Research Article

Supplementation Effect of Concentrate Containing Mineral Mixture on Blood Biochemical and Pregnancy Rate of Limousin Crossbred Cows: A Field Study

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Abstract | An on-farm study was conducted to evaluate the effects of concentrate supplementation containing a mineral mixture on blood biochemical parameters and pregnancy rate of Limousin crossbred cows. A total of 30 Limousin crossbred cows with a mean body weight of 336.63±38.24 kg were randomly divided into two groups (n=15/group). Cows in the control group (CON) were maintained under traditional farmer feeding practices. In the supplemented group (SUP), cows received an additional 1 kg/day of concentrate (HQFS®) containing 0.5% mineral mixture (Agromix Booster®). Blood samples were collected on days 0 and 21 of experimental feeding. Estrous synchronization was performed using 5 ml prostaglandin F2α administered intramuscularly. Artificial insemination (AI) using post-thawed frozen sexed-Y Limousin bull semen was conducted at 2 and 8 h after estrous detection. Pregnancy diagnosis was performed by transrectal palpation 60 days after AI. The results showed that dry matter, organic matter, crude protein, nitrogen-free extract, and total digestible nutrient intake did not differ between groups ($P>0.05$). However, cows in the SUP group had lower crude fiber intake and greater ether extract intake than cows in the CON group ($P<0.05$). Serum high-density lipoprotein (HDL) and phosphorus concentrations on day 21 were significantly greater in the SUP group than in the CON group ($P<0.05$). No significant differences were observed in first-service conception rate or pregnancy rate between treatments under the conditions of this study. The pregnancy rate in both groups was 26.67%. This study concluded that concentrate supplementation containing a mineral mixture increased serum HDL and phosphorus concentrations without adverse effects on other blood biochemical parameters. However, no significant improvement in pregnancy rate was observed under the present experimental conditions.

Keywords | Blood biochemical parameters, Concentrate supplementation, Limousin crossbred cows, Mineral mixture, Pregnancy rate, Smallholder farming systems

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INTRODUCTION

The increasing human population in Indonesia is accompanied by a growing demand for animal-source foods, including beef. Although per capita beef consumption in Indonesia remains relatively low compared with many other countries, domestic beef production is still insufficient to meet national demand (Kusumaningrum *et al.*, 2025). Supply shortages have been estimated to be approximately 40% of total national demand and may reach up to 75% in major urban centers (Chang *et al.*, 2020). Under smallholder farming systems in Indonesia, crop residues and agricultural by-products are commonly used as basal diet components for cattle. However, these feed resources are generally characterized by low nutritional quality, particularly low digestible energy, crude, and mineral contents (Agus and Widi, 2018; Khalil *et al.*, 2019; Woittiez *et al.*, 2018). For example, rice straw is characterized by high fiber and low protein concentrations. The neutral detergent fiber, acid detergent fiber, and hemicellulose contents range from 66.18%–77.22%, 36.33%–49.77%, and 21.67%–33.84%, respectively, whereas crude protein (CP) content is relatively low, ranging from 3.34% to 6.78%. Nutrients play an essential role in supporting reproductive performance, particularly in cattle traditionally reared under smallholder farming systems, whereas inadequate nutrient supply remains one of the major constraints (Kuntariningsih *et al.*, 2025). Reproductive failure is commonly associated with insufficient animal nutrition (Oliveira-Filho *et al.*, 2010). In addition, feed quality also fluctuates with the season, so additional nutrient supplementation may be necessary to meet the nutritional requirements of cows (Tshuma *et al.*, 2019).

