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Research Article
Hematological and Mineral Profiles of Reproductive Failure of Exotic Breed Cattle in Payakumbuh, West Sumatra, Indonesia

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Abstract

Background and Objective: Female exotic breed cattle raised by traditional small farms are susceptible to reproductive disturbances that result in failure or delay to produce calves. The present research investigated the hematologic profiles of exotic breed cattle having reproductive troubles versus heifers and pregnant cows raised by small farms. **Materials and Methods:** A survey was conducted to identify the reproduction performance of 160 female Simmental cows raised at 15 smallholders in Payakumbuh, West Sumatra, Indonesia. Samples of blood were collected from 15 female Simmentals comprised of three different reproductive statues (heifers, pregnant and reproductive failure, n = 5 animals each). Blood plasma was then separated and analyzed for hematological parameters [hemoglobin (Hb), hematocrit, red and white blood cell, mean corpuscular Hb concentration], total protein and mineral content (Ca, P, Mg, Fe, Mn, Cu and Zn). Samples of fodder feed were collected from 15 farms for determination of dominant species and mineral composition. Data were statistically analyzed in a completely randomized 3×5 design for blood parameters and 4×3 design for forage minerals. **Results:** About one-third of female cows found to have reproductive problems. Anestrus was found to be the most important causal factor for reproductive failure, followed by postpartum infertility (poor fertilization). The reproductive failure group had significantly lower ($p<0.05$) Hb levels, hematocrit, red and white blood cell and protein but higher mean corpuscular Hb concentration. Considering the critical levels in the blood and feed, the animals were deficient in protein, Ca, P, Mn and Cu. **Conclusion:** Anestrus and repeated insemination were found to be the major causes of reproductive disorders in exotic breed cattle under small farm conditions that results in delay or failure to produce calves. Reproductive disturbances in Simmental cows were most likely associated with nutrient deficiencies.

Key words: Exotic breed cattle, hematologic profiles, mineral nutrition, predominant forage, insemination

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The Payakumbuh region (includes Payakumbuh city and 50 Kota districts), is a central location for beef cattle breeding in West Sumatra, Indonesia. With a total cattle population of around 44,467 head, this area holds the second largest cattle population after the Dharmasraya district[1]. Beef cattle breeding is dominated by small-scale farms focusing on cow-calf systems. Local government has placed a high priority on increasing beef calf production from smallholder enterprises in West Sumatra. Improving the productivity and competitiveness of these small enterprises is expected to be achieved by a introduction of exotic breeds and development of artificial insemination services to increase the reproductive performance of cows.

Smallholders mostly have shifted from local breeds to raising of exotic ones, especially Simmental and Limousine breeds with greater body size and growth performance. They are supported by the National Breeding Center for Exotic Beef Cattle located at Padang Mangatas in the 50 Kota district[2]. In addition to limited land and capital, raising exotic breeds is constrained by low reproductive rates due to various disorders, such as silent heat periods, delayed conception, poor fertilization and postpartum infertility, which result in failure or delay to produce calves. Smallholder livestock are kept in simple stalls throughout the day and are almost entirely dependent on feeds consisting of wild fodder and crop residues. The intensive breeding system, which is uncompensated by assuring the nutrition and shelter conditions, can lead to malnutrition and other health problems which cause reproductive problems.

Indeed, data on certain blood serum biochemical and hematological parameters could aid in the evaluation of reproductive performance in relation to physiological, nutritional, metabolic and clinical status[3,4]. For example, hemoglobin (Hb) and hematocrit (HCT) concentrations describe the size of erythrocytes and their concentration of Hb, while white blood cell (WBC) and differential leukocyte counts are used to evaluate immunity[5]. Each of these measurements can help to identify and characterize disorders, such as malnutrition, chronic illness, reproductive status, nutrition and management systems[6].

