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Blood biochemical levels of reproductive disorders cases in Limousine Crossbred cows

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ABSTRACT

This study determined the differences in the biochemical blood levels of Limousine crossbred cows with reproductive disorders and cows that successfully become pregnant. Limousine crossbred cows (45) were artificially inseminated, and observed for reproductive disorders after 42 days. The observation was conducted by using rectal palpation and Ultrasonography. The cows were grouped into pregnant cows and cow with reproductive disorder (infertile). The cholesterol level, calcium, total protein, and blood glucose were analyzed and statistically tested by t-test using Microsoft Excel 2019 program. The observation of cows was divided into two groups, eight of 45 were normal or pregnant and 37 of them had reproductive disorder: 8 of repeat breeding, 10 of ovarian dysfunction, 11 of cystic ovary, and 8 of silent heat. The total cholesterol of Limousine crossbred cow varied, pregnant cows demonstrated higher cholesterol than those with the reproductive disorder. The blood protein, calcium, and glucose concentration also showed significantly low concentrations in Limousine cow with reproductive disorders. In conclusion, cows with reproductive disorders were marked by low cholesterol, calcium, total protein, and glucose levels.

Keywords: Biochemical, Blood, Limousine crossbred cow, Reproductive disorders

One of the causes of low reproductive success is the maintenance of cows by the rural farmer, which is generally traditional. Cow rearing involves animals prone to reproductive disorders due to cattle breeds that are not appropriately chosen, and the feed given which is minimal regardless of nutritional value (Lestari *et al.* 2014). Compliance with dietary needs is vital to express genetic potency optimally (Beigh *et al.* 2017).

The role of nutrition is quite important in the reproductive performance of cows which influences fertility especially at various stages of the reproductive cycle (Pradhan and Nakagoshi 2008), for the production of Luteinizing Hormone (LH) which serves to stimulate follicular growth (activated ovary function) so that estrus occurs, if nutrition is lacking it results in delayed onset of estrus (Budiawan *et al*. 2015 and Prasetiani *et al.* 2015). Imbalanced nutrition can adversely affect various stages of the reproductive process (Ramandani and Nururrozi 2015), including post-birth reproductive functional growth (Pradhan and Nakagoshi 2008).

Reproductive disorders usually happen in beef cattle breeding due to nutritional deficiency that causes non-optimal ovary activity, hormone disorders, low body condition score, and long calving interval, which in the end results in low reproductive efficiency (Prihatno *et al.* 2013).

The physiological and nutritional status of cows can be assessed by analyzing biochemical blood parameters (Ashmawy 2015). Abnormalities in blood biochemical values are indicative of reproductive disorders in cows (Bazzano *et al*. 2016). Therefore, blood biochemical components play an important role in maintaining reproductive functional physiology and other body systems. If any component such as cholesterol, protein content, calcium, and glucose changes, it will lead to metabolic disturbances in the body that will eventually lead to pregnancy failure.

MATERIALS AND METHODS

Animals: The experimental animals were 45 Limousine crossbred cows selected randomly, the criteria being that the cow was not pregnant, had never given birth, had a body condition score (BCS) of 4-5 on a scale of 1-9, and was 3-5 years old. Artificial insemination was performed after the cow showed signs of oestrus (discharge of cervical mucus). Rectal palpation and/or ultrasonography (USG) were performed after artificial insemination at 42 days of age to observe pregnancy. Subsequently, the cows that did not become pregnant and the cows that successfully became pregnant were divided into groups.

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Serum collection: The analyzed blood sample consisted of cows that did not become pregnant and cows that did. Blood sampling was performed in the morning (approximately 07:00-09:00 AM). The blood sample was collected through the jugular vein using a Venoject needle, which was then inserted into a Vacutainer tube and placed in a cold box. The blood sample was then centrifuged at a speed of 2500 rpm for 15 min. The resulting serum was then placed in an eppendorf tube and stored at -20°C in the freezer before analysis (Thasmi *et al.* 2021).

Cholesterol analysis using CHOD PAP method (Cholesterol Oxidase-Peroksidase Aminoantipyrine Phenol): Cholesterol is released from lipoproteins by the cholesterol esterase enzyme, which is oxidized to H_2O_2 assisted by the cholesterol esterase enzyme. A positive test turned red, indicating H_2O_2 oxidized reaction with phenol (Panil 2008).

Glucose analysis using Hexokinase method: The Hexokinase method is a gold standard blood sugar level examination, using examination material in the form of blood by glucometer. The procedure was carried out using automatic instruments for blood glucose analysis using 340 nm wavelength; 1 cm diameter cuvette, 20-25°C/37°C temperature; measurement against reagent blank.

