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Subclinal Hypocalcaemia as a Predisposing Factor of Subclinical Mastitis in Dairy Cow in West Java Province, Indonesia

Herwin Pisestyani[a]*, Mirnawati B Sudarwanto[b], Retno Wulansari[c], Afton Atabany[d]

[a]*Bogor Agriculture University Graduate School, Jl. Raya Darmaga, Kampus IPB Darmaga Bogor, Indonesia 16680*

[b]*Department of Animal Diseases and Veterinary Public Health, Faculty of Veterinary Medicine, Bogor Agriculture University, Jalan Agatis Kampus IPB Darmaga Bogor, Indonesia 16680*

[c]*Department of Veterinary Clinic Reproduction and Pathology, Faculty of Veterinary Medicine, Bogor Agriculture University, Jalan Agatis Kampus IPB Darmaga Bogor, Indonesia 16680*

[d]*Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agriculture University, Jalan Agatis Kampus IPB Darmaga Bogor, Indonesia 16680*

[a]*Email: herwinpi@gmail.com*

[b]*Email: mwanto47@hotmail.com*

[c]*Email: rtiwul@gmail.com*

[d]*Email: afton.atabany@yahoo.co.id*

Abstract

Cows with subclinical hypocalcaemia have low calcium level however do not show clinical sign of collapse. Subclinical hypocalcaemia can be a driving factor behind other diseases, both infectious and metabolic diseases.

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* Corresponding author.

This study aims to measure blood calcium levels of dairy cows from various lactation ages (parity) as well as its relations with subclinical mastitis occurrence and milk production from several small dairy farms in West Java Province. The first thing conducted was subclinical mastitis testing to determine the level of blood calcium from 100 dairy cows examined. Respectively as much as 33 dairy cows were positive for subclinical mastitis with degree of severity of 1 and 2, and 34 cows negative for subclinical mastitis were selected randomly. Blood calcium was examined by using Atomic Absorption Spectrofotometer (AAS) method. A large number of dairy cows in small dairy farms in West Java Province experience subclinical hypocalcaemia with an average calcium level of 8.21 mg/dL. The lowest mean found was from a forth parity cow (7.65 mg/dL) that was in Sumedang. This study shows that cows with low calcium level are cows suffering from subclinical mastitis and has a lower milk production compared to healthy cows ($P<0.05$). Conclusion of this study is that subclinical hypocalcaemia can be one of triggering factors for the occurrences of subclinical mastitis which in the end can lowers milk production.

Keywords: calcium level; milk production; subclinical hypocalcaemia; subclinical mastitis.

1. Introduction

Metabolism is the process of generating energy needed for the growth of the body and daily activity. Feed intake with low quality can cause energy deficit during lactation that disturbs the metabolism capacity which then results in metabolic disease. Metabolic disease is defined as a balance disorder of the internal homeostasis due to abnormal changes in the metabolic process.

Hypocalcaemia is divided into 2 categories that is, clinical and subclinical. Clinical signs of cow suffering from clinical hypocalcaemia is clearly seen, that is cow will collapses right after birth (milk fever). Clinical hypocalcaemia in dairy cows is a very important metabolic disease that impacts the economy and can also increase the incidence of mastitis, fetus death, abomasum dysplasia, dystocia and ketosis which can reduce the dairy cows productivity during its lifetime [1]. Subclinical hypocalcaemia cannot be easily detected because the cow have a relatively low calcium level in its blood (<8.5 mg/dL), but does not collapse. Currently, subclinical hypocalcaemia thought to occur often in small dairy farms and are indicated as one of the predisposing factors of health and reproduction problems. Hypocalcaemia decreases calcium transfer into the sarcoplasmic reticulum, so that it causes reduction of all muscle contraction, including the teat sphincter muscle. Teat sphincter is responsible for the closure of the teat hole after milking or suckling. Lack of teat sphincter closure will lead to an increased risk of mastitis [2]. Subclinical mastitis is one of the infectious disease that is also affected by the metabolic status of the individual dairy cow. Lack of calcium can cause a decrease in the ability of the immune cells to response to microbial infections causes of disease such as mastitis [3].

Blood calcium status in cows with subclinical mastitis in small dairy farms in Indonesia has not been widely reported. Therefore, this study is aimed to determine blood calcium levels in dairy cows with subclinical mastitis and analyze the correlation between the two factors and milk production. This research is expected to provide data from Indonesian FH crossbreed dairy cow, that one of the predisposing factors from subclinical mastitis is subclinical hypocalcaemia status.

