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Blood Biochemical Profiles and Pregnancy Rate of Brahman Crossbred Cows Supplemented with Mineral Mixture

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Abstract: The investigation was expected to assess various mineral mixture supplementation impacts on Brahman crossbred cows' blood biochemical, hematological profiles and pregnancy rate. This study used ninety Brahman crossbred cows divided into three dietary treatment groups (n = 30/group). The dietary treatments were: Concentrate and rice straw only as control treatment; control diet + mineral mixture A-formulation as MM-A treatment; and control diet + mineral mixture B-formulation as MM-B treatment. The concentration of MM-B (including calcium, phosphor, cobalt, copper, sulphur, potassium, zinc, manganese, magnesium, sodium, selenium and iron) was higher than MM-A. The mineral mixture was added to 0.5 kg/100 kg of diet. The concentrate mix and rice straw were restricted to 3 kg and 20 kg/day (as-fed basis). Feeding treatment was conducted until four months. Blood sampling and estrous synchronization were carried out after three weeks of dietary treatments. The estrous synchronization aimed to equate the estrous cycle of each cow by using 25 mg prostaglandin F2α (Lutalyse™) (injection via intramuscular). Estrous signs were observed two days after synchronization. Artificial insemination was conducted at ten to twelve hours from estrous detection, inseminated with post-thawed frozen Belgian Blue bull sperm. Transrectal palpation was used to diagnose the cow's pregnancy 90 days after artificial insemination. The serum samples were analyzed for blood biochemical, hematology and mineral status. The results showed that MM-B supplementation significantly increased ($p<0.05$) serum calcium, phosphor, selenium and iron. There were no significant differences in serum glucose, total protein, albumin, cholesterol, triglyceride, creatinine, uric acid, blood urea nitrogen and hematological profile. The pregnancy rate in MM-B treatment was higher than MM-A and control treatment. The pregnancy rate in the MM-B treatment (20.7%) was higher than control (6.67%) and MM-A treatment (3.33%). It was concluded that mineral mixture supplementation improved the pregnancy rate and several blood components on Brahman Crossbred cows.

Keywords: Supplementation, Mineral Mixture, Blood Biochemical, Pregnancy Rate, Brahman Crossbred Cows

Introduction

The increasing meat consumption in Indonesia is in line with the increasing human population every year. However, there is still an imbalance between the supply and demand of meat. Most cattle production comes from smallholder farms, in which reproductive cattle tend to lack minerals, resulting in the low reproductive performance of animals (Agus and Widi, 2018; Khalil *et al*., 2019a). Minerals, both macro and trace minerals, play an essential function in regulating the reproduction and production of animals. Productive livestock most often suffers from nutritional inadequacies because of high production and lack of feed. So, this condition leads to poor reproductive performance in animals (Talukdar *et al*., 2016). The presence of minerals deficiency on feed can cause reproduction problems in animals. Reproduction problems occurred were reported by many researchers, including hypocalcaemia, abortion of a foetus, retained placenta, vaginal prolapse, dystocia, inconsistent estrous and decreasing on reproductive performance of cows (Ahuja and Parmar, 2017; Caixeta *et al*., 2017; Kumar *et al*., 2011;

Mokolopi, 2019; Yatoo *et al*., 2018). Mineral supplementation can also increase pregnancy percentage on repeat breeder animals (Ahmed *et al*., 2010).

Niaz *et al*. (2017) stated that supplementation of minerals in the diet significantly increased serum mineral content, resulting in improved reproductive performance of animals. Many researchers have conducted studies of single mineral components on cows' reproduction, including oral calcium supplementation increased the pregnancy rates (Martinez *et al*., 2016). When phosphor intakes are low, it decreases ovarian activity, irregular oestrous cycles, delayed onset of puberty and low conception rates (Bindari *et al*., 2013). Supplementation of copper, zinc, manganese increased the pregnancy rate in beef cattle (Ahola *et al*., 2004), increasing calf mortality may be caused by deficiency of cobalt concentration in the diet (Patterson *et al*., 2003). Selenium provided to cows at some stage in the pre-and postpartum intervals results in the early recuperation of luteal function and an improved conception rate (Kamada, 2017).

