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Original Article

Effects of mineral supplementation on reproductive efficiency of Simmental heifers

Khalil[1*], Andri Bachtiar[2], Zaituni Udin[3]

[1]Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, Campus II Payakumbuh, West Sumatra, Indonesia

[2]Department of Livestock Business and Social, Faculty of Animal Science, Andalas University, Padang, West Sumatra, Indonesia

[3]Department of Livestock Production, Faculty of Animal Science, Andalas University, Campus II Payakumbuh, West Sumatra, Indonesia.

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**Corresponding author email:*
khalil@ansci.unand.ac.id

Abstract

The objective of the present research was to evaluate the effects of mineral supplementation on estrus onset, growth, and hematological and biochemical parameters in Simmental heifers. The study was initiated by a field survey to determine the reproductive problem of the exotic breed cattle raised by smallholders. Complete minerals were then formulated and fed to 18 Simmental heifers for 14 weeks across three groups as follows: P0, no supplementation (control), P1, supplemented with mineral feed in meal form, and P2, supplemented with mineral in block lick form. Each treatment consisted of six heifers stratified by body weight into six groups for replication. Parameters measured included estrus onset time, body weight gain, mineral intake, blood mineral levels, hematology, total protein, and progesterone levels. Simmental heifers raised by smallholders were delayed in onset of first estrus (20.4 months) and first calving (30.17 months). Heifers supplemented with minerals (P1 and P2) had an earlier estrus in the 2nd and 4th weeks, while in the control groups (P0), the first estrus appeared in the 8th and 9th weeks. Heifers supplemented with block minerals (P2) had lower body weight gain, and Fe, Zn, and progesterone levels than those supplemented with minerals in meal form (P1). The results showed that mineral supplementation in meal form positively affected estrus onset, growth rate, and biochemical parameters in Simmental heifers.

Keywords: Simmental heifer, Mineral supplement, Estrus onset, Hematology

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Introduction

Beef cattle breeding in West Sumatra, Indonesia which are dominated by small-scale farms have shifted from local breed to exotic one, especially Simmental breed with greater body size and growth rate. However, the female of the imported exotic breed raised by the traditional smallholder are susceptible to reproductive disturbance that results in delay or failure to produce calf. Delayed maturity of heifers was found to be the most important causal factor for suboptimal reproductive performance, followed by repeated breeding and postpartum infertility (Yuherman et al., 2017). The reproductive problems of heifer that has lowered breeding efficiency resulting in great economic losses to the small breeding farms might be due to nutrient deficiency, especially protein and mineral.

Supplementation of heifers with concentrates and minerals is uncommon practice, because they are considered as unproductive flock. Therefore, the heifers are almost entirely dependent on feeds consisting of elephant grass, wild vegetation forage derived from diverse sources of non-developed pastures, and crop residues to meet their mineral requirement. These feed vary in nutrient content and are often deficient in mineral (Yuherman et al., 2017; Khalil et al., 2015). The heifers therefore are malnourished and develop health problems that may result in delayed puberty and onset of first estrus.

Age of puberty is important for the reproductive success of heifers in breeding cattle. Puberty is defined as when a heifer first expresses estrus behavior and simultaneously ovulates a fertile oocyte (Ball and Peters, 2004). Young growing heifers require an adequate supply of minerals for normal growth and development of reproductive activities and proper age of first estrus onset, as minerals affect ovarian activity in ruminants (Boland, 2003). Mineral demand of young growing heifers should be increased to support physiological developmental processes that occur during the reproductive phase (NRC, 2003). Minerals play an intermediate role in the action of hormones and enzymes at a cellular level, which ultimately affect the reproductive performance of females (Bearden et al., 2004). Deficiencies of single and combined minerals and their imbalances may cause reproductive disorders or failures (Sharma et al., 2007).

Therefore, there is a need to design specific mineral supplement to make up the mineral deficiency or imbalance and satisfy requirement in improving reproductivity of Simmental heifer raised under traditional smallholders. Mineral supplements could be produced using locally available ingredients. Stone meal of Bukit Kamang and fresh water mussel shell meal in West Sumatra are two local materials that can be potentially used as Ca sources (Khalil et al., 2015). Bukit Kamangs' stone meal is rich in essential micro minerals, including manganese (Mn) (205 ppm), iron (Fe) (295 ppm), and selenium (Se) (388 ppm) (Khalil et al., 2015). These local minerals can be enriched and mixed with other essential macro and micro mineral sources and salts for cattle. Since feeding heifers with concentrates at small breeding farms is uncommon, mineral supplementation should be also be provided in block lick form. Advantages of mineral feed in blocks over supplementation of minerals in concentrated feed are that they are easy to be handled, available 24 hours, and can be consumed slowly.

