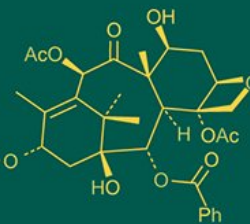
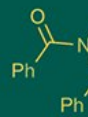


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Effect of different mineral supplementation on reproductive performance in postpartum anoestrus graded Murrah buffaloes

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Abstract

The present study was undertaken to evaluate the effect of different mineral supplementation strategies on estrus induction and reproductive performance in postpartum anestrus graded Murrah buffaloes. A total of 60 buffaloes were randomly divided into four groups ($n = 15$ each): Group I (dicalcium phosphate + trace mineral bolus), Group II (non-chelated mineral mixture), Group III (chelated mineral mixture), and Group IV (control). The supplementation was carried out for 30 days, and animals were monitored for estrus and fertility parameters for 60 days. The estrus response was significantly ($p < 0.05$) higher in Group III (86.67%) followed by Group II (66.67%), Group I (46.67%), and Group IV (33.33%). The mean duration of estrus was also significantly ($p < 0.05$) higher in Group III (32.93 ± 0.81 hours) compared to Group II (26.46 ± 0.98 hours), Group I (22.13 ± 0.83 hours), and Group IV (15.80 ± 0.65 hours). The interval to onset of estrus was significantly ($p < 0.05$) reduced in Group III (25.98 ± 0.98 days) compared to other groups. Although estrus intensity did not differ significantly among groups, higher scores were observed in mineral supplemented animals. The conception rate was highest in Group III (73.33%), followed by Group II (40.00%), Group I (20.00%), and lowest in Group IV (13.33%). The findings indicate that mineral supplementation, particularly in chelated form, significantly improves reproductive performance in postpartum anestrus buffaloes. Chelated mineral mixtures can be effectively utilized under field conditions to enhance estrus expression, fertility, and overall productivity.

Keywords: Buffalo, anestrus, chelated minerals, estrus response, conception rate, reproductive performance, trace minerals, Murrah buffalo

Introduction

The global population of water buffalo (*Bubalus bubalis*) is estimated to exceed 208 million animals, with nearly 97% concentrated in Asia, while comparatively smaller populations are found in Africa and Europe (Food and Agriculture Organization, 2023) [3]. Buffaloes are integral to the livestock-based economy of developing nations, particularly in South Asia, where they contribute substantially to milk production and rural income generation. India possesses the largest buffalo population worldwide, with approximately 109.85 million animals as reported in the 20th Livestock Census (Government of India, 2019) [5], accounting for more than half of the global buffalo population. These animals are predominantly maintained by smallholder and marginal farmers and play a crucial role in livelihood security. Buffalo milk, owing to its higher fat and solids-not-fat (SNF) content compared to cow milk, commands superior market value and consumer preference.

On a global scale, buffaloes contribute over 120 million tonnes of milk annually, forming a significant proportion of total milk output in Asia (FAO, 2023) [3]. The sustainability and profitability of buffalo-based dairy systems are largely dependent on efficient reproductive performance. Disruptions in reproductive cycles often result in extended calving intervals, prolonged dry periods, and reduced lifetime productivity, ultimately causing economic losses (Perera, 2011; Singh *et al.*, 2020) [14, 17]. Conditions such as infertility and anestrus adversely affect milk yield and limit genetic advancement by reducing calf output.

Reproductive efficiency in livestock is influenced by a multifactorial interplay of nutritional, environmental, hormonal, infectious, and genetic factors. Among these, mineral nutrition plays a fundamental role in maintaining physiological equilibrium and reproductive health.

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Minerals are categorized into macro and micro (trace) elements, both of which are essential for normal metabolic and endocrine functions. Imbalances or deficiencies in these minerals can compromise reproductive performance through disruptions in enzymatic activity, hormonal regulation, and cellular metabolism (Kumar *et al.*, 2021) ^[8, 9]. Deficiencies of key minerals such as calcium, phosphorus, copper, zinc, and selenium have been associated with delayed onset of estrus, poor conception rates, and increased incidence of reproductive disorders.

Under field conditions, buffaloes are commonly fed crop residues, dry roughages, and limited green fodder, often without adequate mineral supplementation. Such feeding practices predispose animals to mineral deficiencies, particularly in regions where soils are inherently deficient in essential nutrients (Singh *et al.*, 2019; Das *et al.*, 2020) ^[18, 1, 2]. Continuous nutrient depletion of soils due to intensive agricultural practices further exacerbates region-specific mineral imbalances, thereby directly affecting animal productivity (Underwood and Suttle, 2019) ^[22]. Consequently, suboptimal reproductive efficiency is frequently observed even under otherwise satisfactory management conditions.

