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Blood Glucose and Serum Minerals in Postpartum Cyclic and Acyclic Buffaloes during Hot Humid Season

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ABSTRACT

Background: The postpartum period is a crucial phase in buffalo reproduction involving many physiological and hormonal changes. Postpartum anestrus is a major reproductive challenge in buffaloes, especially during the hot-humid season, due to heightened environmental stress, negative energy balance and micronutrient deficiencies. The present study aimed to evaluate and compare the blood glucose and serum mineral profiles of postpartum cyclic and acyclic buffaloes to understand their role in the postpartum resumption of ovarian activity.

Methods: The study was conducted on 12 pluriparous graded Murrah buffaloes maintained at private dairy farms in Durg (Chhattisgarh) during the hot-humid season. Animals were grouped into postpartum cyclic (n=6) and acyclic (n=6) based on reproductive examination up to 120 days postpartum. Blood samples were collected for estimation of blood glucose using a glucometer and serum macro minerals (Ca, Pi, Mg) in semi auto-analyzer using standard biochemical kits and trace elements (Cu, Co, Fe, Zn, Se) employing atomic absorption spectrophotometry techniques.

Result: The incidence of postpartum anestrus in organised buffalo herds was 22.58% (21/93). The parameters were compared between postpartum cyclic (n=6) and acyclic buffaloes (n=6). A significant difference was observed in concentrations of blood glucose (68.67±1.91 vs. 60.83±4.01 mg/dl), serum calcium (Ca; 8.31±0.65 vs. 6.45±0.37 mg/dl), copper (Cu; 1.47±0.13 vs. 1.08±0.10 ppm), iron (Fe; 2.25±0.16 vs. 1.79±0.11 ppm) and selenium (Se; 0.72±0.03 vs. 0.62±0.03 ppm) between postpartum cyclic and acyclic buffaloes, respectively. However, non-significant variations existed in concentrations of serum inorganic phosphorus (Pi; 6.42±0.49 vs. 5.47±0.66 mg/dl), magnesium (Mg; 2.21±0.48 vs. 1.97±0.44 mg/dl) and zinc (Zn; 5.02±0.74 vs. 4.43±0.58 ppm).

Key words: *Bubalus bubalis*, Buffaloes, Hot-humid stress, Postpartum anestrus.

INTRODUCTION

Buffalo rearing is a key component for rural upliftment in developing nations, including India. The Indian dairy industry is based primarily on buffalo, which contributes maximum total milk production to the country. They are preferred over cattle due to their inherited qualities such as resistance to disease, better feed conversion ratio, milk quality, adaptation to adverse conditions. Despite these strengths, buffaloes are often described as reluctant breeders (Suthar and Dhami, 2010), owing to their vulnerability to environmental stress, which commonly leads to delayed sexual maturity, prolonged postpartum anestrus, weak estrus signs, reduced conception rates and extended intervals between calvings (Barile, 2005).

The postpartum phase represents a critical transitional period in the reproductive life of buffaloes, marked by a series of physiological, gynaecological and biochemical shifts. During this phase, the endocrine, immune and metabolic systems undergo functional alterations to support physiological adaptation, frequently resulting in a negative energy balance (Pande *et al.*, 2016). Various factors such as inadequate nutrition, reduced energy reserves, environmental stress and postpartum illnesses influence the return to ovarian cyclicity. Elevated ambient temperature and humidity are considered the primary stressors affecting this process (El-Wishy, 2007). In the context of India's tropical climate, the prevalence of anestrus tends to increase during the hot-humid season. This is attributed to the buffalo's dark skin and sparse sweat glands, which contribute to excessive heat absorption, thereby impairing estrus expression and making its detection more difficult (Marai and Haeeb, 2010).