One strategy to improve reproductive performance is supplementation with good-quality feed. Providing concentrate supplementation to improve dietary protein concentration is expected to ensure nutrient sufficiency to support optimal animal growth and puberty (Gading *et al.*, 2020). Sonjaya *et al.* (2020) reported that supplementation with concentrate containing 14% CP increased pregnancy rate. Increasing dietary protein concentration from 5.5% to 13% CP also stimulated estrous cycles and estrous expression in cattle (Maquivar *et al.*, 2006). Rodrigues *et al.* (2021) showed that supplementing pregnant Nellore cows with protein during the dry season improved body condition and tended to increase pregnancy rates during the subsequent breeding season. Smuts *et al.* (2019) noted that balanced nutrition is essential for maintaining cows' body condition and directly influences fertility. Nutrient deficiency may impair ovarian activity, resulting in lower conception rates. As micronutrients, minerals are also essential in regulating reproductive performance in cattle (Talukdar *et al.*, 2016; Morsy *et al.*, 2020). Mineral supplementation has been widely reported to improve reproductive performance in some studies (Chester-Jones *et al.*, 2013; Molefe and Mwanza, 2020). Supplementation with mineral mixtures improved pregnancy rate in animals (Anam *et al.*, 2021, 2025; Ray *et al.*, 2012). Trace minerals have specific physiological functions related to reproduction. Zinc is involved in ovarian follicle development (Yatoo *et al.*, 2013), copper contributes to superoxide dismutase enzyme activity associated with progesterone production (Sales *et al.*, 2011), iron plays a role in embryo development (Gao *et al.*, 2007), and selenium is associated with postpartum uterine involution (Arthington and Ranches, 2021).

Several studies have evaluated reproductive performance in cattle supplemented with concentrate diets and single or combined mineral supplementation. However, there is limited research regarding the combination of mineral mixtures with concentrate supplementation, particularly concerning pregnancy rate in Limousin crossbred cows. Accordingly, this study aimed to evaluate the effects of concentrate supplementation containing mineral mixture on blood biochemical parameters and pregnancy rate of Limousin crossbred cows raised under smallholder farming systems.

MATERIALS AND METHODS

Animals and experimental design

An on-farm trial was conducted in Kecopokan and Ngrancah villages, Sumber Pucung Sub-district, Malang Regency, East Java Province, Indonesia (-8.18601 latitude and 112.49985 longitude). The Animal Ethics Committee of the Faculty of Veterinary Medicine, Brawijaya University, Indonesia, approved the research protocol with grant number 128-KEP-UB-2021. Based on the survey, thirty crossbred Limousin × Ongole cows with a mean body weight of 336.63±38.24 kg and a body condition score of 3.85±0.8 were used in this study. Before beginning the study, all experimental animals were examined for reproductive organ normality via transrectal palpation and then randomly allocated into two equal groups (n=15/group). Animals in the control group (CON) were maintained under traditional farmer feeding practices (rice straw and native forage as the basal diet) without nutritional supplementation. In the supplemented group (SUP), cows received 1 kg/day of concentrate along with the control feed. The concentrate was High-Quality Feed Supplement® (PT. Agromix Lestari, Yogyakarta, Indonesia) containing 0.5% Agromix Booster® mineral mixture. The Agromix Booster® consisted of mixed minerals: Ca 243.4 g/kg, Na 24.3 g/kg, Fe 12.5 g/kg, P 3.2 g/kg, Mg 1.8 g/kg, Mn 1.2 g/kg, Zn 439.0 mg/kg, K 277.9 mg/kg, Cu 179.4 mg/kg, S 130.4 mg/kg, Co 5.4 mg/kg, and Se 131 µg/kg. The mineral mixture was included at a relatively low level (approximately 5 g/day/cow), thereby contributing minimally to the overall dietary Ca:P ratio. Concentrate supplementation was provided individually to each cow during feeding to minimize competition and ensure complete consumption. Feed refusals were recorded daily, and no substantial concentrate refusal was observed during the experimental period.