Among macro-minerals, calcium and phosphorus are critically important for reproductive health. Calcium is essential for uterine contractility, ovarian activity, and endocrine regulation, while phosphorus plays a vital role in energy metabolism, rumen microbial activity, and reproductive processes. An imbalance in the calcium-to-phosphorus ratio can impair pituitary and ovarian functions, leading to delayed ovulation and infertility (Goff, 2018) ^[4]. Hypocalcaemia has also been linked with delayed uterine involution and higher incidence of conditions such as dystocia, retained placenta, and uterine prolapse (Lean *et al.*, 2017) ^[10]. Phosphorus deficiency remains one of the most economically significant mineral deficiencies affecting reproduction in livestock, particularly in developing countries (McDowell, 2020) ^[11].

Trace minerals including copper, zinc, and selenium are equally essential due to their roles in enzymatic systems, antioxidant defence mechanisms, immune function, and synthesis of reproductive hormones. Deficiencies may arise either from inadequate dietary intake (primary deficiency) or due to antagonistic interactions that impair absorption and utilization (secondary deficiency) (Suttle, 2022) ^[21].

In recent years, organic (chelated) mineral supplementation has gained prominence due to its superior bioavailability compared to conventional inorganic sources. Chelated minerals are formed by binding metal ions with organic ligands such as amino acids or peptides, resulting in stable complexes that enhance absorption and metabolic utilization. Common chelated trace minerals include copper, zinc, manganese, iron, and cobalt. These forms are less susceptible to dietary antagonisms and anti-nutritional factors, thereby improving their biological efficiency (Spears and Weiss, 2019) ^[20]. Several studies have reported improved reproductive responses, including enhanced estrus expression and higher conception rates, in animals supplemented with chelated minerals (Kumar *et al.*, 2020; Yasothai, 2014) ^[6, 7, 23].

Field investigations have demonstrated significant improvement in estrus induction and conception rates in anestrus buffaloes following mineral supplementation (Meena *et al.*, 2018; Patel *et al.*, 2021) ^[12, 13]. Similarly,

supplementation with balanced mineral mixtures has been shown to enhance reproductive performance in postpartum anestrus buffaloes (Reddy *et al.*, 2020) ^[16]. These findings highlight the critical role of mineral nutrition in improving reproductive efficiency under field conditions.

Despite the acknowledged importance of mineral supplementation, there is limited information on serum mineral dynamics during anestrus and estrus phases in buffaloes. Therefore, the present study was designed to evaluate the comparative efficacy of different mineral supplementation strategies, including dicalcium phosphate with trace mineral bolus, conventional inorganic mineral mixture, and chelated mineral mixture, on reproductive performance in postpartum anestrus graded Murrah buffaloes.

Objectives of the Study

- To evaluate estrus parameters in treated animals.
- To assess improvements in reproductive performance following supplementation.

Materials and Methods

Experimental Location

The present investigation was conducted at the Dairy Experimental Station, College of Veterinary Science, Rajendranagar, Hyderabad, along with selected commercial dairy farms located in Ranga Reddy district of Telangana, India. The study area falls under a semi-arid agro-climatic zone characterized by moderate rainfall, high ambient temperatures, and seasonal fluctuations that influence livestock productivity. The experimental animals were maintained under comparable management conditions, including uniform housing, feeding, sanitation, and health care practices. This standardization was ensured to minimize environmental and managerial variations that could influence the experimental outcomes.

Experimental Animals and Management

A total of sixty graded Murrah buffaloes (*Bubalus bubalis*) were selected for the study. All animals were maintained on a balanced feeding regimen consisting of green fodder, dry roughage, and concentrate mixture formulated according to their physiological and production requirements. Clean and potable drinking water was made available *ad libitum*. Standard herd health practices, including routine vaccination and deworming, were followed throughout the experimental period to maintain optimal health status of the animals.

Selection Criteria of Experimental Animals

The animals were selected based on the following criteria:

- Postpartum pluriparous buffaloes
- Minimum of 60 days post-calving
- Exhibiting true anestrus condition
- Body Condition Score (BCS) ranging from 3.0 to 4.0

The reproductive status of each animal was confirmed through two per rectal examinations conducted at an interval of 8 days. Animals showing inactive ovaries, characterized by absence of corpus luteum and dominant follicles, were diagnosed as true anestrus cases. Buffaloes exhibiting reproductive disorders such as endometritis, ovarian cysts, uterine infections, or any systemic illness were excluded from the study to avoid confounding effects on reproductive performance. Prior to the initiation of the experiment, all

selected animals were dewormed using albendazole at a dose rate of 5 mg/kg body weight administered orally. The experimental trial was commenced after 8-10 days of deworming to ensure stabilization of physiological conditions.

