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Effect of different mineral supplementation on serum mineral profile in postpartum anoestrus graded Murrah buffaloes

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Abstract

The present investigation was conducted on 60 postpartum pluriparous true anoestrus graded Murrah buffaloes (body condition score 3.0-4.0) maintained at the Dairy Experimental Station, College of Veterinary Science, Rajendranagar, Hyderabad and nearby commercial farms. Animals free from reproductive disorders were randomly allotted into four groups ($n = 15$ each): Group I (Cyclomin-7 @ 2 boli/week + dicalcium phosphate @ 30 g/day), Group II (non-chelated mineral mixture @ 50 g/day), Group III (chelated mineral mixture @ 50 g/day) and Group IV (control, no supplementation) for 30 days. Blood samples were collected on day 0, 15 and 30 for haemoglobin and serum mineral analysis.

Haemoglobin concentration showed no significant ($p > 0.05$) variation among groups on day 0 and 15; however, on day 30, all treatment groups recorded significantly ($p < 0.05$) higher values compared to control, with Group III showing maximum improvement (11.89 ± 0.27 g/dL). Serum calcium and phosphorus levels increased significantly ($p < 0.05$) in all treatment groups, with highest values in Group III (8.79 ± 0.07 mg/dL and 6.94 ± 0.05 mg/dL, respectively) on day 30. Similarly, serum iron, copper, manganese and zinc levels were significantly ($p < 0.05$) elevated in supplemented groups, with maximum increase observed in chelated mineral group (iron: 3.680 ± 0.081 ppm; copper: 0.184 ± 0.005 ppm; manganese: 0.182 ± 0.002 ppm; zinc: 3.767 ± 0.042 ppm).

It was concluded that mineral supplementation, particularly in chelated form, significantly improves haemato-biochemical parameters and reproductive performance in postpartum anoestrus buffaloes due to enhanced bioavailability and mineral utilization.

Keywords: Postpartum anoestrus, Murrah buffaloes, chelated minerals, serum mineral profile, trace minerals, mineral supplementation

Introduction

The global population of water buffalo is currently estimated at over 208 million, with approximately 97% distributed in Asia, followed by smaller populations in Africa and Europe. Buffaloes play a pivotal role in the livestock economy of developing countries, particularly in South Asia. India possesses the largest buffalo population in the world, accounting for nearly 110 million animals as per the 20th Livestock Census representing more than 50% of the global population. These animals are primarily reared by small and marginal farmers, significantly contributing to rural livelihoods. Buffalo milk, characterized by higher fat and Solids-Not-Fat (SNF) content compared to cow milk, has greater market value and consumer preference, thereby enhancing farm income. Globally, buffaloes contribute more than 120 million tonnes of milk annually, accounting for a substantial share of total milk production in Asia. The sustainability of buffalo-based dairy production largely depends on optimal reproductive efficiency. Any disruption in reproductive rhythm leads to prolonged calving intervals, increased dry periods, and reduced lifetime productivity, resulting in significant economic losses (Perera, 2011; Gurpreet Singh *et al.* 2020) [44, 45]. Infertility and anoestrus conditions not only reduce milk yield but also hinder genetic improvement by limiting calf crop output. Reproductive performance in livestock is influenced by a complex interaction of environmental, nutritional, hormonal, infectious, and genetic factors. Among these, mineral nutrition plays a crucial role in maintaining metabolic homeostasis and reproductive function. Minerals are broadly classified into macro and

micro (trace) elements, both essential for normal physiological processes. Deficiencies or imbalances of these minerals can impair reproductive efficiency by altering endocrine function, enzymatic activity, and cellular metabolism (Kumar *et al.* 2021) ^[46]. In particular, deficiencies of calcium, phosphorus, copper, zinc, and selenium are associated with delayed estrus, poor conception rates, and increased incidence of reproductive disorders.

Under field conditions, buffaloes are predominantly maintained on crop residues, dry fodder, and limited green forage, often without adequate mineral supplementation. Such feeding practices predispose animals to mineral deficiencies, especially when forages are grown on mineral-deficient soils (Singh *et al.* 2019; Das *et al.* 2020) ^[16, 47]. Continuous soil nutrient depletion due to intensive agricultural practices further aggravates this issue, leading to region-specific mineral imbalances that directly affect animal productivity (Underwood and Suttle, 2019) ^[42]. Consequently, suboptimal reproductive performance is frequently observed despite otherwise adequate management practices.

Calcium and phosphorus are the most critical macro-minerals influencing reproductive health. Calcium is essential for uterine muscle tone, ovarian activity, and hormonal regulation, whereas phosphorus plays a key role in energy metabolism, rumen microbial function, and reproductive efficiency. Imbalance in the calcium-to-phosphorus ratio can disrupt pituitary and ovarian function, resulting in delayed ovulation and infertility (Goff, 2018) ^[21]. Reduced blood calcium levels have also been associated with delayed uterine involution and increased incidence of dystocia, retained placenta, and uterine prolapse (Lean *et al.* 2017) ^[33]. Phosphorus deficiency remains one of the most economically important mineral deficiencies affecting reproduction in developing countries (McDowell, 2020) ^[35]. Trace minerals such as copper, zinc, and selenium are equally important due to their involvement in enzymatic systems, antioxidant defense, immune response, and reproductive hormone synthesis. Deficiencies of these trace elements can lead to reduced fertility and poor reproductive outcomes. These deficiencies may occur either as primary deficiencies due to inadequate intake or as secondary deficiencies due to antagonistic interactions affecting absorption and metabolism (Suttle, 2022) ^[41].