Estrous synchronization, artificial insemination, and pregnancy diagnosis

Estrous synchronization was conducted 21 days after supplemental feeding treatment. Before prostaglandin administration, corpus luteum status was evaluated by transrectal palpation performed by experienced veterinarians to ensure that cows were responsive to the synchronization protocol. Subsequently, cows were injected intramuscularly with 5 ml of prostaglandin F2α (Lutalyse™, dinoprost tromethamine, Zoetis Inc.) to synchronize the estrous cycle. Estrous signs were observed approximately two days after estrous synchronization (Susilowati *et al*., 2021). The semen used to inseminate estrous animals was sexed-Y Limousin bull semen produced by the Singosari National Artificial Insemination Center, Malang, Indonesia. The motility, viability, and abnormality of the semen were 41.0%, 71.0%, and 6.8%, respectively. Sexed semen generally exhibits lower post-thaw motility than conventional frozen semen because the sorting and cryopreservation processes may reduce sperm quality. Artificial insemination (AI) was carried out at 2 and 8 hours after estrous detection (Wiranto *et al*., 2020).

Table 1: Chemical composition of concentrate supplemented to the Limousin crossbred cows.

Item	Value
Dry matter (%)	87.33
% dry matter	
Organic matter	92.87
Crude protein	14.71
Crude fiber	9.04
Ether extract	1.89
Nitrogen free extract	67.23
Total digestible nutrients	60.12

AI in both treatment groups was performed by the same experienced technician using the same batch of sexed-Y Limousin semen to minimize procedural variation among animals. Cows returning to estrus on days 19, 20, and 21 were inseminated for the second AI, and the non-return estrous rate on this cycle (NRR-1) was calculated. Similarly, cows returning to estrus on days 38, 39, and 40 were inseminated for the third AI, and the non-return estrous on this cycle (NRR-2) was calculated. Transrectal palpation at 60 days post-AI was used to confirm pregnancies. The first-service conception rate was calculated using the following formula: (total pregnant cows in the first service/total inseminated cows) × 100%. The pregnancy rate was calculated using the following formula: (total pregnant cows in all services/total inseminated cows) × 100%.

Measurements and laboratory analysis

The diet was provided to the cows twice daily, and diet samples were collected weekly and composited for nutrient analysis. Table 1 shows the chemical composition, including dry matter (DM), organic matter (OM), CP, crude fiber (CF), and ether extract (EE), which was determined according to AOAC (2023). Total digestible nutrients (TDN) was calculated following Jayanegara *et al*. (2019). Feed consumption was measured daily based on the difference between feed offered and feed refused. Nutrient intake was subsequently calculated.

On days 0 and 21 of dietary treatment, approximately 10 ml of blood samples were collected into sterile BD vacutainer® tubes. On day 21, blood samples were collected before prostaglandin F2α administration to avoid potential acute effects of hormonal treatment on blood biochemical parameters. Samples were centrifuged at 2000 rpm for 20 min at 4°C, and serum was separated for further analysis. Serum metabolite concentrations, including glucose, total protein, albumin, cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), creatinine, uric acid, blood urea nitrogen (BUN), calcium, and phosphorus, were analyzed using commercial kits (DiaSys Diagnostic System, Holzheim, Germany).

Statistical analysis

Blood biochemical and nutrient intake data were analyzed using an independent sample T-test. The reproductive parameters, including NRR-1, NRR-2, first-service conception rate, and pregnancy rate, were analyzed using a 2 × 2 Fisher's exact test. No formal adjustment for multiple comparisons was applied; therefore, significant differences in blood biochemical parameters should be interpreted cautiously as exploratory findings. All statistical analyses were performed using IBM SPSS® Statistics version 20.