Experimental Design and Treatment Protocol

The experiment was designed following a Completely Randomized Design (CRD). The selected sixty buffaloes were randomly allocated into four equal groups comprising fifteen animals each.

Group I: Dicalcium Phosphate with Trace Mineral Bolus

Animals in this group were supplemented with a combination of dicalcium phosphate (DCP) and trace mineral bolus for a period of 30 days. Each animal received 30 g of DCP daily along with Cyclomin-7 bolus administered at the rate of two boli per animal once weekly. The trace mineral bolus contained copper, cobalt, iodine, iron, manganese, selenium, and zinc, whereas DCP provided calcium (26%) and phosphorus (16%).

Group II: Non-chelated Mineral Mixture

Buffaloes in this group were supplemented with 50 g per day of a conventional inorganic (non-chelated) mineral mixture for 30 days. The formulation included macro minerals such as calcium and phosphorus, along with essential trace minerals including copper, cobalt, iron, iodine, zinc, selenium, and manganese. The mixture also contained amino acids such as DL-methionine and L-lysine.

Group III: Chelated Mineral Mixture

Animals in this group received 50 g per day of a chelated mineral mixture for 30 days. In addition to macro and micro minerals similar to the non-chelated formulation, the minerals were provided in chelated form to enhance bioavailability. The mixture was further fortified with fat-soluble vitamins (A, D₃, and E), amino acids, and probiotics including *Lactobacillus* spp. and *Saccharomyces cerevisiae* to improve nutrient utilization and gut health.

Group IV: Control Group

Animals in the control group were maintained on a basal diet without any mineral supplementation and served as untreated controls.

Observation of Estrus and Reproductive Parameters

All animals were monitored for a period of 60 days to evaluate estrus expression and reproductive performance.

Estrus Detection

Estrus detection was carried out based on behavioural and physical signs, including restlessness, bellowing, frequent urination, vulvar swelling, and mucus discharge. A teaser bull was used twice daily to aid in detection. Confirmation of estrus was performed through rectal palpation. Farm personnel were adequately trained to observe estrus signs and animals were monitored at least five times daily, particularly during early morning and late evening hours.

Estrus Response

Estrus response was calculated as the percentage of animals exhibiting estrus out of the total number of animals in each group.

Interval to Onset of Estrus

The interval to onset of estrus was defined as the number of days from initiation of treatment to the first observed estrus.

Estrus Intensity

Estrus intensity was evaluated using a standardized scoring system based on behavioral and physiological signs (Rao and Rao, 1981). Based on the score obtained, estrus was categorized as weak, normal, or intense.

Duration of Estrus

The duration of estrus was recorded in hours from the onset of visible estrus signs to their cessation.

Artificial Insemination

Buffaloes detected in estrus were inseminated using frozen-thawed semen from proven Murrah bulls. Insemination was carried out following the AM-PM rule, with two inseminations performed at an interval of 8 hours to improve conception chances.

Pregnancy Diagnosis

Pregnancy was confirmed by per rectal examination at 50 days post-insemination based on standard clinical criteria.

Conception Rate

Conception rate was calculated as the percentage of animals that conceived out of the total number of animals inseminated in each group.

Statistical Analysis

The data generated during the study were subjected to statistical analysis following standard procedures described by Snedecor and Cochran (1994)^[19]. Results were expressed as mean $\pm$ standard error (SE). Differences between treatment groups were evaluated for statistical significance at a probability level of $p < 0.05$.

Results and Discussion

Estrus Patterns in Postpartum Anestrus Buffaloes

Estrus Response

The effect of different mineral supplementation strategies on estrus induction in postpartum anestrus graded Murrah buffaloes is presented in Table 8. The estrus response was highest in Group III (chelated mineral mixture) with 86.67% (13/15), followed by Group II (66.67%; 10/15), Group I (46.67%; 7/15), and the lowest in the control group (Group IV: 33.33%; 5/15). The differences among the groups were statistically significant ($p < 0.05$). The superior estrus response observed in Group III indicates the enhanced efficacy of chelated minerals, likely due to improved bioavailability and metabolic utilization. These findings are consistent with earlier reports indicating improved estrus induction following mineral supplementation in anestrus buffaloes and cattle (Kumar *et al.*, 2020; Patel *et al.*, 2021)^[6, 7, 13]. Similar improvements in estrus response (60-80%) have been documented in buffaloes supplemented with balanced mineral mixtures and trace mineral boluses (Meena *et al.*, 2018; Das *et al.*, 2020)^[12, 1, 2].