The present research investigated the hematologic profile of exotic cattle breeds having reproductive trouble, relative to healthy heifers and normal reproductive cows on traditional smallholder farms in West Sumatra, Indonesia. Conditions for diagnosis of possible pathological factors, metabolic disorders and/or nutrient deficiency related to the reproductive failures were identified and assessed. The current findings may serve as a reference for evaluation of management practices, as well as nutritional and physiological statuses of animals in the region.

MATERIALS AND METHODS

Survey of reproductive performance and feed sampling: The present study was initiated by a rapid survey in August, 2016 to identify the reproductive performance of 160 female Simmental and Limousine raised by 15 small-scale farms located in the Payakumbuh region of West Sumatra, Indonesia. Each farm was sub-divided based on their stock population: Small (<5 head/farms), medium (5-10 head/farms) and large (>10 head/farms). Farm owners were interviewed to collect data on reproductive parameters, including age at first service, anestrus, calving interval, postpartum head period, service/conception, service period, calving interval, postpartum service, postpartum infertility, feed and feeding systems.

Samples of fodder feed in fresh farm were taken at 5 individual sampling points from forage bunches or sacks directly after they arrived at the farms. Individual samples were placed in individual plastic bags, weighed and then sorted by plant species for calculation of botanical composition and identification of dominant species. The percentage of botanical composition was calculated by dividing the fresh weight of individual species with the total weight of individual samples and then multiplying by 100. Four species which had a proportion of 5% or more were considered predominant species.

The predominant species were then chopped and mixed together in the same species. Three representative samples of about 150 g from each species were taken, so that there were in total of 12 samples (4 species and 3 replicates). The representative samples were then dried in a forced draught oven at 60°C for 24 h and then ground into meal form prior to analysis of dry matter (DM) and mineral content (Ca, P, Fe, Cu, Mn and Zn). Samples for mineral analysis were prepared by wet digestion using concentrated sulfuric acid and hydrogen peroxide. The concentration of minerals was determined using an atomic absorption spectrophotometer[7]. All results were reported on a DM basis.

Blood sampling and analyzing: Blood samples were collected from 15 female Simmental and Limousine cattle, including heifers, pregnant and reproductive failure cows (n = 5 animals each). Reproductive failure referred to heifers or cows that were anestrus for long periods or did not become pregnant after repeated artificial insemination services. Anestrus

conditions were obtained from farm owner. Blood samples were collected from the jugular vein using 10 mL disposable syringes. The blood was immediately transferred to heparinized vials in order to avoid clotting and kept on ice. Blood samples were centrifuged at 3000 rpm (LMC-3000, Grant Instrument, UK) for 20 min to separate blood plasma. The plasma was then preserved in refrigerated conditions for determination of minerals and hematological parameters at the Chemical Laboratory of National Veterinary Service Institute in Baso, Bukittinggi, West Sumatra, Indonesia.

Hematological parameters studied were total red blood cell (RBC) and white blood cell (WBC), Hb concentration (HCT), mean corpuscular volume (MCV) and mean corpuscular Hb concentration (MCHb). Determination of the hematological parameters was performed by using Medonic Veterinary Hematology analyzer (Medonic CA 620, Medonic, Sweden). The concentration of total protein, Ca, P and Mg were analyzed by autoanalyzer (Mindray BC-2800) using commercial kits. The concentration of Fe, Cu, Zn and Mn in plasma were determined by standard methods using an atomic absorption spectrophotometer.

Statistical analysis: Data on mineral content of fodder feed were subjected to one-way analysis of variance using a completely random 4×3 design, consisting of 4 main botanical species and 3 replicates/forage, while data on hematological parameters and mineral concentration used a completely randomized 3×5 design. A Duncan's multiple range test was applied to separate means. Differences were considered significant at $p<0.05$[8].

