}\pm0.23$	$2.05^{A}\pm0.30$	$1.42^{A}\pm0.24$
(Non) cyclic)	$0.96^{B}\pm0.12$	$0.77^{B}\pm0.12$	$0.65^{B}\pm0.20$	$0.75^{B}\pm0.20$	$0.58^{B}\pm0.14$

Mean values with different superscripts (A &B) in column differs significantly ($p<0.05$)

The mean serum zinc in the present study was recorded significantly higher concentration of zinc in sub-oestrus/cyclic buffaloes as compared to non-cyclic/true anoestrus buffaloes (Table 3). The concentration of serum zinc in the present study was similar to the findings of Jayachandran *et al*. (2013) [11] Akhtar *et al.* (2009) [2] in cyclic buffaloes however, higher concentration of the same was reported by Kumar *et al.*, (2016) [15]. The higher mean serum zinc level in cyclic buffaloes as compared to anoestrus buffaloes in the present study was in accordance to the finding of Akhtar *et al.* (2009) [2] and Yadav *et al*. (2006) [24] where as Paul *et al*., (2000) [20] and Jayachandran *et al.* (2013) [11] could not finding any significant difference in cyclic and non-cyclic buffaloes. The zinc play an important role in production, storage and secretion of individual hormone and effectiveness of receptor site of end organ adversely affected by zinc deficiency level below 0.62 to 0.84mg/l (Dowell, 1992) [7].

Serum cobalt profile

The mean serum cobalt in the present study was recorded 0.76±0.17 to 1.12±0.mg/l in non-cyclic and 0.76±1.12 to 1.27±0.27mg/l in cyclic buffaloes, However the difference was not statistically significant. The non-significantly higher serum cobalt in cyclic and then non cyclic buffaloes in the present study was in accordance with the previous report (Khasatiya *et al.*, 2015; Singh *et al.*, 2006 and Jayachandran *et al*. 2013) [12, 22, 11], could not find any significant variation in cyclic and anoestrus buffaloes. Similarly, no significant difference in serum cobalt was also found in study of Hedaoo *et al*. (2008) [8] between cyclic and non-cyclic Surti buffaloes. Hidiroglou (1979) [9] reported that cobalt is required to ensure fertility in ruminants. Low cobalt level reduces the storage of copper in liver which interfering the action of Zinc, Iodine and Manganese.

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