However, not many studies have reported the effect of a mixture of various minerals, both macro and micro, on reproductive performance in beef cows through artificial insemination methods. This research was conducted to evaluate the effects of different mineral mixture components on Brahman crossbred cows' blood chemical profiles and pregnancy rate.

Materials and Methods

Ethical Approval

Certificated number 00021/EC-FKH/Eks./2021 approved the experimental procedure, Faculty of Veterinary Medicine, Universitas Gadjah Mada.

Location of Study

The experimental study was conducted at the PT. Widodo Makmur Perkasa, located at Cikalongkulon, Cianjur district, West Java, Indonesia.

Animal and Design

Ninety Brahman Crossbreed cows (mean Body Weight (BW), 394±62.04 kg, aged 3-4 years, had at least calved once) were randomly assigned to three pens. Each pen had 30 cows as replication. The Brahman Crossbred cows were fed according to the dietary treatments. This study had three treatments based on the diets: Concentrate mix and rice straw only as CON treatment, control diet + mineral mixture A-formulation as MM-A treatment and control diet + mineral mixture B-formulation as MM-B treatment. The mineral mixture was formulated with different ratios of mineral content. The mineral content of MM-B was made higher than MM-A (Table 1). The mineral mixture was added to 0.5 kg/100 kg of diet. The concentrate mix and rice straw were restricted to 3 kg and 20 kg/day (as-fed basis). The chemical composition of the feed has been presented in Table 2. Animal always had ad libitum water. The dietary treatments were carried out until pregnancy diagnosis (four months).

Estrous Synchronization

In the third-week feeding treatments, estrous synchronization was carried on each treatment group (n = 30/group). Before it, transrectal palpation was conducted to confirm the existence and condition of an active corpus luteum. The estrous synchronization aimed to equate the estrous cycle of each cow by using 25 mg prostaglandin F2α (Lutalyse™, Dinoprost-T, Zoetis Inc.) (injection via intramuscular). Estrous signs were noticed two days after synchronization based on vulva condition (redness, swelling, warmth), mucus discharge from the vagina, cows' anxiety, or standing to be mounted by other cows (Susilowati *et al*., 2021).

Artificial Insemination and Pregnancy Diagnosis of Brahman Crossbred Cows

The estrous cows were inseminated with post-thawed frozen Belgian Blue bull sperm. The frozen Belgian Blue sperm was imported from Belgian Blue Group (BBG Scrl, Ciney, Belgium). The frozen sperm was thawed using sterile water (37°C) for 30 sec, then was used to inseminate the 30-Brahman crossbred recipients of each treatment. The Artificial Insemination (AI) was carried out from ten until twelve hours after the estrous detection. The 21-day non-return to estrus (Saili, 2017) was used for early pregnancy detection. Pregnancies were confirmed through transrectal palpation at 90 days post-AI (Leigh *et al*., 2019).

Table 1: Mineral component of mineral mixture

Component	MM-A	MM-B
Calcium (mg/100 g)	27806.18	21117.02
Phosphor (mg/kg)	7419.61	58101.81
Sodium (mg/100 g)	2840.24	7244.98
Sulfur (mg/kg)	2093.19	6540.32
Manganese (mg/kg)	817.40	2133.73
Magnesium (mg/100 g)	305.12	699.50
Zinc (mg/100 g)	36.61	171.94
Copper (mg/kg)	49.23	547.30
Potassium (mg/100 g)	20.56	274.49
Iron (mg/100 g)	962.06	1379.15
Cobalt (mg/kg)	3.79	4.65
Selenium (mcg/100 g)	6.35	33.86

Table 2: Chemical composition of feeds fed to Brahman crossbred cows (%, DM basis)

Item	Concentrate	Rice straw
Dry matter	88.79	61.76
Crude protein	13.04	5.25
Ether extract	3.18	0.75
Crude fiber	21.68	28.36
Crude ash	8.43	23.35
Nitrogen-Free Extract (NFE)	53.67	39.79
Total Digestible Nutrient (TDN)	46.34	45.21

The number of pregnant cows divided with inseminated animals in each group was used to calculate the pregnancy rate.