Minerals help in the interplay of hormones and enzymes at cellular level and contribute integrally to cellular energy metabolism. Beyond functioning as enzyme co-

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factors or activators, elements such as calcium enhance tissue responsiveness to hormonal actions. Similarly, trace minerals like copper and zinc are closely associated with reproductive hormones like progesterone and estradiol, as they activate specific enzyme systems that assist in maintaining the activity of hormones in the bloodstream (Georgievskii, 1982). Mineral deficiency in dairy livestock is the primary cause of poor growth rate, suppressed immunity, decreased milk yield and various reproductive disorders (Khadda *et al.*, 2025). A deficiency in one or more of these minerals can impair fertility and contribute to conditions like anestrus. Therefore, the present study aimed to evaluate and compare blood glucose and serum mineral profiles, including both macro and trace elements, in postpartum cyclic and acyclic buffaloes.

MATERIALS AND METHODS

Place of work and selection of animals

The present investigation was undertaken at Veterinary Gynaecology and Obstetrics Department of College of Veterinary Science and Animal Husbandry, Anjora, DSVCKV, Durg (Chhattisgarh) during April to November 2023. Murrah buffaloes stationed in private dairy farms of Durg constituted experimental animals. The buffaloes aged 4 to 8 years, in good body condition, weighing 450 to 500 kg and with history of normal calving were gynaecologically examined. Rectal examination was performed twice at 11-day interval and based on the exhibition of estrus from 90 to 120 days postpartum, the animals were selected. For the incidence study of postpartum anestrus, 187 dairy buffaloes were surveyed over a three-month period. Out of 187, 93 were postpartum buffaloes that calved within two months and were examined for upto 120 days post-calving. A total of twelve pluriparous graded Murrah buffaloes were selected for the study of various blood serum parameters and divided into postpartum cyclic (resumption of estrus within 120 days postpartum; n=6) and postpartum acyclic (anestrus beyond 120 days postpartum; n=6) groups.

Blood collection

Blood (10 ml) was aseptically collected from the jugular vein immediately after signs of heat in postpartum cyclic group and at the end of 120 days in postpartum acyclic group. Serum was separated by centrifugation at 3000 rpm for 15 minutes and stored in a deep freezer at -20°C untill biochemical estimation for blood glucose and serum minerals.

Biochemical estimation of blood glucose and serum minerals

Blood glucose concentrations in freshly collected blood were quantified using a glucometer and blood glucose strips and the results in milligram per decilitre (mg/dl) were recorded. Serum concentrations of macro elements *viz.* Ca, Pi and Mg were estimated by semi auto-analyzer (Microlab 300) using serum diagnostic kit as per specification of manufacturers' protocols and results were expressed in mg/dl. Trace minerals *viz.* Co, Cu, Fe, Zn and Se in blood serum were estimated using Atomic Absorption Spectrophotometer (AAS4141; Electronics Corporation of India Limited, Hyderabad) employing flame atomization techniques after acid digestion of samples and results were expressed in parts per million (ppm).

Statistical analysis

The data obtained were analysed statistically to test the significance of differences between groups. The data generated on various parameters were analysed by t-test and one-way analysis of variance (ANOVA) between different groups, as per the standard statistical method described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Incidence of postpartum anestrus

Buffaloes not exhibiting estrus beyond 120 days postpartum were classified as acyclic. These animals were monitored for postpartum reproductive events, including calving records and reproductive status through per rectal examination, to determine the incidence of anestrus. Out of 93 postpartum buffaloes evaluated, 21 were postpartum acyclic buffaloes and thus the incidence of postpartum anestrus was recorded as 22.58% (21/93) in organized dairy farms (Table 1).

This observation is consistent with the findings of Kumar *et al.* (2013), who reported a 25.84% incidence of postpartum anestrus in buffaloes reared under organized systems. Similarly, Kalsotra *et al.* (2016) noted a 34.4% incidence in Murrah buffaloes in Jammu, while Mishra *et al.* (2023) documented 31.67% anestrus during 90-150 days postpartum and a total of 69.10% between 60 and more than 150 days postpartum in Madhya Pradesh. The relatively lower incidence in the current study may be attributed to scientifically managed feeding practices and optimal summer management in organized dairy farms.