2. Materials and Method

2.1. Sample Determination and Collection

Collection of blood and milk sample was conducted from the month of April until June 2015 in several small dairy farms in West Java Province, they are Bogor, Cianjur, Sukabumi, Garut, and West Bandung. As much as 100 dairy cows on their normal lactation period were assigned as samples. As much as 34 cows that was negative to subclinical mastitis, 33 cows positive 1 and 33 cows positive 2 to subclinical mastitis were chosen randomly to be tested for blood calcium levels. Calcium level testing was conducted in a commercial laboratory using the AAS method with samples of serum from blood collected through *vena cocygea.*

2.2. Measuring the Status of Subclinical Mastitis

Measuring of subclinical mastitis status was conducted through 3 testing methods that is direct somatic cell count (SCC/ml) by using Breed method [4], indirect somatic cell count using IPB-1 *Mastitis test* [4], and examination of existing pathogenic bacteria in blood agar media (*blood agar plate/* BAP) by viewing the clear area around the colony of bacteria. The determination of subclinical mastitis status per quarter were based on standards from International Dairy Federation (IDF 1999), SCC >400 cell/ml, finding pathogenic bacteria and on a normal lactation period. Furthermore, degree of severity from cows with subclinical mastitis were determined by these criteria:

0 : Negative, with criteria off all quarters not affected by subclinical mastitis
1 : Positive 1, with criteria of only 1 quarters affected by subclinical mastitis
2 : Positive 2, with criteria of more than 1 quarters affected by subclinical mastitis

2.3. Secondary Data Collection

Secondary data were obtain through interviews with farmers and review of farmers records. Data needed for this study are: lactation period (parity), month of lactation, and daily milk production.

2.4. Management and Data Analisis

Data were analyzed descriptively and with analysis of variance to see any relations or correlation between variables in the study and to determine the association between variables that are ordinal [5] .

3. Results and Discusion

3.1. Blood Calcium in Dairy Cows in West Java Province

Mineral deficiency tends to be chronic so it is hard to diagnose, does not show any specific clinical signs and causes decrease in production and reproduction. One way to diagnose mineral deficiency in cattle is to examine mineral levels by using blood or serum samples. Figure 1 presents the average blood calcium levels in several region which are the center for dairy farms in West Java Provence, and Figure 2 presents the average blood calcium levels of dairy cows according to parity differences.

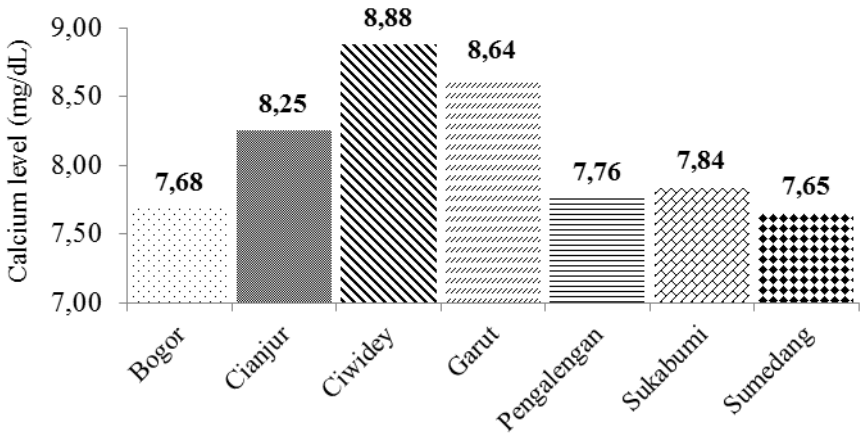

Figure 1: Blood calcium levels of dairy cows in several region from small dairy farms in West Java Province

Average blood calcium level of dairy cows spread in the central of small dairy farms in West Java Provence range from 7.65-8.88 mg/dL (Figure 1). Calcium levels in each region are significantly different ($P<0.05$), with the highest average calcium level (8.88 mg/dL) comes from dairy cows cared for in Ciwidey region, whereas dairy cows with the lowest average calcium level (7.65 mg/dL) comes from dairy cows in Sumedang region. Classification of the health condition of a cow based on the calcium levels, are dairy cow with calcium level under 5 mg/dL will have clinical hypocalcaemia, dairy cow with calcium level 5-8.45 mg/dL is classified as suffering from subclinical hypocalcaemia, and normal calcium levels is within the range 8.5-10 mg/dL [6].

Milk production is greatly influenced by feed consumed and the metabolism. Factors that influence consumption, among others are physical and palatability of feed, while the factor that particularly influence metabolism is absorption of nutrients that are very basic and needed by the body, among others, calcium (1,33%), phosphorus (0,74 %), potassium (0,19%), sodium (0,16%), sulfur (0,15%), chlorine (0,11%), magnesium (0,04%) and ferrum (0,01%). Feed consist of fiber from forage, and consentrate which are source of energy and protein. Low levels of blood calcium of dairy cows in West Java indicates low nutriants in the ration that is given, in terms of both quality and quantity. The main obstical among farmers is irregular availability of good quality forage, so that it is relatively still lacking fullfilment of the cattles need both quality and quantity. Such conditions will affect the production and the quality of milk produced.