RESULTS AND DISCUSSION

There were no significant differences between the two dietary treatments in DM, OM, CP, NFE, and TDN intake ($P>0.05$). However, CF intake was lower, and EE intake was greater in the SUP group than in the CON group ($P<0.05$) (Table 2). Although cows in the SUP group received an additional 1 kg/day of concentrate containing a mineral mixture, the supplementation did not markedly alter overall nutrient intake compared with the CON group. The similarity in total DM intake suggests that concentrate supplementation partially substituted for forage consumption rather than additively increasing total intake, a substitution effect commonly observed when concentrates are introduced into forage-based diets for ruminants (Heublein *et al*., 2017; Quang *et al*., 2015). Individual feed intake measurements indicated that cows in the SUP group consumed lower CF and greater EE than cows in the CON group, a response consistent with the lower CF and greater EE contents of the concentrate diet relative to the basal forage. A similar response pattern was reported by Quang *et al*. (2015), who demonstrated

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that increasing concentrate supplementation in cattle diets reduced fiber intake while increasing ether extract intake. Although CF and EE differed significantly between treatments, overall DM, CP, and TDN intakes remained statistically comparable, indicating that energy and protein supply were broadly equivalent between groups.

Table 2: Nutrient intake of cows as affected by two dietary treatments (mean ± standard deviation).

Item	Treatment		P value
	CON	SUP	
Nutrient intake (kg/day)			
Dry matter	7.39 ± 0.91	8.87 ± 1.15	0.323
Organic matter	5.84 ± 0.69	7.04 ± 0.88	0.291
Crude protein	0.56 ± 0.07	0.72 ± 0.08	0.126
Crude fiber	2.07 ± 0.23[a]	1.38 ± 0.22[b]	0.039
Ether extract	0.12 ± 0.02[b]	1.11 ± 0.28[a]	0.002
Nitrogen free extract	3.09 ± 0.38	3.83 ± 0.47	0.237
Total digestible nutrient	3.39 ± 0.42	4.14 ± 0.50	0.264
Intake as percentage of body weight (% BW)			
Dry matter	2.33 ± 0.30	2.54 ± 0.30	0.634
Crude protein	0.18 ± 0.02	0.21 ± 0.02	0.314
Total digestible nutrient	1.07 ± 0.14	1.19 ± 0.13	0.551

[ab]Superscript letters within the same row indicate statistically significant differences at P<0.05. CON: cows maintained under traditional farmer feeding practices without supplementation; SUP: cows received an additional 1 kg/day of concentrate containing 0.5% mineral mixture.

The reduction in CF intake observed in the SUP group was likely attributable to partial substitution of forage by concentrate rather than severe dietary fiber restriction. Nevertheless, dietary fiber composition can influence rumen fermentation characteristics, nutrient digestibility, and indirectly modulate metabolic processes associated with reproductive physiology (Abramowicz *et al*., 2026). Heublein *et al*. (2017) reported that concentrate supplementation did not markedly alter total DM intake in grazing dairy cows; however, the supplementation level in that study was substantially greater than that used in the present study, and direct quantitative comparisons between studies should therefore be interpreted cautiously. Total nutrient intake in the present study was generally close to the nutrient requirement recommendations for reproductive beef cattle suggested by the NRC (2016), indicating that cows in both treatment groups likely received an adequate nutrient supply for maintenance and reproductive function. Nevertheless, because CF and EE intakes differed significantly between treatments, the possibility that dietary composition influenced rumen fermentation patterns and downstream metabolic responses cannot be fully excluded.

Serum glucose, albumin, protein, triglyceride, cholesterol, LDL, HDL, uric acid, creatinine, BUN, calcium, and phosphorus concentrations on day 0 did not differ between treatments (P>0.05). These parameters also remained similar on day 21, except for serum HDL and phosphorus concentrations, which were greater in the SUP group than in the CON group (P<0.05) (Table 3). Serum glucose concentrations remained within the normal physiological range commonly reported for cattle, indicating a relatively similar energy status between treatments. In ruminants, blood glucose is supplied primarily through hepatic gluconeogenesis from ruminal propionate (Abramowicz *et al*., 2026; Satoh *et al*., 2026); therefore, dietary changes that alter ruminal fermentation patterns can influence circulating glucose concentrations. Tahuk *et al*. (2018) reported that dietary protein levels did not affect pre-feeding serum glucose concentration in Bali cattle, although postprandial glucose concentration increased after feeding. Ray *et al*. (2012) demonstrated that concentrate and mineral supplementation increased blood glucose concentration in grazing goats, possibly through alterations in ruminal volatile fatty acid profiles and propionate availability. However, several studies have also reported inconsistent glucose responses following concentrate or mineral supplementation (Heublein *et al*., 2017; Warken *et al*., 2018). In the present study, the absence of significant differences in serum glucose may indicate that the supplementation level was insufficient to markedly alter energy metabolism under the existing feeding conditions or that the sampling schedule did not coincide with the peak postprandial glucose response.