RESULTS

Reproductive performance: Survey results showed the mean performance parameters of normal reproductive exotic females raised by small farms included a service period of 117.3 day (range: 60-180 day), 1.86 services/conception (range: 1 -4) and a calving interval of 15.0 months (range: 12-18 months) (Table 1). However, about one-third of the animals assessed were found to have reproductive problems. Amongst the reproductive problems, anestrus was the most prominent, followed by postpartum infertility (poor fertilization). After more than 5 insemination services failed to result in a pregnancy, farmers claimed their animals are infertile. The suspected infertile animals were simply culled and then sold to local traders for slaughter houses at much lower prices than bulls.

Hematological, biochemical and mineral values in blood: Hematological values, total protein and mineral concentration in blood samples are presented in Table 2. There was a significant difference in certain hematological values, total

Table 1: Mean performance of normal reproductive exotic cows in Payakumbuh, West Sumatra, Indonesia

	Farm size			
Variables	Small	Medium	Large	Mean
Service period (days)	120.00±30.00*	136.36±31.07	80.00±15.49	117. 27±35.75
Service/conception	1.91±1.04	2.09±0.83	1.67±0.62	1.86±0.82
Calving interval (month)	16. 50±1.08	14.25±1.53	14.75±1.98	14.98±1.83

*Standard deviation

Table 2: Hematological, biochemical and mineral values of exotic cattle with different reproductive statuses

	Reproduction status of cattle			
Parameters	Heifer	Pregnant cows	Reproductive failure	Normal Standard
Hematological values				
Hb (g dL^{-1})	10.12 (0.51)*[a]	9.04 (0.67)[a]	6.42 (1.31)[b]	9.7-13.7
MCHb (g dL^{-1})	39.34 (0.44)[b]	40.88 (0.58)[b]	43.14 (0.80)[a]	33.0-37.0
HCT (%)	25.78 (1.35)[a]	22.20 (1.54)[a]	15.20 (3.16)[b]	23.0-43.0
RBC ($\times 10^6$ μL^{-1})	6.11 (0.39)[a]	5.16 (0.55)[a]	3.38 (0.71)[b]	5.30-7.90
WBC ($\times 10^3$ μL^{-1})	13.86 (2.29)[a]	11.96 (1.24)[a]	6.56 (2.19)[b]	5.2-9.8
Total protein (mg dL^{-1})	7.56 (0.37)[a]	7.22 (0.13)[a]	6.52 (0.45)[b]	6.5-8.5
Serum mineral concentration				
Total Ca (mg dL^{-1})	10.26 (0.43)	10.16 (0.44)	10.56 (1.01)	8.5-11.5
Total P (mg dL^{-1})	7.80 (1.44)	5.04 (0.91)	6.66 (0.68)	3.2-6.0
Total Mg (mg dL^{-1})	2.56 (0.16)	2.72 (0.17)	2.18 (0.34)	1.8-3.2
Fe (ppm)	2.56 (0.18)[a]	2.27 (0.19)[b]	2.68 (0.09)[a]	<1.10**
Zn (ppm)	2.60 (0.44)	2.50 (0.37)	2.26 (0.28)	<0.80

***(SEM): Standard error of the mean, **Critical level suggested for cattle by McDowell[16], [ab]Values in the same row with different superscripts are significantly different ($p<0.05$)

Table 3: Botanical species diversity and composition in forages offered to cattle

Grasses	(%)	Broadleaves and ferns	(%)
Pennisetum purpureum	37.78	*Asystasia gangetica*	2.88
Axonopus compressus	14.93	*Mikania micrantha*	1.77
Panicum maximum	7.89	*Angeratum conyzoides*	0.45
Brachiaria decumbens	3.91	*Phillanthus nirruri*	0.36
Imperata cylindrica	3.35	*Pluchea indica*	0.35
Themeda gigantae	3.32	*Plumeria acaminate*	0.33
Lophaterum gracile brongn	1.75	*Stachytarpheta*	0.30
Cyperus rotundus	1.20	*Wedelia biflora*	0.19
Megathyrsus maximus	0.26	*Crassocephalum crepidioides*	0.17
Leersia nexandra	0.15	*Euphorbia hirta*	0.15
Cynodon dactylon	0.05	*Chorcorus acutangulus*	0.13
Hedyotis corymbosa	0.03	*Cychas rumphii*	0.05
Setaria plicata	0.03	*Peperomia pellucida*	0.04
Cynodon plectostachyus	0.02	*Nerium oleander*	0.03
Synedrella nodiflora	0.01	*Synedrella vialis*	0.03
		Marsilea drummondii L.	0.01
Subtotal	74.68		7.24
Legumes		**Agricultural by product**	
Arachis pintoi	0.52	Rice straw	14.55
Centrosema pubescens	0.01	Cornhusk	3.00
Subtotal	0.53		17.55