Total protein analysis using Biuret method: Total plasma protein was estimated using the Biuret method (Biuret, Colorimetric kit). The Biuret reagent was prepared by adding 0.45 g sodium potassium tartrate, 0.15 g $CuSO_4$. $5H_2O$, 0.25 g potassium iodide and 0.4 g NaOH in 50 ml of water. Furthermore 18 ml was added into 200 µl sample on test tube and beaten until homogeneous (vortex) and was left at room temperature for 20 min. It was then observed at 550 nm wavelength.

Blood calcium analysis using CPC (Cresol Phtalein Complex) method: In the CPC blood calcium test, calcium ions reacted with o-cresolphthalein complexone in the base to establish a purple complex. This absorbance complex was proportional to calcium concentration of sample.

Resultant data of cholesterol levels, calcium, protein total, and blood glucose were analyzed by t-test using Microsoft Excel 2019 program to compare blood biochemical rates of normal cows (pregnant) and reproductive disorders cows (not pregnant). For all of the parameter Tokyo Boeki TMS 50i tool was used.

RESULTS AND DISCUSSION

The reproductive performance of beef cattle was influenced by the amount and quality of feed. Livestock-raising schemes on people's farms with cost feed given depended on feed availability in the field, including climate. The primary need of a pregnant cow was nutrient availability for a fetus. The underweight cow could distemper the fetus. Deficiency of certain nutrients at a critical stage of embryo growth could involve weight disorders at birth (Kerl 1982).

Nutritional value of feed: Nutritional balance of cattle plays an important role in reproductive function and cattle fertility because nutrition effect and/or alternative energy on this reproductive function had been long suspected to be mediated by a metabolic signal which connected adipose supply with neuroendocrine function (El-Khadrawy *et al.* 2011). The display of the consumption data of the feed nutritional value is in Table 1.

Table 1. Nutritional consumption data of Limousine crossbred cow

Nutrition	Value
Dry Matter (DM) (kg/head/day)	10.16±0.98
Crude Protein (CP) (kg/head/day)	0.68±0.02
Total Digistible Nutrient (TDN) (kg/head/day)	4.78±0.29

Nutrients: ME : Metabolism Energy Standard of cows' nutrient needs weight 380.39±7.79 kg ; DM (kg), 8.50; CP (kg), 0.82; TDN(kg), 5.0 (NRC 2001).

The observation result of nutritional value consumption showed that nutritional value (CP and TDN) is under NRC standard. Protein availability in beef cattle feed was crucial as the main component of organ growth and development and hormone synthesis (Kearl 1982). Nutrition directly impacts the reproductive status of ruminants (Bindari *et al.* 2013). Protein, fat, and minerals were used to fulfill the energy. Low-nutrition feed led to pregnancy failure and infertility (Amin 2014). Khan *et al*. (2010) also reported that protein deficiency reduced the functional gonadotropin hormone.

Blood biochemical levels: Blood biochemical levels of cholesterol that circulated in blood flow were very important to build and maintain important parts of cells (like cell membrane) and make some important hormones (sexual hormone, aldosterone, etc.) (Ahmad *et al.* 2004). Table 2 shows cows' identification results that experienced reproductive disorders and those who were pregnant.

Table 2. Blood cholesterol levels (Average ± SEM) in Limousine crossbred cows

Reproductive parameter	N (head)	Cholesterol (mg/dL)	P
Normal			
Pregnant	8	132.00±9.03[a]	p=0.005
Reproductive disorders (not pregnant)			
Repeat Breeding	8	103.63±6.55[b]	p=0.023
Ovarian hypofunction	10	92.30±4.38[b]	p=0.0005
Cystic ovary	11	90.92±4.23[b]	p=0.0002
Silent heat	8	92.38±3.80[b]	p=0.001

[a, b]Different notations in the same column showed significant differences ($p<0.05$) between treatments.

According to the statistical analysis result, pregnant cows' cholesterol levels showed a significant difference ($p<0.05$) from cows with reproductive disorders; serum cholesterol levels of cows with reproductive disorders were lower than pregnant cows. Cholesterol is a compound that functions in the formation of body cells, is the precursor in steroid hormone synthesis, vitamin D, and bile salt (Charlton-Menys and Durrington 2008). Furthermore, Salmanoglu *et al.* (1997) said that cows showed irregular estrus cycles or anestrus cows had lower cholesterol levels than their normal counterparts, as cholesterol is the main ingredient constructed from progesterone.