In recent years, organic or chelated minerals have gained considerable attention due to their higher bioavailability compared to conventional inorganic mineral sources. Chelation refers to the binding of mineral ions with organic ligands such as amino acids or peptides, forming stable complexes that enhance absorption and utilization in the animal body. Commonly used chelated trace minerals include iron, manganese, cobalt, copper, and zinc. These forms are less susceptible to dietary antagonisms and anti-nutritional factors, thereby improving their biological efficiency (Spears and Weiss, 2019) ^[40].

Field studies have reported significant improvements in estrus induction and conception rates in anestrus buffaloes following mineral supplementation (Meena *et al.* 2018; Patel *et al.* 2021) ^[36, 37]. Enhanced reproductive performance has also been observed in postpartum anestrus buffaloes supplemented with balanced mineral mixtures (Reddy *et al.* 2020) ^[38]. These findings underscore the importance of

mineral nutrition in improving reproductive efficiency in buffaloes. Despite the recognized importance of mineral supplementation, information regarding serum mineral profiles during anestrus and estrus in buffaloes remains limited. Therefore, the present study was undertaken to evaluate the comparative efficacy of different forms of mineral supplementation, including dicalcium phosphate with trace mineral bolus, non-chelated mineral mixture, and chelated mineral mixture; changes in serum mineral profiles before and after supplementation were assessed to understand their relationship with reproductive response.

Objectives of the study

- To estimate serum mineral profiles before and during mineral supplementation.
- To determine a cost-effective mineral supplementation strategy under field conditions.

Materials and Methods

Experimental location and animals

The present study was conducted at the Dairy Experimental Station, College of Veterinary Science, Rajendranagar, Hyderabad, and in selected commercial dairy farms located in Rangareddy district of Telangana, India. The experimental area represents a semi-arid agro-climatic zone where buffaloes are maintained under intensive management conditions. A total of 60 graded Murrah buffaloes were selected and maintained under uniform feeding, housing, and health management practices. Animals were fed green fodder, dry fodder, and concentrate mixture according to their production requirements, and clean drinking water was provided *ad libitum*.

Selection of animals

The animals selected for the study were postpartum pluriparous buffaloes, at least 60 days after calving, exhibiting true anestrus condition. The body condition score (BCS) of the selected animals ranged between 3.0 and 4.0. The anestrus condition was confirmed by per rectal examination conducted twice at an interval of 8 days, ensuring absence of ovarian cyclicity. Animals with reproductive disorders such as endometritis, ovarian cysts, or uterine infections were excluded from the study. All animals were dewormed with albendazole (@ 5 mg/kg body weight orally) prior to the experiment, and the study was initiated 8-10 days after deworming.

Experimental Design and Treatment Groups

The experiment was conducted using a Completely Randomized Design (CRD). The selected animals were randomly divided into four groups, each consisting of 15 buffaloes.

- Group I (DCP + Trace mineral bolus): Animals were supplemented with Cyclomin-7 bolus (2 boli per animal, once weekly) along with dicalcium phosphate (30 g/day) for 30 days.
- Group II (Non-chelated mineral mixture): Animals were supplemented with non-chelated mineral mixture at 50 g/day for 30 days.
- Group III (Chelated mineral mixture): Animals were supplemented with chelated mineral mixture at 50 g/day for 30 days.
- Group IV (Control): Animals were maintained on basal diet without any mineral supplementation.

The compositions of mineral mixtures included macro minerals (Ca, P, Mg) and trace minerals (Cu, Zn, Mn, Co, Fe, Se), along with amino acids and vitamins in the chelated formulation.

Blood Sample Collection

Blood samples were collected from all animals via jugular venepuncture on day 0 (before treatment), day 15, and day 30 of the experiment. Approximately 4 mL of blood was collected in EDTA vacutainers for hematological analysis, and 10 mL in plain vacutainers for serum separation. Serum was separated by centrifugation at 3000 rpm for 10 min and stored at -20°C until further analysis.

Haematological Analysis

Haemoglobin concentration was estimated using the acid haematin method as described by Coles (1986) [48], and results were expressed in g/dL.

Serum Mineral Estimation

Serum samples were analyzed for macro-and micro-mineral concentrations using standard procedures.

- **Calcium:** Estimated by the o-cresol phthalein complex one method and expressed in mg/dL.
- **Phosphorus:** Determined using modified molybdate method and expressed in mg/dL.
- **Trace minerals (Fe, Cu, Mn, Zn):** Estimated using Atomic Absorption Spectrophotometer (AAS) following tri-acid digestion as per procedures, and values were expressed in ppm.

Statistical Analysis

The data obtained were analyzed statistically using the methods described by. Statistical analysis was performed using SPSS software (Version 16.0). Results were expressed as mean $\pm$ standard error (SE), and differences were considered significant at $p < 0.05$.

Results

Haemoglobin concentration (g/dL)

The mean haemoglobin (Hb) concentrations in postpartum anestrus buffaloes under different treatment groups at 0, 15, and 30 days are presented in Table 1. In Group I (DCP + Cyclomin-7), Hb levels increased from 11.05 ± 0.26 to 11.55 ± 0.26 g/dL. Similarly, in Group II (non-chelated mineral mixture), values increased from 11.01 ± 0.22 to 11.63 ± 0.22 g/dL, while in Group III (chelated mineral mixture), Hb levels increased from 11.03 ± 0.18 to 11.89 ± 0.27 g/dL. In contrast, Group IV (control) showed negligible variation (11.01 ± 0.16 to 11.06 ± 0.17 g/dL). Statistically, no significant difference ($p > 0.05$) was observed within Groups I, II, and IV across different time intervals. However, in Group III, Hb levels showed a progressive increase, though differences between intervals were not statistically significant.

Between groups, no significant variation ($p > 0.05$) was observed on day 0 and day 15. However, by day 30, all treatment groups (I, II, III) recorded significantly higher ($p < 0.05$) Hb levels compared to the control group, indicating a positive effect of mineral supplementation, particularly chelated minerals.