Serum total protein, albumin, and BUN concentrations were not significantly affected by dietary treatment. Similar observations were reported by Ray *et al*. (2012) and Molefe and Mwanza (2020), who found no significant differences in serum protein metabolites following concentrate or mineral supplementation in small ruminants and cattle. In beef cows, BUN concentration is known to be sensitive to dietary nitrogen supply and the balance between rumen-degradable protein and fermentable energy (Tshuma *et al*., 2019); however, when dietary CP intake is comparable between groups, BUN concentration would not be expected to differ markedly, consistent with the present results. Serum total protein concentrations on day 21 were numerically lower than those observed on day 0 in both treatment groups. Because this reduction occurred similarly in both groups and no clinical signs of disease were observed during the experimental period, the change was unlikely to be directly associated with dietary treatment. Environmental conditions, physiological variation, hydration status, or field-related sampling factors may have contributed to temporal variation in serum protein concentrations.

Table 3: Blood biochemical parameters of cows on days 0 and 21 under two dietary treatments (mean ± standard deviation).

Item	Treatment		P value
	CON	SUP	
Glucose (mg/dl)			
Day 0	47.47 ± 2.88	46.18 ± 4.41	0.812
Day 21	57.92 ± 2.29	57.18 ± 2.31	0.826
Albumin (g/dl)			
Day 0	2.72 ± 0.26	2.83 ± 0.06	0.693
Day 21	2.28 ± 0.23	2.80 ± 0.12	0.074
Protein (g/dl)			
Day 0	7.52 ± 0.23	7.48 ± 0.43	0.923
Day 21	4.91 ± 0.50	5.43 ± 0.44	0.452
Triglyceride (mg/dl)			
Day 0	17.75 ± 3.46	13.00 ± 2.64	0.300
Day 21	8.55 ± 1.37	15.90 ± 5.48	0.223
Cholesterol (mg/dl)			
Day 0	100.93 ± 7.86	108.15 ± 12.15	0.629
Day 21	96.78 ± 7.45	119.82 ± 9.28	0.082
Low-density lipoprotein (mg/dl)			
Day 0	22.03 ± 2.18	24.87 ± 2.18	0.379
Day 21	20.50 ± 2.06	26.17 ± 3.79	0.218
High-density lipoprotein (mg/dl)			
Day 0	50.73 ± 3.12	54.83 ± 5.53	0.533
Day 21	46.25 ± 3.20^{b}	57.10 ± 3.13^{a}	0.036
Uric acid (mg/dl)			
Day 0	1.03 ± 0.10	1.61 ± 0.54	0.317
Day 21	1.71 ± 0.34	1.42 ± 0.07	0.417
Creatinine (mg/dl)			
Day 0	1.28 ± 0.08	1.72 ± 0.29	0.172
Day 21	1.31 ± 0.18	1.27 ± 0.13	0.860
Blood urea nitrogen (mg/dl)			
Day 0	9.30 ± 2.03	8.74 ± 1.15	0.815
Day 21	7.42 ± 1.65	7.47 ± 0.49	0.979
Calcium (mg/dl)			
Day 0	10.69 ± 0.66	10.89 ± 0.48	0.816
Day 21	11.79 ± 1.06	11.56 ± 0.93	0.874
Phosphorus (mg/dl)			
Day 0	1.95 ± 0.16	2.03 ± 0.23	0.783
Day 21	1.66 ± 0.27^{b}	2.50 ± 0.20^{a}	0.030

^{ab}Superscript letters within the same row indicate statistically significant differences at $P<0.05$. CON: cows maintained under traditional farmer feeding practices without supplementation; SUP: cows received an additional 1 kg/day of concentrate containing 0.5% mineral mixture.