Blood calcium levels of pregnant cows showed a significant difference (p<0.05) (Table 3). Magnus and Lali (2009) said that blood calcium levels as much as 7.0±0.43 mg/dL affected hypocalcemia. Blood calcium had an important role in physiological process settings and biochemicals, including neuromuscular excitability, blood coagulation, secretion process, membrane integrity, membrane transport plasma, enzyme reaction, hormone release, and neurotransmitter also intercellular numbers of hormones (Granner 2003). The main source of calcium was feed. The mineral contained in the feed is absorbed by the intestine from the mucosal surface by cells in particular from a number of microphilia, then entered intestinal cells' cytoplasm (Cunningham *et al*. 2005).

Table 3. Blood calcium levels (Average ± SEM) of Limousine crossbred cows

Reproductive parameter	N (Head)	Calcium (mg/dL)	p
Normal			
Pregnant	8	9.26±0.20[a]	p=0.05
Reproductive disorders (not pregnant)			
Repeat Breeding	8	8.69±0.17[b]	p=0.048
Ovary hypofunction	10	8.58±0.23[b]	p=0.040
Cystic ovarium	11	8.60±0.18[b]	p=0.024
Silent heat	8	8.48±0.28[b]	p=0.034

[a, b] Different notations in the same column showed significant differences (p<0.05) between treatments.

Table 4. Protein total levels (Average ± SEM) of Limousine crossbred cows

Reproductive Parameter	N (head)	Protein total (g/dL)	p
Normal			
Pregnant	8	7.72±0.36[a]	p=0.05
Reproductive disorders			
Repeat breeding	8	6.70±0.19[b]	p=0.028
Ovary hypo-function	10	6.79±0.16[b]	p=0.024
Ovary Cystic	11	6.94±0.11[b]	p=0.035
Silent heat	8	6.86±0.11[b]	p=0.046

[a, b,] Different notations in the same column showed significant differences (p<0.05) between treatments.

According to statistical analysis, protein total levels (Table 4) in pregnant cows' blood and cows experiencing reproductive disorders showed significant differences (p<0.05). According to Cheeke (2005) when cows gave birth, a lack of protein ratio could affect long anestrus postpartum. Furthermore, it may impact on the emergence of silent estrus, anestrus, repeat breeder, death of embryo, and the birth of a weak cow or premature birth (Boland and Lonergan 2003). As per Barson *et al.* (2019), cows that had a protein total lower (6.815 mg/dl) than normal cows would have reproductive disorders.

Blood glucose levels (Table 5) in cows' serum experiencing reproductive disorders (not pregnant) and those pregnant, showed significant difference (p<0.05). Cows that experienced reproductive disorders had blood glucose levels lower than pregnant cows. Cows experiencing repeat breeding had serum glucose levels (43.00 mg/dL) lower than normal cows (Barson *et al.* 2019). A low glucose concentration exacerbates the reproductive hormone function disorders. The disruption of hormone has domino effect on reproductive tract and then caused follicular, oocyte and embryo development failure. Furthermore, this condition would affect embryo death and lack of fertilization caused repeat breeding (Ramandani and Nururrozi 2015).

Table 5. Blood glucose levels (Average ± SEM) of Limousine crossbred cows

Reproductive parameter	N (head)	Glucose (mg/dL)	p
Normal			
Pregnant	8	54.11±1.48[a]	p=0.05
Reproductive disorders			
Repeat breeding	8	46.88±1.60[b]	p=0.005
Ovary hypo-function	10	47.80±1.25[b]	p=0.0044
Ovary Cystic	11	49.45±1.26[b]	p=0.0267
Silent heat	8	49.50±1.21[b]	p=0.0311

[a, b,] Different notations in the same column showed significant differences (p<0.05) between treatments.

Glucose is an essential macromolecule in cattle for promoting energy sources such as ATP (Boland and Lonergan 2003). Low serum glucose levels in cows inhibited producing gonadotropins releasing hormone (GnRH). Low glucose also obstructs follicle-stimulating hormone (FSH), luteinizing hormone (LH), which interferes in follicular development. Lack of nutrition reduces the steroid hormone in the ovarium. This triggers the disruption of the ovum, embryo, and foetus death (Prihatno *et al.* 2013). Cholesterol, calcium, blood glucose, and protein, indicate low nutrient intake with the ratio administered, both in quality and quantity. This condition strongly affects the reproductive system (Thasmi *et al.* 2021).

In conclusion, Limousine crossbred cows that were not pregnant due to reproductive disorders had lower cholesterol, calcium, total protein, and glucose levels than pregnant cows (normal).

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