The present study aimed to determine reproductive problems in Simmental heifers raised on traditional smallholders and to evaluate the positive impact of mineral supplementation on growth rate, estrus activities, and hematological, biochemical, and mineral profiles in Simmental heifers.

Material and Methods

Survey on reproductive performance

The study used a field survey in May 2017 to determine reproductive problems of imported heavy-breed cattle raised under smallholders with flock size under 10 animals/farms. Data on reproductive performance were collected from 48 small farms in the Payakumbuh region of West Sumatra (60 Simmental cows). The selected farms were distributed across seven villages (Padang Ambacang, Sibaladuang, Indobaleh Barat, Subarang Sawah, Pandan, Koto Harau, and Payonibung) in Payakumbuh city and the Limapuluh Kota regency. There was an average of 1.37 cows/farm with an age range of 20-156 months and an average 1.9 calving number. Table 1 shows the flock size and reproductive history of the Simmental cows.

Table 1. Mean flock size and reproductive history of Simmental cows raised by 48 smallholders in the Payakumbuh region

Parameter	Mean±SD	Range
Cattle population (head/farm)	2.69±1.53	2-10
Cows	1.37±0.57	1-3
Heifers	0.61±1.20	0-7
Calves	0.67±0.60	0-2
Cow age (months)	46.21±28.06	20-156
Calving number	1.86±2.01	1-10

Farm owners were interviewed using a structured data format to obtain information on the following: cattle population number, feed and feeding practices, age of animals, age at first estrus, service per conception, age at first birth, calving number, calving interval from the 1st-to-5th birth, and reproductive disorder history (anestrus, post-partum anestrus, and repeated breeding). Cows were examined rectally to determine the status of reproductive organs, including the cervix, uterus, and ovary.

Preparation of mineral formulas

Mineral supplementation was formulated using Bukit Kamangs' stone powder and roasted fresh water mussel shell meal, which are locally available materials. The formula was enriched with dicalcium phosphate, micro mineral compounds ($ZnSO_4.7H_2O$ and $CuSO_4.5H_2O$), iodized kitchen salt, limestone, and commercial mineral premix. Mineral concentrations were formulated to complement the mineral deficiency of forage and concentrate feed (Khalil et al., 2016) to meet the standard of mineral supplement for cattle according to NRC (1996) and Weinreich et al. (1994). The formulas were prepared in meal and block lick forms and then analyzed for Ca, P, Mg, Fe, Zn, Cu, Mn, Se, and Co levels by standard methods using an atomic absorption spectrophotometer. Adhesive materials of cement were added to the lock lick formula; the mixture was prepared according to procedures to make *urea molasses block* described by Haili et al. (2008). The mineral composition of the formulas is shown in Table 2.

Table 2. Mineral composition of grass, concentrate, and mineral formulas in heifer feed

Minerals	Grass	Concentrate	Meal mineral formula	Block mineral formula
Macro minerals (g/kg DM)				
Ca	6.60	7.10	99.89	115.25
P	1.40	12.10	5.10	3.70
Mg	2.16	1.56	1.72	3.25
Trace minerals (mg/kg DM)*				
Fe	100.66	119.92	360.10	354.55
Zn	0.25	15.38	1002.51	932.06
Cu	6.41	6.29	1801.12	1335.07
Mn	70.52	108.03	114.91	121.13

*Se and Co were not detected by standard concentration of 0.02 and 0.005 mg/L, respectively

Feeding trial

The minerals were fed to Simmental heifers across three groups as follows: P0 (no supplementation) (control), P1 (supplemented with mineral in meal form), and P2 (supplemented with mineral in block lick form). This experiment was carried out at the National Breeding Center for Exotic Breed Cattle of Padang Mangatas in Payakumbuh, West Sumatra. Eighteen Simmental heifers with an average age of 18-24 months and body weight of 247.4±41.1 kg/head were used in this experiment. The heifers were stratified by body weight into six groups and groups were randomly assigned to three treatments; each treatment included six animals as replicates.