Concentrate supplementation did not significantly affect serum cholesterol, triglyceride, or LDL concentrations. However, serum HDL concentration on day 21 was greater in the SUP group under unadjusted statistical analysis. In ruminants, HDL is the predominant circulating lipoprotein fraction, and the majority of plasma cholesterol is carried in HDL particles, whose composition and abundance depend strongly on hepatic apolipoprotein A-I production (Duran *et al.*, 2021). Cholesterol serves as a precursor for steroid hormone biosynthesis; consequently, alterations in HDL concentration may reflect changes in lipid transport, lipid metabolism, and nutrient utilization associated with concentrate supplementation rather than cardiovascular health (Xiao *et al.*, 2024). Duran *et al.* (2021) further reported that HDL profiles in dairy cattle are linked to reproductive outcomes and milk yield, suggesting a physiologically meaningful, although still mechanistically incomplete, role for HDL in ruminant productive performance. Because multiple blood biochemical parameters were evaluated without formal correction for multiple comparisons, the observed differences in HDL concentration should be interpreted cautiously as exploratory findings rather than definitive physiological responses and require confirmation in future studies.

The supplemented concentrate containing a mineral mixture significantly increased serum phosphorus concentration in the SUP group compared with the CON group, whereas serum calcium concentration remained unchanged. The relatively low serum phosphorus concentrations observed in both groups may have been associated with the low phosphorus content of the basal diet, which consisted mainly of rice straw and native forage commonly used under smallholder farming conditions and is known to be marginal in available phosphorus for ruminants (Shah *et al.*, 2025; Pinit *et al.*, 2023; Agus and Widi, 2018; Anam *et al.*, 2020). Although serum phosphorus concentration was significantly greater in the SUP group than in the CON group, the magnitude of the response was relatively modest, and serum phosphorus concentrations in both groups remained below the normal physiological range commonly reported for adult cattle. Therefore, the observed increase should be interpreted as a partial improvement in phosphorus status rather than complete correction of phosphorus deficiency. Phosphorus flow from the digestive tract into the blood pool increases with phosphorus intake when intake is below requirement, with apparent absorption typically ranging between 60% and 70% in cattle, and large fractions of intake undergoing endogenous recycling through saliva (Abramowicz *et al.*, 2026). Similar increases in serum phosphorus concentration following mineral supplementation have been reported in Brahman cross cows and grazing goats (Anam *et al.*, 2021; Ray *et al.*, 2012). Phosphorus has an established role in reproductive physiology, and sustained phosphorus deficiency has been associated with altered estrous expression, lower conception rates, and embryonic mortality (Ali *et al*., 2014; Zhou *et al*., 2021). In grazing yak heifers, 30 days of premating supplementation with monocalcium phosphate, but not calcium chloride, increased serum phosphorus concentration, body weight gain, and subsequent conception and calving rates, indicating that improving phosphorus status before breeding can enhance reproductive efficiency when basal phosphorus status is deficient (Zhou *et al*., 2021). However, dietary phosphorus in excess of requirement does not improve reproductive performance and may increase environmental phosphorus excretion (Cerosaletti *et al*., 2004; Tallam *et al*., 2005). As with HDL, the observed increase in serum phosphorus concentration should be interpreted cautiously because no formal correction for multiple comparisons was applied. Therefore, this finding should be regarded as exploratory and requires confirmation in future studies with larger sample sizes and predefined primary outcomes.