Table 1: Haemoglobin (mg %) levels in post-partum anoestrus graded Murrah buffaloes of different mineral supplementation groups

S. No.	Name of the Group	Days of sample collection		
		0 day	15 th day	30 th day
1	Group I	11.05 ± 0.26	11.31 ± 0.27	$11.55^{\text{AB}} \pm 0.26$
2	Group II	11.01 ± 0.22	11.32 ± 0.23	$11.63^{\text{AB}} \pm 0.22$
3	Group III	$11.03^{\text{a}} \pm 0.18$	$11.46^{\text{ab}} \pm 0.26$	$11.89^{\text{Bb}} \pm 0.27$
4	Group IV	11.01 ± 0.16	11.03 ± 0.17	$11.06^{\text{A}} \pm 0.17$

a, b, c,...differ significantly ($p < 0.05$) within a row

A, B, C, D,... differ significantly ($p < 0.05$) within a column

Serum mineral profile

Serum calcium (mg/dL)

Serum calcium levels increased significantly in all treatment groups over time (Table 2).

- **Group I:** $7.20 \pm 0.23 \rightarrow 8.08 \pm 0.22$
- **Group II:** $7.15 \pm 0.21 \rightarrow 8.63 \pm 0.16$
- **Group III:** $7.24 \pm 0.23 \rightarrow 8.79 \pm 0.07$
- **Control:** $7.31 \pm 0.10 \rightarrow 7.35 \pm 0.07$

Within-group analysis revealed significant increases ($p < 0.05$) from day 0 to day 30 in Groups I, II, and III, whereas no significant change was observed in the control group.

Between groups, no significant difference was noted on day 0. On day 15 and 30, treatment groups showed significantly higher ($p < 0.05$) calcium levels compared to control. On day 30, Group III and II exhibited comparable but significantly higher levels than Group I and control, indicating superior efficacy of chelated minerals.

Table 2: Serum Calcium levels (mg/dl) in post-partum anoestrus graded Murrah buffaloes of different mineral supplementation groups

S. No.	Name of the Group	Days of sample collection		
		0 day	15 th day	30 th day
1	Group I	$7.20^{\text{a}} \pm 0.23$	$7.69^{\text{ABb}} \pm 0.23$	$8.08^{\text{Bc}} \pm 0.22$
2	Group II	$7.15^{\text{a}} \pm 0.21$	$7.85^{\text{Bb}} \pm 0.12$	$8.63^{\text{Cc}} \pm 0.16$
3	Group III	$7.24^{\text{a}} \pm 0.23$	$7.97^{\text{Bb}} \pm 0.12$	$8.79^{\text{Cc}} \pm 0.07$
4	Group IV	7.31 ± 0.10	$7.34^{\text{A}} \pm 0.06$	$7.35^{\text{A}} \pm 0.07$

a, b, c,...differ significantly ($p < 0.05$) within a row

A, B, C, D,... differ significantly ($p < 0.05$) within a column

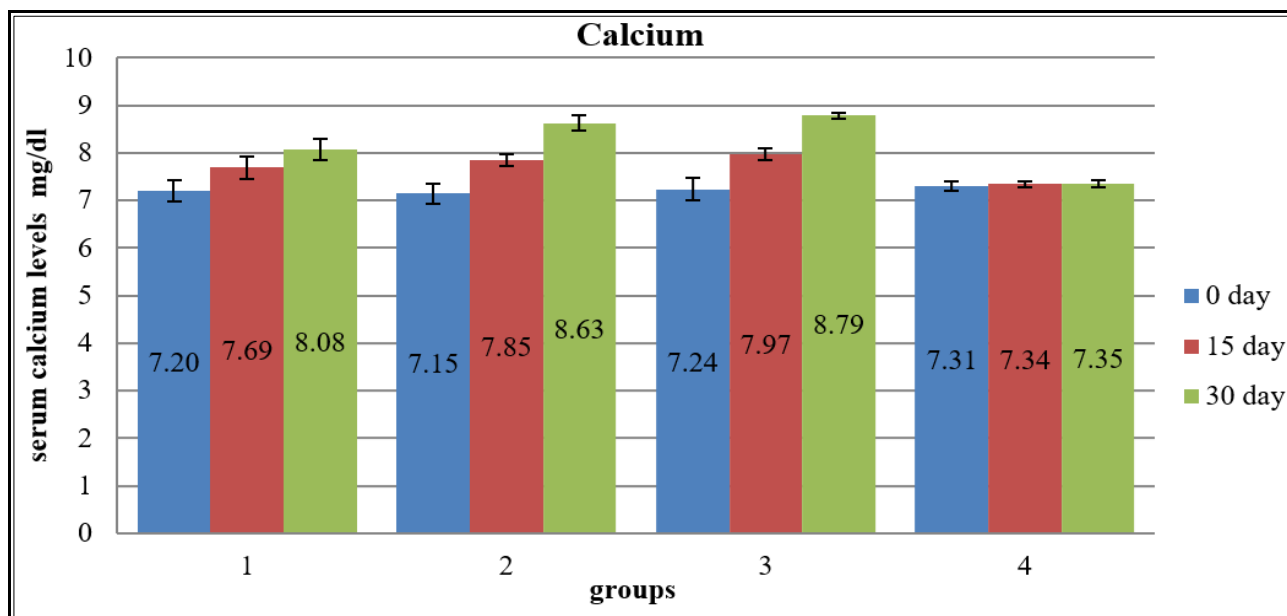


Fig 1: Serum Calcium levels (mg/dl) in postpartum anoestrus graded Murrah buffaloes of different mineral supplementation groups

Serum Phosphorus (mg/dL)

Serum phosphorus levels showed improvement across treatment groups (Table 3):

- Group I: $5.46 \pm 0.29 \rightarrow 6.24 \pm 0.18$
- Group II: $5.78 \pm 0.18 \rightarrow 6.30 \pm 0.25$
- Group III: $5.56 \pm 0.07 \rightarrow 6.94 \pm 0.05$
- Control: $5.56 \pm 0.07 \rightarrow 5.80 \pm 0.10$

A significant increase ($p < 0.05$) was observed only in Group III, with progressive elevation across all time points. Groups I and II showed non-significant increases, while control exhibited minimal change.