Heifers were kept in a pen with individual stalls and fed chopped Kings' grass with a nutrient content of 10% crude protein (CP), 37% crude fiber (CF), 0.66% Ca, and 0.26% P based on dry matter (Table 2). They were also supplemented with concentrates of 2 kg/head/day. The concentrates used were composed of commercial concentrate, rice bran, coconut meal, urea, salt, and mineral premix with nutrient content of 13% CP, 22% CF, 0.71% Ca, 1.21% P, and 66% total digestible nutrient (TDN) (Table 2). Heifers in the control group (P0) were fed a basal diet composed of forages and concentrates as the normal daily ration used in the breeding center. The P1 group was provided with mineral formulas (100 g/head/day) in meal form by mixing it with concentrate. Block minerals for P2 were kept in special mineral boxes and placed on the feed trough, so that it could be accessed and licked by heifers at any time. All heifers were given 15 days of adaptation until the consumption of mineral block reached approximately 100 g/head/day.

Parameters

The feeding trial lasted for 14 weeks from June to September 2017. The parameters measured included the following: body weight, block mineral intake, hematological and mineral profiles, total protein, and progesterone concentration. During the trial, reproductive activities and other performances related to mineral supplementation, such as estrus, hair color, and health status, were also observed. Estrus was detected by visual observation of physical signs (the tumefaction of vulva, reddening of vulva into a bright cherry color, and excess mucus discharge) and by measuring vaginal temperature. Heifers observed in estrus were artificially inseminated.

During the last week of the trial period, two blood samples from each heifer were collected from the jugular vein into vacutainer tubes. The first samples were collected in 10 mL plain vacutainer and centrifuged at 3000 rpm (LMC-3000, Grant Instrument, UK) for 20 min to separate blood serum. The serum was collected in a sterile vial and preserved at-20°C until analysis for total protein, minerals, and progesterone. The concentration of total protein, Ca, P, and Mg were analyzed by auto analyzer (Mindray BC-2800) using commercial kits. Serum micro minerals of Fe, Mn, Cu, Zn, Se, and Co were determined using AAS. Progesterone concentrations were measured using an ELISA kit (Monobind, Lilac). The second blood samples were collected in vacutainer tubes (Becton Dickinson vacutainer) containing EDTA and used for analysis of hematological parameters. Hematological data measured included hemoglobin (HGB), total red blood cell (RBC) and white blood cell (WBC), Hb concentration (HCT), mean corpuscular volume (MCV), and mean corpuscular Hb concentration (MCHB). Hematological parameters were analyzed using Medonic Veterinary Hematology analyzer (Medonic CA 620; Medonic, Sweden) at the Chemical Laboratory of National Veterinary Service Institute in Baso, Bukittinggi in West Sumatra, Indonesia.

Statistical Analysis

Data were statistically analyzed using variance analysis (ANOVA) in a complete block design of 3 x 6, consisting of three treatments and six blocks as replicates. Duncan's Multiple Range (DMRT) was applied to separate means. Differences were considered significant at $P<0.05$ (Steel et al., 1997).

Results

Reproductive performance of Simmental cows raised by smallholders

The results of the field survey on reproductive performance of Simmental cows raised by smallholders are shown in Table 3. The mean age at first estrus was 20.4 months, ranging from 14-45 months, with a mean service/conception of 1.3. The delayed onset of puberty resulted in a delay of first calving at 30.17 months. The time period of estrus after first calving was four months, and the calving interval after the first calving was 14.9 months.

Effects of mineral supplementation

Eight out of the total 24 heifers showed signs of estrus during the 14-week' feeding trial, including three heifers each in the supplemented groups (P1 and P2), and two in the control group (P0). The first estrus sign appeared in the 2nd and 4th weeks in the P1 and P2 groups, respectively; however, in the P0 group, the first estrus sign appeared in 8th and 9th weeks. Heifers supplemented with minerals in meal form (P1) had the highest body weight gain, followed by those supplemented with block minerals (P2) (415.7 g/head/d) and the control (P0) group (410.4 g/head/d); there were no statistically significant differences across groups (Table 4).

Table 3. Reproductive performance of Simmental cows raised by smallholders in the Payakumbuh region

Parameter	Mean±SD	Range
- Age at first estrus (months)	20.40±5.08	14-45
- Service/conception	1.30±0.56	1-3
- Age at 1st birth (months)	30.17±5.96	23-54
- Estrus after 1st birth (months)	4.04±1.79	1.5-8
- Service/conception	1.19±0.47	1-3
- Calving interval of 1st and 2nd birth (months)	14.84±7.15	12-42
- Estrus after the 2nd birth (months)	3.03±1.14	2-5
- Service/conception	1.15±0.38	1-2
- Calving interval of the 2nd and 3rd birth (months)	12.82±1.08	12-15
- Estrus after the 3rd birth (months)	3.66±2.28	1.3-7
- Service/conception	1.00±0.00	1-2
- Calving interval of the 3rd and 4th birth (months)	13.00±1.73	12-16
- Estrus after the 4th birth (months)	2.50±1.00	2-4
- Service/conception	1.50±1.00	1-2
- Calving interval of the 4th and 5th birth (months)	13.00±2.00	12-16

Mineral concentration, total protein, and hematological values ranged within normal levels, except for WBCs and P, Fe, and Zn levels. WBCs were above the normal standard in the P2 group (Table 4), and P levels were also above the normal standard across all groups, presumably due to high P content in the concentrates (Table 2).