Blood glucose

Table 2 illustrate the average blood glucose concentrations in postpartum cyclic and acyclic buffaloes. In this investigation, the postpartum cyclic group exhibited a significantly higher ($p<0.05$) mean blood glucose level (68.67±1.91 mg/dl) compared to the acyclic group (60.83±4.01 mg/dl).

These results are in line with those of Sutaria *et al.* (2022) who observed the blood glucose concentration was significantly lower in Mehsana buffaloes exhibited estrus at more than 75 days of calving compared to the buffaloes

Table 1: Incidence of postpartum anestrus in organised farms of Durg-Bhilai.

Total no. of animals surveyed	No. of postpartum animals	No. of postpartum acyclic animals (%)
187	93	21 (22.58%)

exhibited estrus at ≤75 days of calving. In a similar study by Shrivastava and Kharche (1985) a notable difference in glucose levels between cyclic and acyclic (79.75 vs. 62 mg/dl) buffaloes were observed. Similarly, Tiwari *et al.* (2012) reported elevated glucose concentrations in postpartum cyclic animals compared to acyclic ones. In contrast, Kavani *et al.* (2005) found slightly lower plasma glucose levels in fertile (53.46±3.99 mg/dl) than in infertile Surti buffaloes (54.80±3.69 mg/dl). Meanwhile, Jayachandran *et al.* (2013) noted no significant difference in blood glucose levels between cyclic and acyclic buffaloes.

Glucose is considered as an indicator of energy status in ruminants and lowering blood glucose level was associated with increasing parity of animals (Sriranga *et al.,* 2023). Reduced blood glucose due to sub-maintenance energy stress elevates plasma estrogen, which may lead to CL regression via uterine prostaglandin release (Wenntzel, 1987). Conversely, higher glucose levels indicate better energy status, supporting reproductive function by raising progesterone levels-possibly through increased LH secretion (Richards *et al.*, 1989).

Sharma *et al.* (1998) suggested that low blood glucose levels impair signal transmission along the hypothalamic-pituitary-ovarian axis, contributing to anestrus in buffaloes. The significant variation in blood glucose levels observed between cyclic and acyclic animals in the current study further supports the role of glucose concentration in influencing reproductive cyclicity in the experimental subjects.

Serum minerals

Major elements

Mean serum concentrations of major elements (Ca, P, Mg) and Ca:P ratio measured in postpartum cyclic and acyclic buffaloes are presented in Table 2.

Calcium

In the present study, postpartum cyclic buffaloes exhibited significantly higher mean serum calcium levels (8.31±0.65 mg/dl) compared to postpartum acyclic buffaloes (6.45±0.37 mg/dl; p≤0.05).

These findings are in concordance with Tiwari *et al.* (2012), who observed higher calcium levels in postpartum cyclic buffaloes (10.31±0.28 mg/dl) compared to acyclic ones (9.62±0.02 mg/dl) during summer. Kumar *et al.* (2010) reported mean serum calcium of 8.31±1.36 mg/dl in normal cyclic and 7.91±0.48 mg/dl in acyclic Murrah buffaloes. Later, Kumar *et al.* (2016) found significant differences between postpartum cyclic and acyclic (11.75±0.86 vs. 7.42±0.62 mg/dl) buffaloes from organized farms, consistent with the present study. Bohara and Devkota (2009) recorded lower calcium levels in cyclic and acyclic (7.36±0.61 vs. 5.70±0.41 mg/dl) buffaloes than seen here. In contrast, Khasatiya *et al.* (2005) and Pant *et al.* (2015) reported higher calcium concentrations in both cyclic and acyclic buffaloes. The mean calcium in postpartum cyclic buffaloes was within the normal range of 8-12 mg/dl (Hidiroglou, 1979), while the postpartum acyclic group of buffaloes showed signs of hypocalcemia.