Between groups, Group III recorded significantly higher ($p < 0.05$) phosphorus levels on day 30 compared to all other groups, indicating enhanced bioavailability of chelated minerals.

Table 3: Serum Phosphorus levels (mg/dl) in post-partum anoestrus graded Murrah buffaloes of different mineral supplementation groups

S. No.	Name of the Group	Days of sample collection		
		0 day	15 th day	30 th day
1	Group I	5.46 ± 0.29	5.79 ± 0.32	$6.24^{AB} \pm 0.18$
2	Group II	5.78 ± 0.18	5.87 ± 0.28	$6.30^B \pm 0.25$
3	Group III	$5.56^a \pm 0.07$	$5.96^b \pm 0.17$	$6.94^{Cc} \pm 0.05$
4	Group IV	$5.56^a \pm 0.07$	$5.83^b \pm 0.11$	$5.80^{Aab} \pm 0.10$

a, b, c,... differ significantly ($p < 0.05$) within a row

A, B, C, D,... differ significantly ($p < 0.05$) within a column

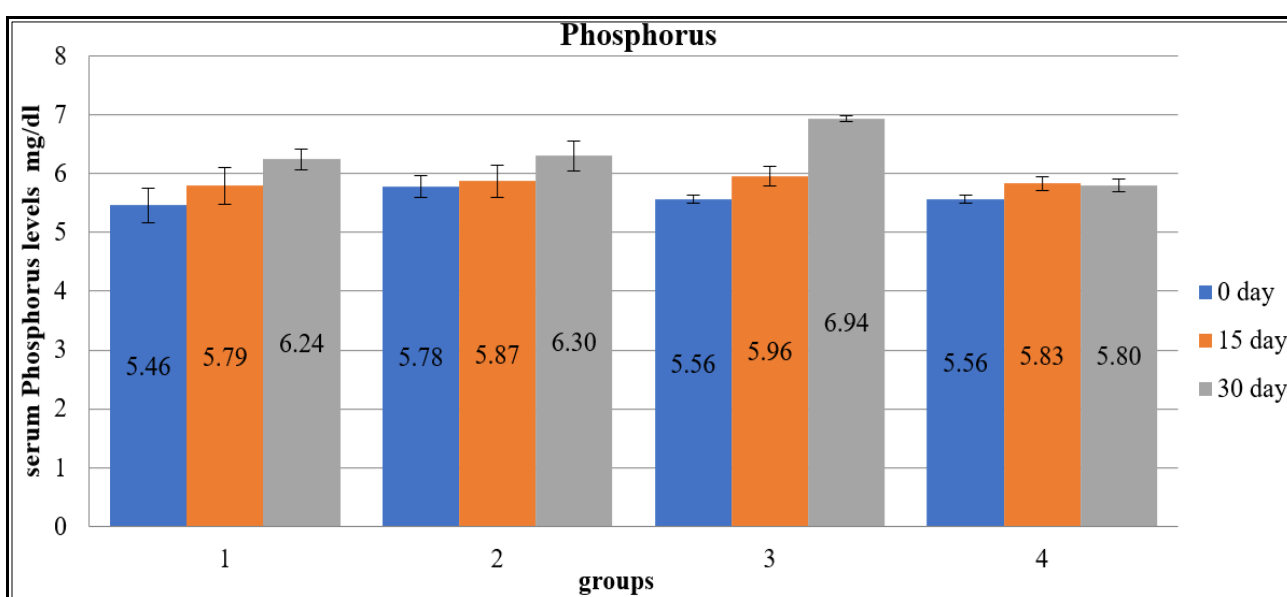


Fig 2: Serum Phosphorus levels (mg/dl) in postpartum anoestrus graded Murrah buffaloes of different mineral supplementation groups

Serum iron (ppm): Serum iron levels increased in all treatment groups (Table 4):

- **Group I:** $2.716 \pm 0.112 \rightarrow 3.180 \pm 0.110$
- **Group II:** $2.656 \pm 0.058 \rightarrow 3.246 \pm 0.140$
- **Group III:** $2.689 \pm 0.047 \rightarrow 3.680 \pm 0.081$
- **Control:** $2.679 \pm 0.039 \rightarrow 2.702 \pm 0.060$

Within-group analysis showed significant increases ($p < 0.05$) from day 0 to day 15 and 30 in Groups I and II, while Group III showed highly significant progressive increases ($p < 0.05$) across all intervals. Between groups, Group III recorded significantly higher iron levels ($p < 0.05$) on day 30 compared to Groups I, II, and IV.