No significant differences were observed in NRR-1, NRR-2, first-service conception rate, or pregnancy rate between treatments (Table 4). Although NRR-1 and NRR-2 were numerically greater in the SUP group than in the CON group, these differences were not statistically significant, and the final pregnancy rate was identical between groups (26.67%). The absence of detectable reproductive responses may have been associated with several factors. First, the supplementation period before AI was relatively short (21 days), which may have substantially limited the opportunity for nutritional improvement to influence reproductive physiology and conception outcomes. Nutritional interventions designed to improve reproductive performance generally require sufficient time to enhance body condition, replenish mineral reserves, support follicular development, and modulate endocrine function before breeding. This consideration may be particularly important under smallholder farming conditions, where cattle are commonly fed low-quality basal diets based on rice straw and native forage. Consequently, the 21-day supplementation period used in the present study may have been insufficient to generate detectable improvements in reproductive performance. Zhou *et al*. (2021) found that 30 days of premating supplementation were sufficient to produce measurable improvements in serum phosphorus concentration, body weight gain, and conception rate in grazing yak heifers, suggesting that supplementation duration is an important determinant of detectable reproductive responses. Second, although concentrate supplementation numerically improved nutrient intake, the supplementation level may still have been insufficient to markedly improve the overall nutritional status of cows maintained under smallholder farming systems. Third, the basal diet consisted mainly of rice straw and native forage, which are generally characterized by low nutritional quality and marginal mineral content (Shah *et al*., 2025; Pinit *et al*., 2023; Agus and Widi, 2018). Another factor that may have contributed to the absence of significant reproductive responses was the relatively low level of mineral supplementation provided in the present study. Although the concentrate contained a mineral mixture, the actual amount supplied to each cow was limited because the mineral mixture represented only 0.5% of the concentrate and concentrate supplementation was provided at 1 kg/day. Consequently, the intake of several trace minerals may have been insufficient to substantially improve mineral status beyond basal dietary levels. Under such conditions, improvements in reproductive performance may be difficult to detect, particularly when baseline nutritional limitations, semen quality, and other field-related factors simultaneously influence conception outcomes.

Table 4: Reproductive performance of Limousin crossbred cows following artificial insemination.

Item	Number (%) of cows in each treatment		P value[1]
	CON	SUP	
Inseminated on day 0	15 (100.00)	15 (100.00)	-
Non-return rate (NRR-1)	12 (80.00)	14 (93.33)	0.598
Non-return rate (NRR-2)	11 (73.33)	13 (86.67)	0.651
First-service conception rate	4 (26.67)	3 (20.00)	1.000
Pregnancy rate	4 (26.67)	4 (26.67)	1.000

[1]P-value for comparison between treatments using a 2 × 2 Fisher's exact test. CON: cows maintained under traditional farmer feeding practices without supplementation; SUP: cows received an additional 1 kg/day of concentrate containing 0.5% mineral mixture.

In addition, the use of sexed-Y semen with relatively low post-thaw motility may have contributed substantially to the low pregnancy rates observed in both treatment groups. The post-thaw motility of the semen used in the present study was only 41%, which is lower than values generally considered optimal for artificial insemination programs. Consequently, semen quality may have represented a major limiting factor affecting conception success irrespective of dietary treatment. Under such conditions, overall fertility may have been constrained by the reduced fertilizing capacity of the semen, thereby contributing to the low pregnancy rates observed in both treatment groups. Sex-sorted sperm generally exhibit lower fertility than conventional semen because the sorting and cryopreservation processes can compromise sperm membrane integrity, motility, and viability (Magopa *et al*., 2023; Sharma *et al*., 2024). In field comparisons of timed artificial insemination, conception rates with X-sexed semen have been reported to be similar to or lower than those achieved with unsexed semen, depending on breed and parity, with greater pregnancy and embryo losses observed in beef cows than in dairy cows (Magopa *et al*., 2023). Recent work has also indicated that delayed insemination of sexed semen relative to estrus onset can substantially improve pregnancy per AI in beef heifers (Kasimanickam *et al*., 2025), implying that AI timing relative to estrus onset is an important moderating factor that may not have been optimally captured by the routine AI protocol used in this study. Despite the standardization of semen source and insemination technician across cows, reproductive outcomes under field conditions remain influenced by semen quality, estrous detection accuracy, environmental stress, and individual animal variation. Artificial insemination in both treatment groups was performed by the same experienced technician using the same batch of sexed-Y Limousin semen to minimize procedural variation among animals.

