

Changes in Blood Calcium Concentrations in Bali Cattle During the Periparturient Period

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Abstract. Calcium is a mineral that plays an important role in animal health, production, and reproduction. Calcium requirements depend on breed, age and parity, pathophysiological status, body condition score, gestation status, and lactation. During the periparturient period, the cows struggle to maintain calcium homeostasis, and if unsuccessful, the animal will have hypocalcemia. Hypocalcemia has known as a predisposing factor for reproductive disorders and infectious diseases. This study aimed to investigate changes in blood calcium levels in Bali cattle during the periparturient period. Three ml blood samples from nine months healthy pregnant cows were taken through the jugular vein for three times periods, namely in the last three weeks of gestation, on day parturition, and three weeks after calving. Blood samples were processed by the wet digestion method and calcium concentrations were measured by the flame atomic absorption spectrometric method. The results showed that the average blood calcium levels were 9.57 ± 1.26 mg/dL, 4.11 ± 0.63 mg/dL, and 8.32 ± 1.23 mg/dL, respectively. These results indicate that changes in calcium levels in the blood, normal value for the last three weeks of gestation were decreased significantly, and increased after three weeks of calving. New evidence regarding the physiological data of Bali cattle was founded with subclinical hypocalcemia.

Keywords: Blood calcium, Periparturient period, Bali cattle, Hypocalcemia

I. INTRODUCTION

Bali cattle (*Bos sondaicus*), as livestock, is a beef cattle native to Indonesia, with good growth, adaptable to the microclimate environment, good infertility and conception rates, and good quality meat [1], [2], [3]. Farmers were keeping their livestock in cages to get good quality meat. The main feed sources are fresh grass and legumes from the surrounding of the stall and are usually of the same type. Lack of diversity in feed sources raises the risk for livestock that have insufficient nutrients, such as minerals [4].

The livestock needs many minerals known as essential minerals, which are composed of macro-mineral and micro-mineral. Macro-mineral is a nutritional component that has an important role in the growth, health, production, reproduction and immunity of animals. The macro-minerals include; calcium, phosphorus, magnesium, potassium, sodium, chloride and sulfur [5]. Reproductive function is directly affected by calcium and phosphorus. Almost 99% of calcium were stored in the bones, and the remaining 1% of calcium ions which are bound to serum proteins form complexes with organic and inorganic acids [6]. The normal total blood calcium concentration for

Holstein-Friesian breeds are 8.42-11.2 mg/dl [7], adult dairy cows 8.5-10 mg/dl [8], [9], and in adult Bali cattle 11.88 ± 3.03 mg/dl [10]. The level of mineral needs of cattle varies depending on breed, age and parity, pathophysiological status, body condition score, pregnancy status and lactation [11]. In periparturient cattle are needed feed with high calcium which is a transition period that starts three weeks of pre-parturition until three weeks post-parturition [12]. In the last three weeks of pregnancy were a process of fetal bone calcification and the formation of colostrum milk. Meanwhile, dairy cows need calcium at least 20 gram to produce 10 kg of colostrum in 24 hours [6]. In case the mineral intake in the ration is insufficient and the animal fails to maintain blood calcium balance, hypocalcaemia will occur [8] [13].

Hypocalcaemia can be subclinical and clinical symptoms. Subclinical hypocalcaemia increases the risk of dystocia, fetal membrane retention, uterine prolapsed and prolonged uterine involution, abomasal displacement, and decreased immune system as well as being a predisposing factor for infectious disease [14]-[17]. Similar events are often found in Bali cattle, where cases characterize

hypocalcaemia. However, it is unfortunate that there are no publications that investigate changes in calcium levels in Bali cattle blood at each stage of the birth period. This study aims to investigate changes in blood calcium concentrations in Bali cattle during the periparturient period. The benefits of this study add to the physiological data information of Bali cattle so that it can be used as supporting data in preventing reproductive disorders related to mineral balance. Improved reproductive health will have a direct impact on increasing the population of Bali cattle.

II. MATERIAL AND METHODS

Ethical Approval

This research was accepted in the Ethical Commission for the Use of Animals in Research and Education of the Faculty of Veterinary Medicine, Udayana University, Indonesia with approval number is 3239a/UN14.2.9/PD/2019.

The Animals and Locate of Research

Ten healthy Bali cattle in nine months of pregnancy were selected by purposive sampling. All cows from smallholder farms kept in cages, fed with conventional feed sourced from horticultural plantations. Sampling location was in

Badung Regency, Bali Province, Indonesia. Examination of serum calcium levels in Bali cattle was carried out at the Analytical Laboratory of Udayana University.

Collection of Blood Samples

Samples were taken three times, namely at three weeks last of gestation, on the day of parturition and three weeks postpartum. A 3 ml blood sample was collected aseptically using a vacutainer through the jugular vein. During transportation to the laboratory blood samples were stored in a cool box.

Blood Sample Development

Destruction of blood samples using acid wet digestion method was adopted by Yahaya *et al.* [18], and also the development of models by Pujiastari *et al.* [19]. A 3 ml blood sample was inserted into the kjeldal digestion. Then added 20 ml acid mixture of 97% H₂SO₄ (Brata Chemical, Indonesia) and 97% HNO₃ (Merck, Germany) in a ratio of 1:1 (v/v), and left it for 10 minutes. This acid liquid was then heated until a half-dark dry mass was formed, cooled and diluted by adding 1-2 ml of HNO₃. The digestion was then evaporated by heating at a temperature of 60-70 °C for 1-2 hours until all organic matter was oxidized or the solution changes colour to light, let it cool. Next dilute with

10 ml of aquadest and heat until smoky, and let it cool. Furthermore, again diluted with 5 ml of aquadest and heated until smoky, and let it cool. The cold digestion liquid was then diluted, and blood calcium levels measured using the flame atomic absorption absorbance method. The results of blood calcium measurements were in units of mg/dl.