Table 4: Serum Iron levels (ppm) in post-partum anoestrus graded Murrah buffaloes of different mineral supplementation groups

S. No.	Name of the Group	Days of sample collection		
		0 day	15 th day	30 th day
1	Group I	$2.716^a \pm 0.112$	$3.122^{Bb} \pm 0.084$	$3.180^{Bb} \pm 0.110$
2	Group II	$2.656^a \pm 0.058$	$3.147^{Bb} \pm 0.068$	$3.246^{Bb} \pm 0.140$
3	Group III	$2.689^a \pm 0.047$	$3.174^{Bb} \pm 0.194$	$3.680^{Cc} \pm 0.081$
4	Group IV	2.679 ± 0.039	$2.690^A \pm 0.061$	$2.702^A \pm 0.060$

a, b, c,...differ significantly ($p < 0.05$) within a row

A, B, C, D,... differ significantly ($p < 0.05$) within a column

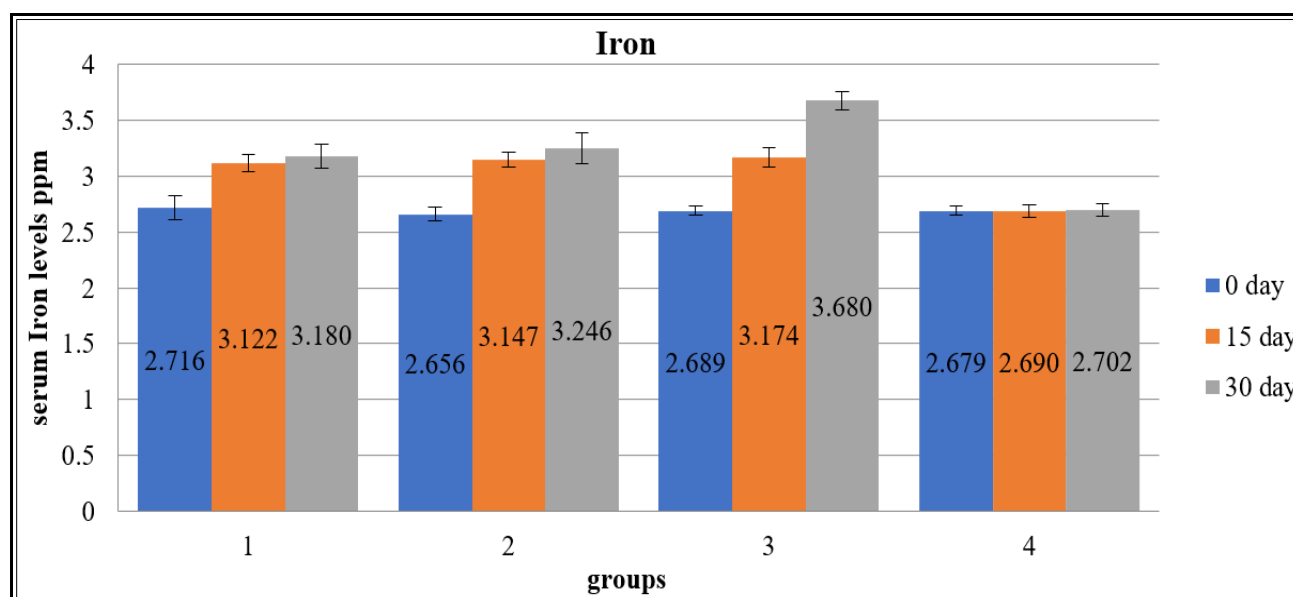


Fig 3: Serum Iron levels (ppm) in postpartum anoestrus graded Murrah buffaloes of different mineral supplementation groups

Serum copper (ppm): Serum copper levels increased significantly in all treatment groups (Table 5):

- **Group I:** $0.047 \pm 0.003 \rightarrow 0.142 \pm 0.006$
- **Group II:** $0.049 \pm 0.005 \rightarrow 0.163 \pm 0.005$
- **Group III:** $0.052 \pm 0.002 \rightarrow 0.184 \pm 0.005$
- **Control:** $0.049 \pm 0.002 \rightarrow 0.054 \pm 0.003$

All treatment groups showed significant increases ($p < 0.05$) over time, whereas control group showed no significant variation.

Between groups, significant differences ($p < 0.05$) were observed on days 15 and 30, with Group III showing highest values, followed by Group II and Group I.

Table 5: Serum Copper concentration (ppm) in post-partum anoestrus graded Murrah buffaloes with mineral supplementation in different groups

S. No.	Name of the Group	Days of sample collection		
		0 day	15 th day	30 th day
1	Group I	$0.047^a \pm 0.003$	$0.097^{Bb} \pm 0.003$	$0.142^{Bc} \pm 0.006$
2	Group II	$0.049^a \pm 0.005$	$0.127^{Cb} \pm 0.005$	$0.163^{Cc} \pm 0.005$
3	Group III	$0.052^a \pm 0.002$	$0.149^{Db} \pm 0.003$	$0.184^{Dc} \pm 0.005$
4	Group IV	0.049 ± 0.002	$0.052^A \pm 0.002$	$0.054^A \pm 0.003$