Duration of Estrus

The mean duration of estrus was significantly different ($p < 0.05$) among the treatment groups (Table 9). The duration recorded was 22.13 ± 0.83 hours in Group I, 26.46 ± 0.98

hours in Group II, 32.93 ± 0.81 hours in Group III, and 15.80 ± 0.65 hours in Group IV. The longest duration of estrus was observed in Group III, which was significantly higher than all other groups, whereas the control group exhibited the shortest duration. The prolonged estrus duration in mineral-supplemented animals may be attributed to improved follicular growth, steroidogenesis, and endocrine balance. Comparable findings have been reported in earlier and recent studies, where estrus duration in buffaloes ranged between 18 to 30 hours depending on nutritional and physiological status (Perera, 2011; Singh *et al.*, 2020) [14, 17]. Enhanced estrus duration following mineral supplementation has also been linked to improved levels of trace minerals such as zinc and copper, which are essential for hormone synthesis and reproductive function (Suttle, 2022) [21].

Estrus Intensity

The mean estrus intensity scores were 10.57 ± 0.69 , 12.30 ± 0.76 , 13.15 ± 0.72 , and 11.40 ± 1.36 in Groups I, II, III, and

IV, respectively (Table 9). Although Group III recorded the highest intensity score followed by Groups II, IV, and I, the differences among the groups were statistically non-significant ($p > 0.05$). Based on the estrus scoring system, animals in all treatment groups exhibited normal estrus intensity (score range: 10-15), whereas the control group tended toward weaker estrus expression. The improved estrus intensity in supplemented groups could be attributed to better mineral status, which enhances behavioural and physiological manifestations of estrus. Trace minerals such as copper, zinc, and manganese play a critical role in enzymatic activity, steroid hormone synthesis, and neuromuscular coordination, thereby influencing estrus behaviour (Spears and Weiss, 2019; Kumar *et al.*, 2021) [20, 8, 9]. Nutritional deficiencies are known to contribute to silent estrus, a common problem in buffaloes under field conditions (Das *et al.*, 2020) [1, 2]. The present findings suggest that mineral supplementation, particularly in chelated form, may help mitigate silent heat conditions.

Table 1: Details of the Estrus response, Conception rate in mineral supplemented post-partum anoestrus graded Murrah buffaloes.

S. No.	Name of the group	No. of animals treated	No. of Animals Exhibited Estrus	No. of Animals found Positive	Percent of Estrus response	Conception rate on treated animals (%)	Conception rate on Estrus exhibited animals (%)
1	Group I	15	7	3	(7/15) 46.66%	(3/15) 20%	(3/7) 42.85%
2	Group II	15	10	6	(10/15) 66.66%	(6/15) 40%	(6/10) 60%
3	Group III	15	13	11	(13/15) 86.66%	(11/15) 73.33%	(11/13) 84.61%
4	Group IV	15	5	2	(5/15) 33.33%	(2/15) 13.33%	(2/5) 40%

*Figures indicating in the parenthesis were number of animals exhibited estrus, conceived in respective groups.

Table 2: Details of Estrus patterns in mineral supplemented post-partum anoestrus graded Murrah buffaloes.

S. No	Name of the group	No. of animals in the group	No. of animals exhibited Estrus	No. Days to estrus (Mean $\pm$ S.E., Days)	Duration of Estrus (Mean $\pm$ S.E., Hrs)	Intensity of Estrus (Mean $\pm$ S.E., Points)
1	Group I	15	7	$46.67^b \pm 0.95$	$22.13^b \pm 0.83$	$10.57^b \pm 0.69$
2	Group II	15	10	$35.20^c \pm 1.06$	$26.46^c \pm 0.98$	$12.30^c \pm 0.76$
3	Group III	15	13	$25.93^d \pm 0.98$	$32.93^d \pm 0.81$	$13.15^d \pm 0.72$
4	Group IV	15	5	$70.13^a \pm 1.79$	$15.80^a \pm 0.65$	$9.40^a \pm 1.36$

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

Table 3: The Details of estrus pattern in mineral supplemented post-partum anoestrus graded Murrah buffaloes.