Heifers supplemented with local mineral in meal form (P1) had the highest concentration of progesterone (2.40 ng/mL), followed by the control (P0) (1.90 ng/mL), and those supplemented with block mineral (P2) (1.55 ng/mL); there was no statistical difference across groups (Table 4).

Table 4. Body weight gain, hematological and biochemical values and mineral concentration of heifers supplemented with minerals

Parameter	Control (no mineral supplement)	Supplemented with meal minerals	Supplemented with block minerals	Normal range
	P0	**P1**	**P2**	
Body weight gain (g/head/day)	410.35±139.59	427.83±54.10	415.65±97.49	
Hematological values:				Normal standard*)
- HGB (g/dL)	11.54±3.36	9.62±0.67	10.16±1.37	8.4 -12.0
- HCT (g/dL)	28.84±9.49	23.60±1.39	24.78±3.53	21.00 - 30.00
- MCHC (%)	40.34±1.63	40.88±0.83	41.16±2.16	38.00 - 43.00
- RBC ($^{X}10^{6}\mu L^{-1}$)	7.94±2.17	6.27±1.11	6.53±1.02	4.90 - 7.50
- WBC ($^{X}10^{3}\mu L^{-1}$)	12.28±2.45^{a}	11.28±1.19^{a}	15.04±2.56^{b}	5.10 -13.30
Total protein ($mg.dL^{-1}$)	7.47±0.72	7.17±0.86	7.43±0.68	6.50 - 8.50
Blood minerals:				Critical level**)
- Ca ($mg.dL^{-1}$)	9.26±0.76	9.78±1.74	8.86±2.75	8.50 - 1.50
- Mg ($mg.dL^{-1}$)	1.87±0.90	1.97±0.32	1.90±0.20	1.80 - 3.20
- P ($mg.dL^{-1}$)	8.14±1.79	8.68±0.99	9.32±1.34	3.20 - 6.00
- Fe (ppm)	1.65±0.35^{b}	1.76±0.30^{b}	1.24±0.78^{a}	0.89 - 2.53***)
- Zn (ppm)	0.81±0.17	0.77±0.10	0.60±0.10	0.70 - 1.30
Progesterone (µg/mL)	1.90±0.88	2.40±1.31	1.44±0.33	

*) Normal standard according to Wood and Quiroz-Rocha (2010)

) Critical level suggested for cattle by McDowell (1997); *) Underwood (1977) for cows

a,b Values in the same row with different superscripts are significantly different (P<0.05)

Se, Co, Mn and Cu were not detected by standard concentration of 0.02, 0.004 and 0.005 mg/L, respectively

Heifers supplemented with mineral in block lick form (P2) had the lowest Fe, Zn, and progesterone concentrations. The mean WBC or leucocytes values of heifers supplemented with block minerals (P2) the WBC values exceeded the normal reference range suggested by Wood and Quiroz-Rocha (2010) and were significantly higher (P<0.05) compared to groups P0 and P1.

Discussion

Simmental cattle is an increasingly popular breed for small-scale beef cattle farmers in Indonesia due to their large body size, better growth performance, and calm behavior; however, heifers of the imported exotic breed are susceptible to delay in onset of puberty, resulting in delay of first calving at 30.17 months. Ideally, Simmental heifers should be pubertal by 11 to 12 months to ensure first calving by 24 months of age (Ball and Peter, 2004). Yuherman et al. (2017) suggested that delayed sexual maturity and anestrus of Simmental cows raised by small breeding farms in the Payakumbuh region are most likely caused by deficiency in protein and several essential minerals. They found that animals were deficient in Ca, P, Mn, Zn, and Cu (Yuherman et al., 2017). Deficiencies in Ca, P, Cu, Mn, and Zn are associated with repeat breeding, reduced conception, prolonged labor, and lower birth weight (Kumar et al., 2011). Common symptoms of Cu, Mn, and Zn deficiency in cattle include delayed or suppressed estrus, abnormal estrus, reduced conception, immature ovaries, infertility, and embryo death (Yasothai, 2014). Delayed maturity and reproductive problems related to mineral deficiencies were also reported in cross-bred cattle raised in smallholder's dairy production systems in the Kakatpur block of Odisha, India (Satapathy et al., 2018). Khan et al. (2016) reported a high incidence of anestrus cases in dairy cattle raised in a semi-intensive system in Odisha and North-Eastern India. Several incidents of mineral inadequacies in forages have also been reported, which are principal causes of reproductive failure of imported dairy cattle raised by smallholders in tropical countries (Rukkwamsuk, 2011, Swai et al., 2005; Lyimo et al., 2004).