a, b, c,...differ significantly ($p < 0.05$) within a row

A, B, C, D,... differ significantly ($p < 0.05$) within a column

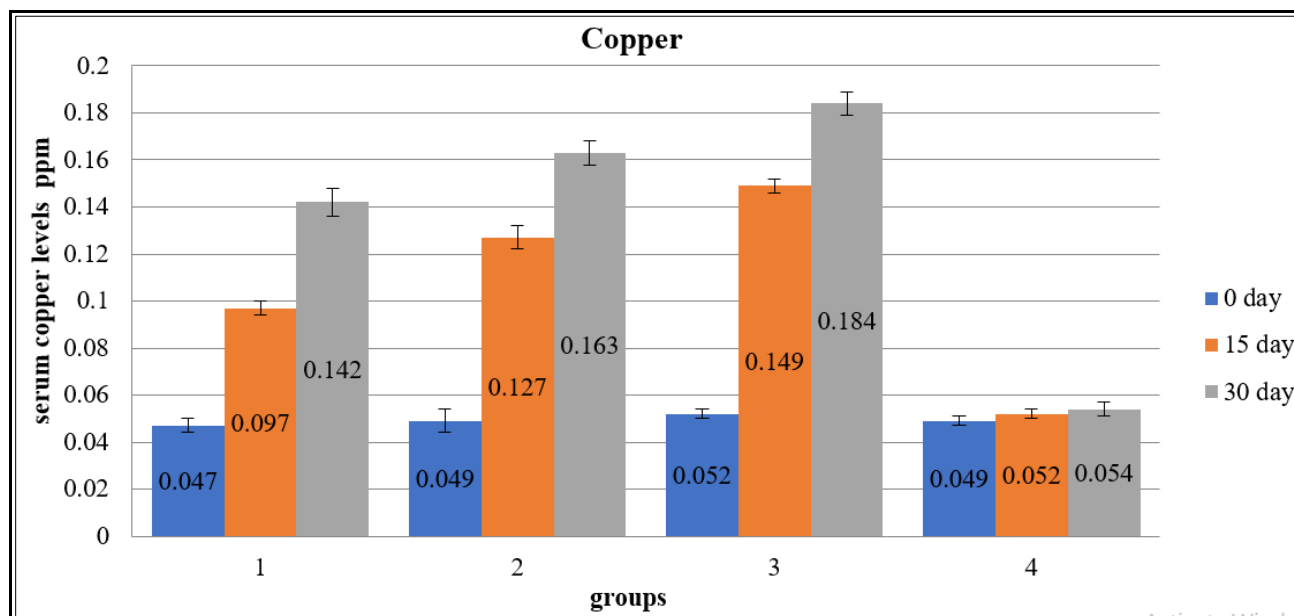


Fig 4: Serum Copper levels (ppm) in post-partum anoestrus graded Murrah buffaloes of different mineral supplementation groups

Serum manganese (ppm)

Serum manganese levels improved markedly in treated groups (Table 6)

- Group I: $0.056 \pm 0.003 \rightarrow 0.151 \pm 0.007$
- Group II: $0.058 \pm 0.002 \rightarrow 0.165 \pm 0.006$
- Group III: $0.054 \pm 0.002 \rightarrow 0.182 \pm 0.002$
- Control: $0.055 \pm 0.002 \rightarrow 0.055 \pm 0.003$

A significant increase ($p < 0.05$) was observed in all treatment groups across time, whereas control group remained unchanged. Between groups, Group III recorded significantly higher manganese levels ($p < 0.05$) on day 30, followed by Group II and Group I.

Table 6: Serum Manganese levels (ppm) in post-partum anoestrus graded Murrah buffaloes of different mineral supplementation groups

S. No.	Name of the Group	Days of sample collection		
		0 day	15 th day	30 th day
1	Group I	$0.056^a \pm 0.003$	$0.076^{Bb} \pm 0.003$	$0.151^{Bc} \pm 0.007$
2	Group II	$0.058^a \pm 0.002$	$0.112^{Cb} \pm 0.002$	$0.165^{Cc} \pm 0.006$
3	Group III	$0.054^a \pm 0.002$	$0.148^{Db} \pm 0.002$	$0.182^{Dc} \pm 0.002$
4	group IV	0.056 ± 0.002	$0.056^A \pm 0.003$	$0.055^A \pm 0.003$

^{a, b, c, ...} differ significantly ($p < 0.05$) within a row

^{A, B, C, D, ...} differ significantly ($p < 0.05$) within a column

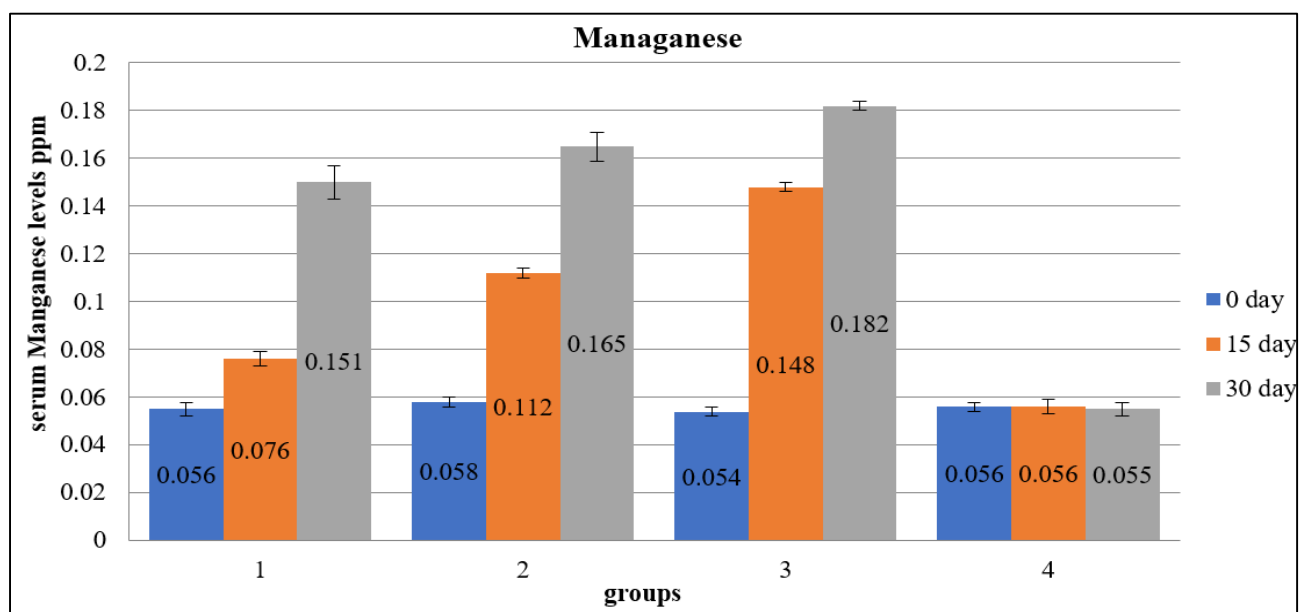


Fig 5: Serum Manganese levels (ppm) in post-partum anoestrus graded Murrah buffaloes of different mineral supplementation groups