Dietary and Blood Collection-Analysis

The amount of feed given (rice straw and concentrate) was recorded every day. The dietary samples were collected every 3-weeks during the research period. Chemical composition, including Dry Matter (DM), Organic Matter (OM), Crude Protein (CP), Crude Fibre (CF) and Extract Ether (EE), was determined according to the method reported by AOAC (2005). Blood samples from all cows in each treatment group were taken in the morning before feeding. To determining blood biochemical and mineral profiles, we used blood serum. The amount of 10 mL of blood samples was taken from the caudal vein of Brahman Crossbred cows using the BD vacutainer® blood collection needle. The blood samples were directly transferred to 10-mL BD vacutanier® serum tubes. The blood samples were left for 15 min then were centrifuged at 2000 rpm for 20 min. The serums that had been separated from the blood cells were then taken and subjected to further testing. Serum biochemical and macromineral were analyzed by DiaSys Diagnostic System, Holzheim, Germany, using commercially available kits: Glucose, total protein, albumin, cholesterol, triglyceride, creatinine, uric acid, Blood Urea Nitrogen (BUN), calcium and phosphor. The selenium and iron serum concentration were determined utilizing the Atomic Absorption Spectrophotometer (AAS).

For the blood hematology analysis, 3 mL of whole blood was transferred into a 3 mL K3 EDTA blood collection tube (Valucab®, OneMed, Surabaya). Samples were directly refrigerated at ~4°C within 24 h of blood collection for hematology analysis. The hematological parameters included: Hemoglobin concentration, hematocrit, total erythrocyte count, total leucocyte count, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Red cell Distribution Width (RDW), total count and percentage of granulocyte, lymphocyte and monocyte. Progesterone hormone analysis was determined by ELISA kit (DRG Progesterone EIA-1561, DRG International, Inc., USA) and estrogen hormone analysis was determined by ELISA kit (DRG estradiol EIA-2693, DRG International, Inc., USA).

Statistical Analysis

All data were analyzed of variance in SAS University Edition® Software (www.sas.com). The mean differences were tested using Duncan Multiple Range Test at the significant $p<0.05$.

Results

This study showed that mineral mixture supplementation had no significant differences ($p>0.05$) in biochemical blood profiles (glucose, total protein, albumin, cholesterol, triglyceride, creatinine, uric acid, BUN) among different groups (Table 4). Likewise, mineral mixture supplementation did not significantly differ with blood hematology from the hemoglobin concentration, hematocrit, erythrocyte count, leukocyte count, MCV, MCH, RDW, granulocyte count, granulocyte percentage and lymphocyte percentage (Table 5).

Table 3: Feed intake of Brahman crossbred cows supplemented with mineral mixture (kg DM/head/day)

Nutrient ntake	CON	MM-A	MM-B
Dry matter	17.43	17.51	17.44
Organic matter	13.76	13.82	13.78
Crude protein	1.10	1.08	1.16
Ether extract	0.20	0.19	0.20
Crude fiber	4.83	4.78	4.72
Nitrogen-Free Extract (NFE)	7.26	7.37	7.33
Total Digestible Nutrient (TDN)	7.75	7.77	7.81

Table 4: Serum biochemical and mineral profile on Brahman crossbred cows supplemented with mineral mixture