Table 2: Serum levels of blood glucose and major minerals (mg/dl, Mean±SE, n=6) in postpartum cyclic and acyclic buffaloes.

Parameter	Groups		p value
	Cyclic	Acyclic	
Blood glucose	68.67±1.91	60.83±4.01	0.04*
Calcium	8.31±0.65	6.45±0.37	0.03*
Phosphorus	6.42±0.49	5.47±0.66	0.27
Ca:P ratio	1.31±0.08	1.31±0.21	0.19
Magnesium	2.21±0.48	1.97±0.44	0.42

*Significant variation (p≤0.05).

Phosphorus

Mean serum inorganic phosphorus concentration in present study was non-significantly higher in postpartum cyclic buffaloes as compared to postpartum acyclic group of buffaloes (6.42±0.49 vs. 5.47±0.66 mg/dl).

The present results align with the observations of Chaurasia (1999), Tiwari *et al.* (2012) and Kumar *et al.* (2016), who also reported elevated inorganic phosphorus concentrations in cyclic buffaloes, with respective values of 5.29±0.14, 5.37±0.14 and 6.98±0.35/ mg/dl, compared to lower concentrations in acyclic animals (3.96±0.20, 3.25±0.02 and 5.94±0.30/mg/dl). Campanile *et al.* (1997) reported a physiological serum phosphorus value of 6 mg/dl in adult buffaloes across various reproductive stages. In contrast, Jani *et al.* (1995) recorded a comparatively higher mean value of 8.50±0.58 mg/dl in normal cyclic buffaloes than that observed in the current study.

Calcium-to-phosphorus (Ca:P) ratio

In the current investigation, the calcium-to-phosphorus (Ca:P) ratio did not differ significantly between postpartum cyclic and acyclic buffaloes, with values recorded at 1.31± 0.08 and 1.31±0.21, respectively.

In contrast to calcium, serum inorganic phosphorus levels did not exhibit significant variation in the present study. However, reduced calcium concentrations contributed to a shift in the Ca:P ratio. According to Carnahan (1974), an optimal Ca:P ratio ranging between 1.5:1 and 2.5:1 is essential for maintaining reproductive efficiency in livestock. Morrow (1969) emphasized that imbalances in this ratio could delay the onset of first ovulation. Such disruptions may partially explain the decline in reproductive performance observed during the summer. Deviations in this ratio might impair ovarian activity by inhibiting pituitary function, resulting in delayed estrus and ovulation, thereby diminishing overall fertility. The issue is further exacerbated in the summer months due to the limited availability of green fodder, which can intensify nutritional imbalances.

Magnesium

The mean serum magnesium concentration was higher in postpartum cyclic group (2.21±0.48 mg/dl) compared to

postpartum acyclic (1.97±0.44 mg/dl) buffaloes. These findings are in alignment with those of Newer *et al.* (1999), who reported significantly higher magnesium levels during estrus (3.37±0.02 mg/dl) compared to the anestrus period (2.20±0.01 mg/dl) in swamp buffaloes ($p<0.05$). However, the magnesium levels observed in the present study were lower than those documented in previous reports. For instance, Takkar *et al.* (1992) recorded serum magnesium concentrations of 3.30±0.10 mg/dl on the day of estrus in buffaloes. Similarly, Khattab *et al.* (1995) observed magnesium levels of 3.34 mg/dl in Egyptian buffaloes with regular estrous cycles, whereas animals with irregular cycles and inactive ovaries exhibited lower but identical values (2.33 mg/dl).

Trace minerals

Overall mean concentrations of trace minerals (Cu, Co, Fe, Zn and Se) estimated in serum samples of postpartum cyclic and acyclic buffaloes are presented in Table 3.