Serum zinc (ppm)

Serum zinc levels showed the most pronounced increase (Table 7):

- **Group I:** $1.062 \pm 0.023 \rightarrow 2.639 \pm 0.061$
- **Group II:** $1.089 \pm 0.008 \rightarrow 3.038 \pm 0.017$
- **Group III:** $1.075 \pm 0.010 \rightarrow 3.767 \pm 0.042$
- **Control:** $1.032 \pm 0.012 \rightarrow 1.054 \pm 0.010$

Within-group analysis showed:

- **Group I:** Significant increase from day 0 to 15 and 30 ($p < 0.05$)
- **Groups II & III:** Significant progressive increase ($p < 0.05$)
- **Control:** No significant change

Between groups, Group III showed significantly highest zinc levels ($p < 0.05$) followed by Group II and Group I, while control group had the lowest values.

Table 7: Serum Zinc levels (ppm) in post-partum anoestrus graded Murrah buffaloes of different mineral supplementation groups

S. No.	Name of the Group	Days of sample collection		
		0 day	15 th day	30 th day
1	Group I	$1.062^{ABa} \pm 0.023$	$1.162^{Bb} \pm 0.033$	$2.639^{Bb} \pm 0.061$
2	Group II	$1.089^{Ba} \pm 0.008$	$1.248^{Cb} \pm 0.018$	$3.038^{Cc} \pm 0.017$
3	Group III	$1.075^{ABa} \pm 0.010$	$1.427^{Db} \pm 0.039$	$3.767^{Dc} \pm 0.042$
4	Group IV	$1.032^A \pm 0.012$	$1.050^A \pm 0.010$	$1.054^A \pm 0.010$

a, b, c,... differ significantly ($p < 0.05$) within a row

A, B, C, D... differ significantly ($p < 0.05$) within a column

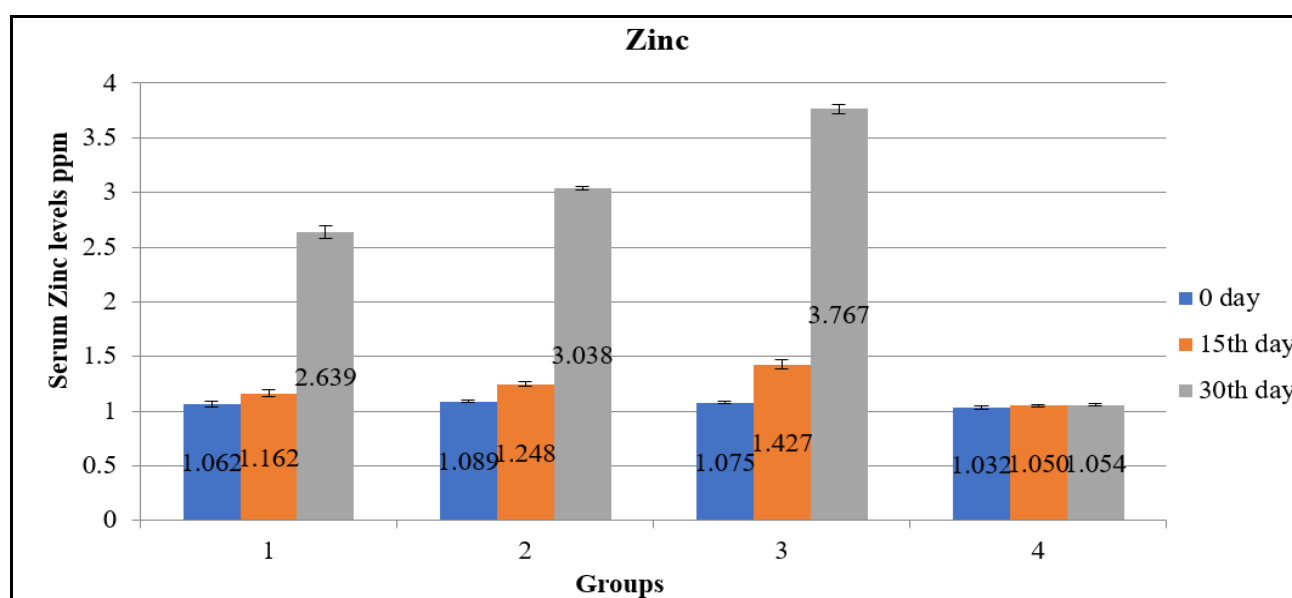


Fig 6: Serum Zinc levels (ppm) in post-partum anoestrus graded Murrah buffaloes of different mineral supplementation groups

Overall interpretation

Mineral supplementation significantly improved serum mineral status in postpartum anestrous buffaloes. Among treatments, chelated mineral mixture (Group III) consistently showed superior improvement ($p < 0.05$) in all mineral parameters compared to non-chelated and conventional supplementation, indicating better bioavailability and efficacy.

Discussion

The present investigation evaluated the effect of different forms of mineral supplementation on haemoglobin levels, serum mineral profile, estrus characteristics, and conception rate in postpartum anoestrus graded Murrah buffaloes. The findings clearly indicate that mineral supplementation, particularly in chelated form, significantly improved metabolic and reproductive parameters.