Item	CON	MM-A	MM-B	*p*-value
Biochemistry				
Glucose (mg dl^{-1})	54.47±7.53	70.57±8.20	66.77±1.69	0.220
Protein (g dl^{-1})	7.26±0.56	8.18±0.26	9.07±0.62	0.068
Albumin (g dl^{-1})	4.21±0.48	4.04±0.22	3.76±0.17	0.602
Cholesterol (mg dl^{-1})	289.18±11.51	296.28±21.70	303.75±21.41	0.862
Trygliceride (mg dl^{-1})	37.17±7.38	41.97±7.67	58.32±13.02	0.300
Creatinine (mg dl^{-1})	2.35±0.65	2.43±0.21	2.55±0.21	0.946
Uric acid (mg dl^{-1})	3.11±0.94	4.49±0.94	3.63±0.92	0.448
BUN (mg dl^{-1})	15.35±0.78	15.13±0.59	16.16±0.81	0.590
Mineral				
Calcium (mg dl^{-1})	9.83±0.17[a]	10.69±0.29[ab]	11.60±0.42[b]	0.004
Phosphor (mg dl^{-1})	2.79±0.14[a]	3.80±0.33[b]	3.97±0.34[b]	0.220
Selenium (mcg/100 mL)	6.62±0.81[a]	8.05±0.40[ab]	9.97±1.03[b]	0.029
Iron (mg/100 mL)	1.27±0.41[a]	2.68±0.61[ab]	3.51±0.64[b]	0.039

[a,b] On the same row indicate a significant difference ($p<0.05$)

Table 5: Blood hematological on Brahman crossbred cows supplemented with mineral mixture

Parameter	CON	MM-A	MM-B	*p*-value
Hemoglobin (g/dL)	9.47±0.17	10.60±0.49	10.32±0.38	0.113
Hematocrit (%)	11.95±3.96	6.32±0.87	5.03±0.69	0.125
Erythrocyte ($\times 10^6$/µL)	2.62±0.82	2.38±0.17	1.14±0.12	0.108
Leukocyte ($\times 10^3$/µL)	6.67±0.68	9.13±0.81	7.03±1.40	0.213
MCV (fL)	44.68±0.85	45.23±1.05	43.62±1.03	0.511
MCH (pg)	57.92±15.47	82.22±10.18	96.65±10.72	0.116
RDW (%)	30.63±1.90	32.05±1.30	28.98±1.17	0.371
Granulocyte ($\times 10^3$/µL)	2.22±0.35	2.55±0.30	2.03±0.67	0.740
Lymphocyte ($x10^3$/µL)	3.58±0.41	5.92±0.66	4.42±0.75	0.054
Monocyte ($\times 10^3$/µL)	0.88±0.11	0.68±0.07	0.55±0.11	0.086
Granulocyte (%)	33.40±4.08	28.32±3.17	27.15±4.05	0.481
Lymphocyte (%)	53.28±4.25	64.43±2.98	64.95±4.12	0.082
Monocyte (%)	13.32±1.30[a]	7.25±0.37[b]	7.92±0.37[b]	0.000

[a,b] On the same row indicate a significant difference (p<0.05)

Table 6: Hormone and pregnancy rate on Brahman Crossbred Cows supplemented with mineral mixture

Item	CON	MM-A	MM-B	*p*-value
Estrogen (pg/mL)	75.08±11.83	68.12±14.54	77.19±19.80	0.914
Progesterone (ng/mL)	4.57±0.73	13.88±4.65	10.75±1.60	0.098
Pregnancy rate (%)	6.67 (2/30)	3.33 (1/30)	20.69 (6/29)	-

However, the percentage of monocyte in the treatment MM-B decreased significantly (p<0.01). For serum minerals, MM-B supplementation significantly increased the serum concentrations of calcium, phosphorus, selenium and iron compared to the CON treatment (Table 4). The result also showed that mineral supplementation had no significant differences in serum progesterone and estrogen (p>0.05) (Table 6). However, the pregnancy rate in the MM-B treatment (6/29; 20.7%) was higher than CON (2/30; 6.67%) and MM-A treatment (1/30; 3.33%) (Table 6). The pregnancy rate in Brahman Crossbred cattle using artificial insemination in this study was low in general.