Data Analysis

The data of blood calcium concentrations will be presented in Mean (\pm SD), and analyzed using One Way Analysis of Variance followed by Duncan's test.

III. RESULT

The mean of serum calcium concentration of Bali cattle in the prepartum, puerperium and postpartum was 9.57 ± 1.26

mg/dl, 4.11 ± 0.63 mg/dl, and 8.32 ± 1.23 mg/dl, respectively (Tabel 1). Statistical analysis showed that there was significantly different ($P < 0.01$) between the normal value of blood calcium at the last three weeks of gestation with the day of parturition. The normal value of blood calcium in the last three weeks of gestation was decreased. However, there was not significantly different ($P < 0.01$) from the three weeks after calving. Meanwhile, blood calcium concentrations after three weeks of postpartum were significantly different ($P < 0.05$) and higher than the time of calving. The confirmation based on available references, calcium levels on the day immediately after delivery indicated hypocalcaemia [7][8].

Tabel 1. Bloods calcium concentrations of Bali cattle during the periparturient periods (mg/dL)

Various of parturient period	Mean of blood calcium (mg/dL)	Sig.
3 weeks last of gestation	9.57 ± 1.26^a	0.000
On parturition day	4.11 ± 0.63^c	
3 weeks after parturition	8.32 ± 1.23^{ab}	

Note: Different superscript letters towards the column show significant differences ($P < 0.05$)

IV. DISCUSSION

Calcium is an essential macro-mineral which has the most availability in

animals. This mineral plays several vital roles in maintaining metabolism, health, growth, production, reproduction and

immune system. During the transition of the period, cattle struggled to maintain calcium homeostasis. Blood calcium balance is mediated by parathyroid hormone and calcitonin. Decreased extracellular calcium ions are responded to by parathyroid gland through parathyroid hormone secretion to stimulate the formation of dihydroxycholecalciferol (calcitriol) to increase calcium absorption in the intestinal mucous and re-absorption in the kidney's tubules [6][16]. Calcium imbalance is related to dietary factors and is indicated by a low level of calcium in the blood (hypocalcemia), because of not enough calcium intakes then the body has compensated for the resorption of calcium ions from bones. Whereas, the calcitonin provides negative feedback, when calcium is increased in the blood it will respond to increase deposition in bones and prevent to reabsorption from kidneys [20].

In this study, calcium blood levels in the last three weeks of parturition were obtained 9.57 ± 1.26 mg/dL. These levels were within a normal range, as in Holstein-Friesian cows [7], [8]. However, the levels are lower than heifer of Bali cattle [10]. In the last weeks of gestation, the calcium needs increase three-fold for fetal bone

calcification, birth preparation, and colostrum's production. It is apparent in the process of fetal bone calcification of the role of calcium with phosphorus in 1.5-2:1 ratio to form the Hydroxyapatite. Cows will secrete 2-3 g/kg of calcium in the production of milk. It takes plentiful of calcium about 10-15 kg/day in dairy cows and 1.5-2 kg/day in beef cattle to produce of milk, but only 3 g of calcium is circulated in animal blood [6][9]. In a traditional farm, calcium needs are obtained from forage, grass, legumes, and mineral supplements.

Cows with 24-72 hours postpartum can occur hypocalcaemia which is subclinically or clinically. Hypocalcaemia depends on the calcium homeostatic ability of cattle. About 4.11 ± 0.63 mg/dL blood calcium level of Bali cattle on a day of postpartum. The analysis shows the blood calcium has significantly different and has declined compared with parturition which is as sub-clinical hypocalcaemia and have 5.5-8.0 mg/dL of blood calcium levels [8]. Sub-clinically hypocalcaemia is a predisposing factor for dystocia, uterine prolapsed, retained fetal membranes and placenta, abomasal displacement, metritis, mastitis and immune depression [21][22]. The sudden hypocalcaemia causes nervous

system hyperexcitability, reduce the strength of muscle contraction, and resulting in both tetany and paresis. The occurrence of muscle tetany can also be caused by low serum magnesium level. Paresis may occur in cows any ages but is most common in high-producing cows entering in high parity and milk production [8][23].

Clinical hypocalcaemia or parturient paresis usually occurs within 7-14 days. In healthy cows, the process can be faster and is accompanied by an increase in phosphorus serum. In this study, blood calcium levels in Bali cattle after three weeks obtained at 8.32 ± 1.23 mg/dL. Calcium levels were increased significantly until normally (8.8-10 mg/dL) compared to hypocalcemic condition [7]. Maintaining livestock health is very important for reproductive performance during the parturient period. Poor management was caused decrease postpartum recovery and economic losses [24]. Therefore, good livestock management during the transition period contributes to animal health and reproduction. Mineral as a feed supplement can be added to the ration according to the level of livestock needs [25].

V. CONCLUSION

The calcium levels of Bali cattle blood during the periparturient period are fluctuating. The normal value of blood calcium in the last three weeks of pregnancy has significantly decreased on parturition time and three weeks postpartum, then were increased around the normal range. In subclinical hypocalcaemia that cattle were looked normal even though their blood calcium levels decreased. This research data adds new evidence regarding the dynamics of blood calcium in Bali cattle during the periparturient period. Furthermore, more in-depth research needs to be done on the subclinical effects of hypocalcaemia on Bali cattle and appropriate prevention efforts.

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