Haemoglobin levels

In the present study, haemoglobin concentrations showed a gradual improvement in all treatment groups, with comparatively higher values observed in Group III (chelated

mineral mixture). Although intra-group differences were not statistically significant in Groups I, II, and IV, Group III exhibited a progressive numerical increase from 11.03 ± 0.18 g/dL (day 0) to 11.89 ± 0.27 g/dL (day 30). Furthermore, on day 30, all treatment groups recorded significantly ($p < 0.05$) higher haemoglobin levels than the control group. The improvement in haemoglobin concentration may be attributed to enhanced availability of trace elements such as iron and copper, which are essential for erythropoiesis. Similar improvements following mineral supplementation have been reported in dairy animals (Kumar *et al.* 2020; Singh *et al.* 2021) [32, 39]. Chelated minerals, owing to their higher bioavailability, facilitate better absorption and utilization, thereby improving hematological status. Improved haemoglobin levels enhance tissue oxygenation, including reproductive tissues, which may positively influence ovarian activity and estrus expression (Goff, 2018) [21].

Serum mineral profile

Serum calcium: The present study recorded significantly ($p < 0.05$) increased serum calcium levels in Groups I, II, and

III, with the highest concentration in Group III (8.79 ± 0.07 mg/dL on day 30). Initial calcium levels across groups were below the critical threshold (~ 8 mg/dL), indicating a pre-existing deficiency state. The marked improvement, particularly in chelated mineral supplementation, can be attributed to enhanced absorption and reduced antagonistic interactions in the gastrointestinal tract. Similar findings have been reported by Das *et al.* (2020)^[16] and Patel *et al.* (2021)^[37], who observed improved calcium status in supplemented buffaloes. Calcium plays a critical role in follicular development, steroidogenesis, and uterine contractility. Deficiency may delay ovulation and prolong postpartum anoestrus (Lean *et al.* 2017)^[33]. Thus, improved calcium levels in treated groups likely contributed to better reproductive performance observed in this study.

Serum phosphorus

Serum phosphorus levels showed significant improvement in Group III, reaching 6.94 ± 0.05 mg/dL on day 30, while Groups I and II showed moderate increases. Control animals exhibited minimal variation. Phosphorus is essential for energy metabolism (ATP synthesis), which directly influences ovarian function and embryo survival. Deficiency leads to irregular estrus and poor conception rates (McDowell, 2020)^[35]. The superior response in chelated mineral group may be due to improved mineral utilization efficiency. These findings are consistent with reports by Meena *et al.* (2018)^[36] and Reddy *et al.* (2020)^[38], who observed enhanced reproductive performance with improved phosphorus status.

Serum iron

A significant ($p < 0.05$) increase in serum iron was observed in all treatment groups, with Group III showing the highest levels (3.680 ± 0.081 ppm on day 30). Control group values remained unchanged. Iron plays a vital role in haemoglobin synthesis and oxygen transport. The higher increase in Group III suggests better bioavailability of chelated iron. Similar improvements have been reported in supplemented buffaloes (Kumar *et al.* 2020)^[32]. However, it is important to note that excessive iron may interfere with copper absorption. Balanced mineral supplementation, as in chelated formulations, helps mitigate such antagonistic effects (Suttle, 2022)^[41].

Serum copper

Serum copper levels increased significantly ($p < 0.05$) in all treatment groups, with Group III recording the highest value (0.184 ± 0.005 ppm). Despite improvement, values remained near or slightly below critical levels, indicating persistent marginal deficiency. Copper plays an essential role in enzyme systems, reproductive hormone synthesis, and iron metabolism. Low copper levels are associated with delayed estrus and poor fertility (Underwood and Suttle, 2019)^[42]. The better response in chelated mineral group may be due to protection from antagonists like iron and sulphur. Recent studies (Singh *et al.* 2021; Yasothai, 2014)^[39, 43] also confirm improved copper status with chelated supplementation.

Serum manganese

Significant ($p < 0.05$) improvement in serum manganese was observed in all treatment groups, with Group III showing the highest levels (0.182 ± 0.002 ppm). Control

group remained unchanged. Manganese is crucial for steroidogenesis and follicular development. Deficiency is associated with silent estrus, delayed ovulation, and reduced conception (Kreplin and Yaremicio, 1992)^[31]. The low baseline levels in this study indicate regional deficiency, likely due to soil and fodder mineral imbalance. Chelated mineral supplementation improved manganese status effectively, supporting earlier reports (Ward *et al.* 1996; Cao *et al.* 2000)^[14, 49].

Serum zinc

Serum zinc levels increased significantly ($p < 0.05$) in all treatment groups, with the highest levels observed in Group III (3.767 ± 0.042 ppm), followed by Group II and Group I. Control group showed no significant change. Zinc is involved in enzyme activity, immune function, and reproductive hormone regulation. It also plays a role in uterine repair and early resumption of ovarian cyclicity (Graham, 1991; Spears and Weiss, 2019)^[23, 40]. The higher levels in chelated group indicate superior bioavailability and retention. These findings are in agreement with recent studies demonstrating improved zinc status and reproductive performance with organic mineral supplementation (Kumar *et al.* 2020; Patel *et al.* 2021)^[32, 37].

Conclusion

The present study was undertaken to evaluate the effect of different mineral supplementation strategies on haemoglobin levels, serum mineral profile, estrus characteristics, and conception rate in postpartum anoestrus graded Murrah buffaloes. The findings clearly demonstrated that mineral supplementation had a significant positive influence on both metabolic and reproductive performance. Haemoglobin levels showed a gradual improvement in all treatment groups, with comparatively better enhancement observed in buffaloes supplemented with chelated minerals. Although changes were not significant within groups over time, the overall higher values in treated animals compared to control on day 30 indicate improved hematological status due to mineral supplementation.

Serum mineral profile analysis revealed that calcium, phosphorus, iron, copper, manganese, and zinc levels increased significantly ($p < 0.05$) in all supplemented groups, whereas no appreciable change was observed in the control group. Among the treatments, the chelated mineral mixture (Group III) consistently exhibited superior improvement in all mineral parameters, indicating higher bioavailability and better utilization of nutrients.

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