Discussion

Blood Biochemical, Hematological and Mineral Profile

Serum glucose concentrations were 54.47; 70.57 and 66.7 mg dl- in the CON, MM-A and MM-B treatments, respectively and there were no differences between mineral supplementation treatments. Similar findings with (Warken *et al*., 2018) that mineral supplementation on cows diet among 3, 15, 30, 45 and 60 days of treatment did not affect blood glucose. Blood glucose concentration is one of the biochemical indicators of body energy supply (Xuan *et al*., 2018). Furthermore, mineral mixture supplementation in this study did not affect the serum total protein and serum albumin concentration. Serum protein is part of the collection of amino acids in the body and indicates the nutritional status of livestock (Xuan *et al*., 2018). Molefe and Mwanza (2020) showed no difference in total blood protein by giving several doses of mineral. Khalil *et al.* (2019b) mentioned that mineral supplementation on Simmental cows using the different forms (meal and block) did not affect blood protein. Kumar and Dass (2006) also stated that niacin supplementation did not affect serum total protein, glucose, globulin and albumin on female animals fed by wheat straw and concentrate mixture as basal diets. Albumin content in the blood could reflect protein status. Its concentration could be affected by liver function, age, intake of protein and energy and protein dropping during specific illnesses. In addition, the level of several constituents in the blood (total protein, globulin, albumin and N-urea) is a representation of nitrogen adequacy or insufficiency in animal diets (Hammond, 1983).

In the animal body, cholesterol was synthesized from fatty acids. The blood cholesterol level indicates the body's lipid metabolism. In this study, supplementation of mineral mixture did not affect the serum cholesterol and serum triglyceride concentrations. A similar trend by Satapathy *et al*. (2016) showed no significant dissimilarity in serum cholesterol and serum triglyceride concentrations on 0 and 60 days after mineral supplementation. The serum cholesterol and serum triglycerides concentrations on the 60-day were 108.45 and 80.84 mg dl-, respectively. However, Oliveira *et al*. (2014) reported an increase in hypocholesterolemic status after 60 days of mineral mixture supplementation. Mineral mixture supplementation did not generally affect blood hematology in Brahman Crossbred cattle, including hemoglobin, erythrocyte, leukocyte, MCV, MCH and RDW. The same trend was reported by Khalil *et al.* (2019b) that mineral supplementation in Simmental cattle did not affect hemoglobin, hematocrit, MCHC, red blood cells but increased white blood cells. Overall, the blood hematology in this research was in the normal range, as showed by Weiss and Wardrop (2011).

The amount of granulocyte, lymphocyte and monocyte cells was not affected by treatments. However, mineral supplementation of MM-B and MM-A significantly decreased (p<0.01) the percentage of monocytes in blood hematology. The monocyte percentage in the CON group was 13.31% but lower in the MM-A and MM-B groups, 7.25 and 7.92%, respectively. Whereas Roland *et al*. (2014) stated that the normal range of monocyte presentation was 0 until 8% of total white blood, so the monocyte percentage in the CON group was higher than the normal range. Monocyte count tends to increase also in the CON group (p = 0.086). It might be suspected that during the blood collection process, the cattle had experienced stress, increasing monocytes. Van Engen *et al*. (2014) stated that monocyte profiles followed a similar stress response. So, animals had increased blood monocyte numbers. Monocytosis had been seen during intense stress and in the recuperating period of acute and persistent infections (Latimer, 2011). However, some studies mentioned that mineral supplementation reduced stress activity; Gao *et al*. (2018) explained that zinc on high concentration decreased the viability of monocyte cell line. Supplementation of zinc also reduced inflammatory cytokines and oxidative stress (Prasad, 2014). Ahrens *et al*. (2008) found that selenium supplementation induced metalloproteinase-dependent L-selectin, resulting in decreased monocyte rolling and adhesion under shear stress conditions. Trace mineral supplementation (e.g., zinc, selenium and copper) improves the immune system and production of superoxide (Batistel *et al*., 2017).