S No	Name of the group	Significance of Estrus			Significance of Conception rate (based on treated animals)			Significance of Conception rate (based on estrus animals)		
		No. of animals treated	No. of animals exhibited estrus	Chi square test	No. of animals treated	No. of Animals found positive	Chi square test	No. of animals came to heat	No. of animals found positive	Chi square test
1	Group I	15	7	10.08 ** Chi Square Value	15	3	14.06 ** Chi Square Value	7	3	4.98 ** Chi Square Value
2	Group II	15	10		15	6		10	6	
3	Group III	15	13		15	11		13	11	
4	Group IV	15	5		15	2		5	2	

** Among the groups significant ($p < 0.01$) level.

Interval from Treatment to Onset of Estrus

The interval between initiation of treatment and onset of estrus differed significantly ($p < 0.05$) among the groups (Table 9). The mean intervals recorded were 46.67 ± 0.95 days in Group I, 35.20 ± 1.06 days in Group II, 25.98 ± 0.98 days in Group III, and 70.13 ± 1.79 days in Group IV. The corresponding ranges were 44-49, 33-37, 24-28, and 66-74 days, respectively. Group III exhibited the shortest interval to estrus onset, followed by Groups II and I, whereas the control group showed the longest interval. The reduction in interval suggests early resumption of ovarian cyclicity in

supplemented animals. These findings are in agreement with previous studies reporting reduced postpartum anestrus duration following mineral supplementation (Kumar *et al.*, 2020; Reddy *et al.*, 2020) [6, 7, 16]. Organic trace minerals have been shown to improve follicular dynamics and hasten ovarian activity compared to inorganic sources and control groups (Spears and Weiss, 2019) [20].

Conception Rate

The conception rate was significantly influenced ($p < 0.05$) by mineral supplementation (Table 8). The conception rates

were 20.00% (3/15), 40.00% (6/15), 73.33% (11/15), and 13.33% (2/15) in Groups I, II, III, and IV, respectively. Group III recorded the highest conception rate, followed by Groups II and I, while the control group exhibited the lowest conception rate. The enhanced conception rate in the chelated mineral group may be attributed to improved oocyte quality, fertilization rate, and uterine environment. Similar improvements in conception rates following mineral supplementation have been reported in buffaloes and cattle, with values ranging from 60 to 75% in supplemented groups compared to lower rates in controls (Patel *et al.*, 2021; Meena *et al.*, 2018) ^[13, 12]. Organic trace minerals, in particular, have been shown to improve reproductive efficiency by enhancing antioxidant status, immune function, and embryo survival (Spears and Weiss, 2019; Suttle, 2022) ^[20, 21].

Overall Interpretation

The present study clearly demonstrates that mineral supplementation significantly improves estrus expression, reduces the interval to onset of estrus, and enhances conception rates in postpartum anestrus buffaloes. Among the different supplementation strategies, chelated mineral mixture proved to be the most effective, highlighting the importance of bioavailable mineral sources in improving reproductive performance under field conditions.

Conclusion

The present study clearly demonstrates that mineral supplementation plays a crucial role in improving reproductive performance in postpartum anestrus graded Murrah buffaloes. Among the different supplementation strategies evaluated, chelated mineral mixture proved to be the most effective in enhancing estrus expression, reducing the interval to onset of estrus, and improving conception rate.

Buffaloes supplemented with chelated minerals exhibited the highest estrus response (86.67%), longer duration of estrus, reduced interval to estrus onset, and superior conception rate (73.33%) compared to non-chelated mineral mixture, dicalcium phosphate with trace mineral bolus, and control groups. Although estrus intensity did not differ significantly among groups, a trend towards improved expression was observed in supplemented animals.

The improved reproductive performance observed in mineral-supplemented groups may be attributed to better bioavailability of essential macro-and micro-minerals, leading to enhanced ovarian activity, hormonal balance, and metabolic efficiency. In particular, chelated minerals, due to their higher absorption and reduced antagonistic interactions, were more effective than conventional inorganic sources.

The findings of the present study emphasize the importance of strategic mineral supplementation under field conditions, especially in regions where feeding practices are predominantly based on crop residues and mineral-deficient soils. Supplementation with chelated mineral mixtures can be recommended as a practical and efficient approach to overcome anestrus, improve fertility, and enhance overall productivity in buffaloes.

Further studies involving larger sample sizes and economic analysis are warranted to validate these findings and to establish cost-effective mineral supplementation protocols for field application.

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