In the present study, serum copper levels were found to be significantly elevated ($p<0.05$) in postpartum cyclic buffaloes compared to those of acyclic buffaloes (1.47± 0.13 vs. 1.08±0.10 ppm). Although the mean cobalt concentration was also higher in the postpartum cyclic group (1.18±0.10 ppm) than in the acyclic group (0.88± 0.13 ppm), the difference was not statistically significant. A significant increase ($p<0.05$) in mean serum iron levels was observed in cyclic buffaloes (2.25±0.16 ppm) compared to acyclic buffaloes (1.79±0.11 ppm). The average serum zinc concentration was also higher in postpartum cyclic (5.02±0.74 ppm) than in the acyclic group (4.43±0.58 ppm) of animals under study. The mean serum selenium levels exhibited a statistically significant difference ($p\leq0.05$) between the two groups, with postpartum cyclic buffaloes showing higher concentrations (0.72±0.03 ppm) than postpartum acyclic animals (0.62± 0.03 ppm).

In the present investigation, postpartum cyclic buffaloes exhibited significantly higher mean concentrations of copper and iron compared to the buffaloes in postpartum acyclic group. Although the mean levels of cobalt, zinc and selenium were also greater in the cyclic group, the differences were not statistically significant. These results are in agreement with those of Parshad *et al.* (1979), who reported plasma copper, iron and zinc levels of 0.81±0.02, 2.60±0.05 and 1.80±0.05 ppm, respectively, in postpartum lactating buffaloes. Desai *et al.* (1982) recorded serum copper concentrations during ovulation, estrus and on days 9 and 15 thereafter, ranging from 1.90 to 2.05 ppm, which are comparable to the current findings. Additionally, the present results align with the observations of Kumar *et al.* (2016), who noted significantly ($p\leq0.05$) lower serum iron concentrations in postpartum acyclic buffaloes compared to cyclic counterparts (1.76±0.02 vs. 2.33±0.01 ppm).

Table 3: Serum levels of trace minerals (ppm, Mean±SE) in postpartum cyclic and acyclic buffaloes.

Parameter	Groups		p value
	Cyclic	Acyclic	
Copper	1.47±0.13	1.08±0.10	0.04*
Cobalt	1.18±0.10	0.88±0.13	0.09
Iron	2.25±0.16	1.79±0.11	0.04*
Zinc	5.02±0.74	4.43±0.58	0.26
Selenium	0.72±0.03	0.62±0.03	0.05*

*significant variation ($p\leq0.05$).

Over recent decades, growing interest in the nutritional role of trace minerals has emerged due to advancements in clinical and analytical techniques, along with the development of functional indicators of trace element status (Setia *et al.*, 1994). Various factors including species, breed, sex, age, health, nutritional status and physiological condition can influence the serum mineral profile (Swanson *et al.*, 2004). Blood mineral concentrations generally reflect dietary mineral intake, as livestock derive essential trace elements primarily from feed (Underwood, 1977). Since the animals in this study were housed in organized dairy farms with periodic mineral supplementation in addition to regular feeding, their serum mineral profiles indicated adequate intake. However, the potential influence of interactions such as copper-molybdenum antagonism or the copper-to-zinc ratio on reproductive physiology may warrant further investigation.

CONCLUSION

The present study underscores the pivotal role of blood glucose and serum minerals in the resumption of postpartum ovarian activity in buffaloes. Postpartum cyclic buffaloes showed significantly higher levels of glucose, calcium, copper, iron and selenium than acyclic ones. These elements play critical roles in timely uterine involution and ovarian cyclicity. In hot-humid climatic conditions, nutritional deficits-especially in key minerals-can disrupt hormonal pathways and immune responses essential for reproductive function. Thus, strategic management focusing on energy and mineral supplementation during stressful seasons can enhance reproductive performance and reduce the incidence of postpartum anestrus in dairy buffaloes.

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Disclaimers

The opinions and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors

are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Institutional Animal Ethics Committee (IAEC).

Conflict of interest

On behalf of all authors of the manuscript, I declare that we have no conflict of interest.

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