Mineral supplementation had a significant effect on blood mineral levels. Serum calcium and phosphor levels in the MM-B treatment were higher than CON treatment. Sahoo *et al*. (2017) reported similar findings, increasing serum mineral in cattle supplemented with the mineral combination in feed. Venjakob *et al*. (2017) showed that increased blood calcium concentration was linear with calcium levels given to animals. It might be vitamin D, which acts as 1,25-dihydroxycholecalciferol, stimulates calcium channels and facilitates calcium absorption in the intestinal mucosa. Inorganic phosphorus plays an essential role in accelerating ovulation and fertilization in cows (Wu and Satter, 2000). Phosphorus is often associated with energy metabolism, which is necessary for fertilization and embryo growth. Therefore, phosphorus deficiency can lead to premature embryonic death leading to repeated breeding or anestrous conditions (Ali *et al*., 2014).

Serum selenium and iron levels in the MM-B treatment were higher than those in CON treatment. MM-B has a higher concentration of selenium and iron, thus increasing absorption in the body. Hall *et al*. (2014) reported that cows were given super nutritional selenium-yeast supplementation during 8-weeks before calving had higher blood selenium concentrations at 48 h and 14 days of lactation. Chelated mineral mixture supplementation in buffaloes could increase serum iron 45 and 90 days after supplementation (Joshi *et al*., 2019).

Pregnancy Rate

The pregnancy rate in the MM-B treatment (6/29; 20.7%) was higher than CON (2/30; 6.67%) and MM-A treatment (1/30; 3.33%). However, the pregnancy rate in Brahman Crossbred cattle in this study was low in general. It might be caused by the low level of nutrients consumed by animals; it could be seen in Table 3 that the feed consumed has a relatively low balance of energy and protein. The dry matter intake of all treatment groups was around 17.46 kg/day. The results were following the NRC (2000), in which the dry matter intake for reproductive cattle was above 3% of Body Weight (BW). However, the TDN and protein intake in this research was still far below the range of Kamada (2017) in reproductive cattle, which was around 17.23 kg/day for TDN intake (2.51% of BW) and 4.03 kg/day for crude protein intake (0.59% of BW), while this finding for the TDN intake was only 7.77 kg/day (1.97% of BW) and 1.11 kg/day for crude protein intake (0.28% of BW). Spicer *et al*. (1993) stated that the reproductive performance of cows could be affected by protein and energy intake. Kang *et al*. (2020) suggested feeding concentrate and hay until 120% of cows' nutrient requirement had many transferable and high-quality embryos.

The results obtained were similar to Ervandi *et al*. (2020) that Brahman Crossbred cows fed low-quality feed will produce a low pregnancy rate. Haque *et al*. (2015) also suggested that the variety of feed given to breeding cows (fresh forage, straw and concentrate mixture) increased reproductive hormone, resulting in a better pregnancy percentage. In this study, early detection of pregnancy was assumed to be using 21-NRR. The results showed more excellent results (CON: 60.00%, MM-A: 66.67%, MM-B: 72.41%) than confirmation of pregnancy using rectal palpation on the 90th-day post-AI. We also suspected that low nutrient intake could lead to premature embryonic death. Unfortunately, the direct embryo examination was not carried out. Nutrient deficiency can reduce fertility (Boland and Lonergan, 2003).