protein, certain minerals (Ca, P, Mg and Zn) between healthy heifers and pregnant cows. Cattle in the reproductive failure group showed significantly ($p<0.05$) lower Hb, HTC, RBC and WBC but higher in MCHb values compared to heifers and pregnant cows. Mean Hb, HCT, RBC and total protein content in the reproductive failure group were under the normal range, while the MCHb of heifers, pregnant and reproductive failure groups was higher.

Ca (10.16-10.56 mg dL^{-1}), P (5.04-7.80 mg dL^{-1}) and Mg (2.18-2.72 mg dL^{-1}) levels in blood plasma were also found to be in the normal range in all animals. In terms of trace elements, mean Fe (2.27-2.68 ppm) and Zn (2.26-2.60 ppm) concentrations in blood plasma were above critical levels of 1.1 and 0.80 ppm, respectively (Table 2). The Mn and Cu trace mineral content in blood plasma was below detectable limits compared to the accepted standard concentration of 0.005 mg L^{-1}.

Diversity and mineral composition of predominant forages: The diversity of fodder feeds is presented in Table 3. Breeding cattle in the Payakumbuh region largely depend on wild grasses and agricultural by-products. About 35 plant species were identified in forage feed to the smallholder cattle in the current study. Forages were mainly composed of grasses (75%), broadleaves and ferns (7%) and agricultural byproducts (18%) and very low proportion of legumes (about 0.5%).

Table 1 shows the mineral content of the four predominant plant species fed to beef cattle in Payakumbuh. There was no statistically significant difference in the content of Mg, Fe, Zn, Mn, or Cu amongst the predominant forages. *Panicum maximum* (*P. Maximum*) contained the highest Ca concentration (11.2 g kg^{-1} DM, $p<0.05$), while *Axonous compressus* and *P. maximum* had the highest concentration of P (7.08 and 7.05 g kg^{-1} DM, respectively). However, the overall Ca, P and Mn concentrations in forage samples were much lower than the standard requirements of breeding cattle.

DISCUSSION

Reproduction is a basic economic parameter of productivity and competitiveness of the small cattle farms. In particular, calving intervals are the most important reproductive parameter. Here in, the mean calving interval over a 15-month period in normal reproductive cows (Table 1) was found to be in range with that reported in Australia (13-15 months)[9] and the Sukoharjo district of Central Java, Indonesia (13-14 months)[10]. The reproductive performance of normal exotic breeds raised by small farms is better than that at the National Breeding Center for Exotic Beef Cattle in Padang Mangatas[2]. Talukder *et al.*[3] reported that the mean calving interval of Holstein-Frisian crosses at University Dairy Farm in Bangladesh was about 530.9 days (17.7 months).

However, the female exotic breeds raised by traditional small farms are susceptible to reproductive disturbances that result in failure to produce calves. The reproductive failure of exotic females not only causes serious economic loss, but also hinders the government efforts to achieve national beef self-sufficiency. These cases of reproductive failure are suspected to be a key factor causing high numbers of exotic cows to be slaughtered in the productive-age period in the