Supplementation of minerals in meal form enhanced the growth, nutritional status, and reproduction of Simmental heifers as indicated by better blood hematological values, blood mineral profiles, and accelerate estrus onset. Heifers supplemented with mineral in meal form also had better body condition and looked healthy and shiny hair. Similar results were reported by Khalil et al. (2015), who showed that Simmental heifers supplemented with local minerals enriched with commercial premix have higher body weight gain, lower feed conversion ratio, and higher net return than cattle fed only grass. Supplementation of specific mineral mixture comprising Ca, P, Zn, Zu, and Mn enhanced the growth, estrus expression and conception rate in crossbred cattle in Jatani block of Khurda district, Odisha, India (Mohapatra et al., 2012; Sahoo et al., 2016).

The present results have also demonstrated that there were no beneficial effects of mineral feed in blocks over supplementation of minerals in concentrated feed. The lower performance of heifers supplemented with block minerals compared to those supplemented with a meal form was mainly caused by unstable and limited block mineral intake. The average block mineral intake for the 14-week trial was 68.9 g/head/day, which is much lower than the targeted 100 g/head/d. At the beginning of the trial, block minerals were consumed normally and increased from 80 to 103 g/head/day at week 3, which achieved the targeted intake amount. These data suggest that the block mineral formula was palatable. However, starting at week 4, mineral intake steadily decreased from 86 to 42 g/head/day to week 11 due to a limited supply of drinking water caused by drought season at the end of July to August in the study sites of the Payakumbuh region. Drinking water was not available *ad libitum* due to water scarcity. The animals were just supplied drinking water twice a day, in the morning and afternoon during feeding of forages and concentrates.

Current data suggested limited water supply had negative effect on mineral intake, although feed intake was not measured because of the difficulty of on farm condition. The limited mineral intake was directly reflected in the serum mineral profiles of the animals. The lowest levels of the micro mineral concentration (Fe, Zn, and Mn) might be related to the lowest progesterone concentration in the serum of heifers supplemented with block minerals (Table 4). Progesterone levels directly reflect the activity of the corpus luteum and could be used as a precise indicator of ovarian function to monitor estrus cycles (Ball and Peter, 2004; Barui et al., 2015). Iron plays an important role in ovarian activity (Qian et al., 2001). Yatoo et al. (2016) reported a positive correlation between Fe, Zn, and Mn with progesterone in heifers. Copper and zinc are involved in regulating progesterone production by luteal cells via superoxide dismutase, and manganese acts as a cofactor in the synthesis of cholesterol, which is a precursor to steroids such as estrogen and progesterone (Nocek et al., 2006).

Trace element deficiency and infectious diseases are often in association each other, as trace elements play an important role in the defense against infectious organisms (Bhaskaram, 2002; Fouda et al., 2011). Trace elements serve as structural components of several metalloenzymes, such as glutathione catalase (Fe) and superoxide dismutase (Cu and Zn). Metalloenzymes are required for a wide range of metabolic activities, such as energy production, protein digestion, cell replication, antioxidant activity, and wound healing (McDowell, 1992).

This study demonstrated that reproductive disturbances problems in Simmental cows are most likely associated with nutrient deficiencies. Mineral supplementation positively affected health, reproduction, and growth performance of Simmental heifers. The findings of this study provide supportive evidence on the importance of mineral supplementation in resuming delayed sexual maturity of imported heavy-breed cattle fed limited and low quality forage at tropical small-scale farms. Adequate nutrition of minerals could encourage the imported heavy-breed types to reach their genetic potential and minimize the negative effects of poor feeding and management practiced by traditional smallholders.

Conclusion

Simmental heifers raised on small farms had delayed reproductive activities. Mineral supplementation produced positive effects on reproduction and growth performance. Mineral supplementation offered in meal form was more effective than in block lick.

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Contribution of Authors

Khalil: Conceived Idea, Data Collection, Data Analysis, Data Interpretation, Manuscript Writing
Bachtiar A: Statistical Analysis, Data Interpretation, Literature Search
Udin Z: Designed Research Methodology, Literature Review, Manuscript Final Reading and Approval.

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