Regarding the timing of artificial insemination, Ervandi *et al*. (2020) also performed artificial insemination at almost the same hour (8-10 h after estrous detection); they showed that Brahman cows had a few numbers of pregnancy rate (27.5%, 11/40). In contrast, Sutiyono *et al*. (2018) showed that the others breed cattle, Ongole Grade and Simmental-Ongole Crossbred, had a higher pregnancy rate (70.59 and 50.00%). In addition, fertility rates in beef cattle pasture-grazing with natural mating systems were usually very high. Under normal conditions, 90% of ovulated ovarium were successfully fertilized. However, this condition did not occur in crossbred cattle using artificial insemination with varying failure rates, according to the composition of the genotype (Diwyanto and Inounu, 2009). Ervandi *et al*. (2020) also examined the pregnancy rate using the ultrasonography observation of Brahman crossbred cows. The results showed that those crossbred cows had several ovarian abnormalities. Many factors should be evaluated, including the high cases of ovarian hypo-function in productive cattle due to crossbreeding (Dibia *et al*., 2015).

However, among the various factors affecting the percentage of pregnancy in Brahman Cross cattle by artificial insemination, the MM-B treatment showed a better response to pregnancy percentage than other treatments. Numerous investigations linking mineral supplementation with successful pregnancy are related to increasing progesterone in the blood. Although in this study, serum progesterone levels did not show a significant difference. That was possible with a different blood sampling process, where sampling for progesterone testing had been done before artificial insemination. As research by Khalil *et al*. (2019a), who took blood samples for progesterone testing at 16 weeks in female goats, had a linear increase in serum progesterone levels with the addition of mixed minerals in the feed. However, Khalil *et al.* (2019b) also reported that mineral supplementation did not affect serum progesterone levels of female cattle taken at 14 weeks. Ganie *et al.* (2014) showed that selenium supplementation did not significantly differ initially and during estrous but increased estrogen and progesterone hormones at the end of treatment.

Behera *et al*. (2012) found linear results with mineral supplementation with an increase in pregnancy rates. In cows, lack of standard copper, manganese and zinc lead to postponed estrous, unusual estrous, decreased conception, juvenile ovaries, infertility and embryonic decease (Yasothai, 2014). Supplementation of certain mineral mixtures of calcium, phosphor, zinc, manganese improved growth performance, estrous quality and pregnancy rate in crossbred cows (Mohapatra *et al*., 2012; Sahoo *et al*., 2016). Zinc assumes a fundamental part in the sexual turn of events and is concerned with developing ovarian follicles, which might source the progesterone hormone (Yatoo *et al*., 2013). Copper is an essential part of superoxidase dismutase enzyme activity, controlling luteal cells to produce progesterone hormone (Sales *et al*., 2011). Ahmed *et al*. (2010) stated that copper deficiency showed low ovarian activity correlated with oxidative stress. Iron plays an essential function in embryo development; as a result, Gao *et al*. (2007), oocyte maturation was not affected by iron supplementation, but it improved the development rate to the 8-cell, morula and blastocyst phases. Sales *et al*. (2011) also reported that the adequacy of some trace minerals (copper, zinc, manganese and selenium) reduced early embryonic death, resulting in an increased pregnancy rate on crossbred cows.

Conclusion

The supplementation of mineral mixtures affected the mineral serum concentration and did not affect the blood biochemical and hematological profiles. The supplementation of mineral mixture B-formulation (high mineral concentration) increased the pregnancy rate on Brahman Crossbred cows.

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Author's Contributions

Mohammad Sofi'ul Anam and Ali Agus: Contributed to the designing, conducting the experiment and preparing the first draft.

Lies Mira Yusiati, Chusnul Hanim and Sigit Bintara: Conducted the laboratory analysis and interpreted the obtained data.

Andriyani Astuti and Muhsin Al Anas: Contributed to the correction of the manuscript and data interpretation.

Ethics

This article was original, no ethical issues were involved and it contained unpublished material. The corresponding author ensured that the final manuscript had been read and approved by all authors.

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