Table 4: Mineral composition of predominant forages offered to cattle

	Plant species				
Minerals	*Pennisetum purpureum*	*Axonopus compressus*	*Rice straw*	*Panicum maximum*	Requirement Standard*
%	37.78	14.93	14.55	7.49	
Ca (g kg^{-1} DM)	8.96 (5.17**)^{b}	8.79 (5.07)^{b}	8.27 (4.77)^{b}	11.18 (6.46)^{a}	18-24
P (g kg^{-1} DM)	4.67 (2.70)^{b}	7.08 (4.09)^{a}	2.84 (1.64)^{c}	7.05 (4.07)^{a}	13-17
Mg (g kg^{-1} DM)	4.22 (2.44)	4.42 (2.48)	4.72 (2.72)	4.58 (2.64)	<1.0
Fe (mg kg^{-1} DM)	53.00 (30.60)	58.17 (33.58)	50.47 (29.14)	67.46 (38.89)	<50
Zn (mg kg^{-1} DM)	44.87 (25.98)	34.84 (20.12)	47.26 (27.29)	40.80 (23.55)	<30
Mn (mg kg^{-1} DM)	26.55 (15.33)	25.46 (14.70)	26.52 (15.31)	29.05 (16.77)	<40
Cu (mg kg^{-1} DM)	54.15 (31.26)	49.50 (28.58)	51.49 (29.7[illegible])	67.36 (34.10)	<8

*Requirement standard suggested for cattle by National Research Council (NRC)[13] and McDowell[16], **(SEM): Standard error of the mean, [ab]Values in the same row with different superscripts are significantly different (p<0.05)

Payakumbuh region. The results of a survey on 234 female cattle slaughtered in Payakumbuh conducted in 2015 found that about 79% were productive female Simmental and Limousine[11], while the results of another survey on the availability of inedible bones from 30 slaughtered cows conducted in 2016 at the same location found that 80% (24 heads) were Simmental and Limousine[12].

Current data suggest that reproductive failure assessed here in was likely not caused by microbial infections leading to endometritis or anestrus[3], because the mean WBC value of the reproductive failure group was the lowest. Reproductive disturbances were most likely associated with lack of proper feed and deficiency of essential nutrients, especially proteins and minerals. Hematological data, total protein and mineral content in blood samples revealed that cows in the reproductive failure group had significantly (p<0.05) lower Hb, HTC, RBC and WBC but higher in MCHb values compared to heifers and pregnant cows. Furthermore, the mean concentration of total protein in the reproductive failure group was significantly lower than that in heifers and pregnant cows (Table 2).

Livestock raised by small farms are almost entirely dependent on feeds consisting of grasses and crop residues (Table 3). Supplementation of ruminants with concentrated feeds or minerals is not a common practice. The reproductive failure cows were normally fed more low-quality forages, such as rice straw, which contain low protein and high fiber. Rice straw accounted for about 14.6% of all 35 plant species identified in forages (Table 3), containing about 6.0% crude protein and 41.6% ADF (acid detergent fiber)[13]. This is much lower compared to the minimum standard of crude protein required for breeding beef cattle (8-11%)[14]. The other species identified in forages included napier grass, wild grasses, broadleaves and ferns (Table 3), which mostly come from diverse sources of undeveloped pastures, like plantation areas, river banks, rice fields, forest edges and roadsides. These wild grasses vary in nutrient content and are often of poor quality, usually being high in fiber and deficient in protein and minerals[15,16]. Moreover, fodder feeds also contained a very limited amount of legumes (0.5%, Table 3).

Various essential minerals (Ca, P, Cu, Co, Se, Mn, I, Zn and Fe) can influence the reproductive performance of ruminants[17]. Reproductive failure may be induced by deficiencies of a single or multiple trace elements[17]. Considering the mineral composition of predominant forages in the current study (Table 4) and standard requirements of breeding cattle[14], the results suggest that these animals were deficient in Ca and P. The normal levels of Ca and P in the blood are due to Ca-P homeostasis[18]. The mean concentrations of Ca and P in the predominant forages ranged from 8.3-11.2 g kg^{-1} DM for Ca and 2.8-7.1 g kg^{-1} DM for P, while the standard requirement of dietary Ca and P is 18-24 and 13-17 g kg^{-1} DM, respectively, for proper maintenance and production[14] (Table 4). Deficiency of these minerals in breeding cattle are often associated with the incidence of reproductive disorders, such as dystocia, retention of placenta, prolapse of uterus and embryonic death[17,19,20]. Previous studies on the mineral composition of wild forages conducted by Khalil *et al.*[15] showed that Ca content ranged between 7 and 7.7 g kg^{-1} DM, Na (11-14 g kg^{-1} DM) was the most abundant, followed by Mg (8.7-9.5 g kg^{-1} DM) and K (8.3-8.9 g kg^{-1} DM), while P was found to be the lowest (0.5-1.3 g kg^{-1} DM).