Although NRR-1 and NRR-2 were numerically greater in the SUP group than in the CON group, these differences were not statistically significant. The relatively small sample size (n= 15 cows per treatment) and the low number of pregnancies recorded in the present study may have limited the statistical power of Fisher's exact test to detect modest but biologically meaningful differences between treatments; consequently, the absence of significant differences in conception and pregnancy rates should be interpreted cautiously. Previous studies have reported positive effects of concentrate or mineral supplementation on reproductive performance in beef and dairy cattle, buffaloes, and yak heifers (Joshi *et al*., 2019; Sonjaya *et al*., 2020; Zhou *et al*., 2021); however, differences in supplementation level, supplementation duration, basal diet quality, management system, and semen quality may explain the inconsistent reproductive responses observed across studies. Reproductive responses to nutritional intervention are generally more pronounced when baseline nutritional or mineral status is severely deficient and the intervention period is sufficiently long to allow improvements in body condition and follicular dynamics before breeding (Abramowicz *et al*., 2026; Zhou *et al*., 2021).

NRR was included in the present study because it is commonly used as an indirect field indicator of estrous return following artificial insemination. However, NRR can systematically overestimate the true pregnancy rate because not all open cows are accurately detected as returning to estrus, and pregnancy diagnosis by transrectal palpation or ultrasonography remains a more accurate method for confirming conception success (Magopa *et al*., 2023). Therefore, the NRR findings in the present study should be interpreted cautiously as supportive reproductive observations rather than definitive indicators of pregnancy outcome. Further studies using larger sample sizes, longer supplementation periods, optimized AI timing relative to estrus onset, and improved overall nutritional management are needed to clarify the potential reproductive benefits of concentrate supplementation containing a mineral mixture in beef cattle raised under smallholder farming systems.

CONCLUSION

The present study demonstrated that concentrate supplementation containing a mineral mixture improved serum HDL and phosphorus concentrations in Limousin crossbred cows raised under smallholder farming systems without adverse effects on other blood biochemical parameters. However, supplementation did not significantly improve first-service conception rate or overall pregnancy rate under the present experimental conditions. The absence of reproductive improvement may have been associated with the relatively short supplementation period, low supplementation level, low nutritional quality of the basal diet, and the use of sexed-Y semen with relatively low post-thaw motility. Therefore, further studies using larger sample sizes, longer supplementation periods, and improved nutritional management are warranted to clarify the potential reproductive benefits of concentrate supplementation containing mineral mixtures in beef cattle under smallholder production systems.

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NOVELTY STATEMENT

Previous studies have primarily evaluated the effects of concentrate supplementation or mineral supplementation separately on reproductive performance in cattle. However, limited information is available regarding the combined use of concentrate supplementation containing mineral mixtures under practical smallholder farming conditions, particularly in Limousin crossbred cows. Therefore, this study provides field-based evidence regarding the effects of concentrate supplementation containing a mineral mixture on blood biochemical responses and reproductive performance in Limousin crossbred cows raised under smallholder production systems.

AUTHOR'S CONTRIBUTION

MSA and CH: conceptualization, investigation, methodology, software, writing original draft, writing review and editing; AA: conceptualization, methodology, supervision, writing review and editing, TS and JK: data curation, supervision, writing review and editing.

Generative AI and AI assisted technology statement

No generative AI tools were employed for data analysis, interpretation, or the generation of original scientific content. AI assistance was strictly limited to refining English grammar and enhancing language clarity.

Conflict of interest

The authors have declared no conflict of interest.

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