Even though the predominant forages contained relatively high Fe and the Fe concentration in reproductive failure cows was higher than that in pregnant cows and not significantly different from heifers (Table 2), reproductive failure animals were suspected of anemia in the current study. The mean concentrations of HBG, HCT and RBC in the reproductive failure group were also found to be much lower than the normal standard values (Table 2). According to Rosenfeld and Dial[5], an anemic patient will have decreased

Hb, HCT and erythrocyte counts. Moreover, Fe deficiency anemia is associated with hypochromasia (decreased MCHb) and in most cases of nonregenerative anemia, the MCHb is within normal limits because there are few reticulocytes present[5].

Moreover, Mn and Cu in blood plasma were undetectable compared to the standard concentration of 0.005 mg L^{-1}. Both of these trace minerals play an important role in cattle reproduction. In females, Mn functions to properly regulate synthesis of ovarian hormones[21]. Signs of Mn deficiencies include poor growth and skeletal deformities in newborn calves and reproduced fertility and abortion, in adult cows[21-23].

Cu deficiency is reported to be responsible for early embryonic death and resorption of the embryo, increased incidence of retained placenta and placental necrosis and low fertility associated with delayed or depressed estrus[17]. The Mn concentration (25.5-29.1 mg kg^{-1} DM) in the predominant forages in the current study was found below the critical level of 40 mg kg^{-1} DM (Table 4). Marginal deficiency of Mn was also observed in developed grazing cattle and below the critical level at the Padang Mangatas National Breeding Center for Exotic Beef Cattle[2] and natural pastures in South Sulawesi, Indonesia[24].

Furthermore, reproductive failure, especially anestrus problems was suspected to be caused by ovarian abnormalities resulting from the deficiency of some essential dietary nutrients. Talukder *et al.*[3] observed that about 80% of anestrus cow in Bangladesh were caused by ovarian abnormalities, such as hypoplasia, deformed ovaries and cystic ovaries. Hematological, biochemical parameters and mineral profiles of reproductive failure cattle in the present study may aid evaluation of the normal development function of reproductive organs in relation to nutrition, housing, environment, disease and management. Prevention and treatment of reproductive abnormalities are essential to ensuring the productivity of small farming enterprises. Farm owners need to be empowered to feed their livestock with adequate nutrition to encourage heavy-breeds to reach their genetic reproductive potential, alleviate the negative effects of a harsh physical environment and minimize the effects of poor management techniques. Therefore, comprehensive investigation of the relationship between reproductive organs with the feeding practices and health status of imported heavy breeds raised by traditional smallholders is needed.

CONCLUSION

Anestrus and repeated insemination were found to be the major causes of reproductive disorders in exotic breed cattle under small farm conditions that results in delay or failure to produce calves. Cows having reproductive troubles showed lower Hb, HCT, WBC and total protein and higher MCHb levels, as well as deficiencies in Ca, P and Mn compared to heifers and pregnant cows.

SIGNIFICANCE STATEMENTS

This study shows convincing evidence that reproductive disturbances in exotic cattle raised on traditional small Indonesian farms were most likely caused by malnutrition which adversely affect reproduction. Current results imply that comprehensive investigation on ovarian dysfunction, health, nutrition and metabolic status are needed for better understanding of reproduction problems.

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