Generally, in rainy season, availability of forage is in abundant with a low dry matter, whereas the dry season forage production decreases, in certain areas even do not produce at all. Mineral contents in cattle forage in areas where water availability depends on rainfall really rely on climate and soil conditions in that area. Forage has high contents of calcium during the rainy season, on the contrary in dry season calcium contents in forage are low. Mineral contents in forage also depends on soil condition. Areas with sandy soil have acidic conditions that causes minerals and nutrients to descend under the soil, so that grass that grows have low contents of minerals. Conditions such as this will cause cattle that consume the forage in this area to have mineral deficiency [7] and most of feed substance in Indonesia lack minerals [8]. [9] stated that obstacles faced by dairy farmers in Indonesia is the availability of forage, especially during the dry season, whereas continuity of availability of forage as a source of fiber throughout the year will determine the productivity and reproducibility

of cattle.

3.2. Status of Blood Calcium Levels Based on Parity Differences

Cow normally has enough calcium reserved in the bones (6000 g) as well as from feed intake through digestion (100 g) and only a small amount present in the blood circulation (8 g). Normally, calcium loss will always happen through excretion of urine and feces, this cannot be avoided by dairy cow. The need of calcium during lactation period is 1 g/hour, while cow with high milk production can reach 2 g/hour. Cow that has just given birth and secrete colostrum requires calcium 10 times more compared to normal milk. Calcium in milk originates from calcium in the blood which supplies into the udder to become part of the composition of milk and colostrum. The role of calcium in the body is very important, therefore blood calcium concentration that are lost after being supplied to the udder and exit the body together with milk must be maintained (homeostasis) by a calcium metabolism mechanism. In the event of an interruption in maintaining the concentration of calcium in the blood, decrease in blood calcium concentration will happen. Blood calcium level of dairy cow with lactation age (parity) 1 until 6 is presented in Figure 2

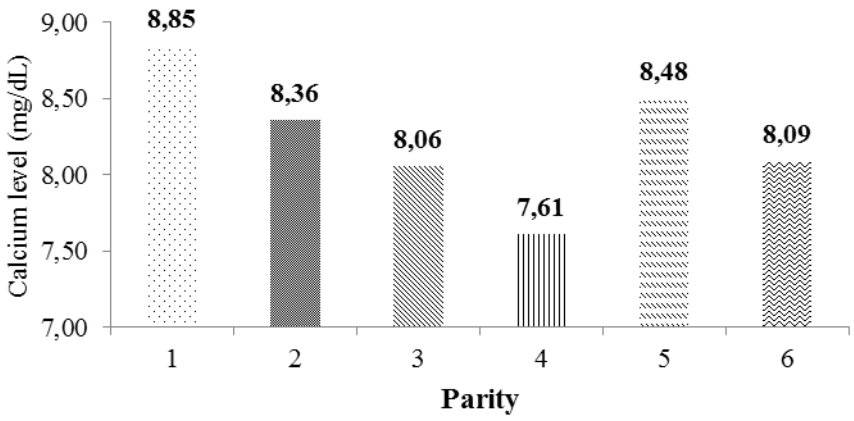

Figure 2: Blood calcium levels of Indonesian dairy cow with different parity in West Java Province

Results of this study shows that most of the dairy cows have an average calcium level below the normal limit, except cows in its first lactation (Figure 2). The lowest calcium level is found in dairy cows in its 4th lactation (7.61 mg/dL), but did not show significant differences between parity groups (p.0.05). This study is in line with the study by [10] that shows the calcium level is lowest in cows in the 4th lactation. Incidence of subclinical hypocalcaemia experience an increase from 2nd lactation to 4th. First parity dairy cows had a normal calcium level (8.85 mg/dL), then decreased starting from the second parity until the sixth, although on the fifth parity calcium level slightly increased, however still below normal. This study shows that the average blood calcium level bellow the normal limit (8.21 mg/dL), therefor dairy cows in West Java can be classified as having subclinical hypocalcaemia.

Normally cows will adapt by adjusting the inflow and outflow pace of calcium, but this adaptation process is not perfect because of transient hypocalcaemia as the cause of the fall of normal calcium from 8.5 mg/dL to <8.0

mg/dL. This incident is experienced by many older cows, during the third and subsequent births. As an animal ages, its general metabolism declines. Older cows bone mineral turnover and also the capacity of the stomach in absorbing calcium, will decrease. The flow rate of food passing through the digestive tract in older cows will also decline.

Subclinical hypocalcaemia cannot be easily detected because cows with relatively low blood calcium levels does not experience collapse. Subclinical hypocalcaemia can be one of the causes of reproduction problems and decrease in productivity in dairy cows in Indonesia. Lack of nutrients has been reported as the main factor that inhibits the production system of cows in tropical areas. Lack of nutrients or insufficient nutrient intake can directly effect the efficiency of reproduction [11], such as low reproduction performance and productivity.

3.3. Subclinical Hypocalcaemia as a Predisposition Factor for Subclinical Mastitis

One of the factors that can affect the success of subclinical mastitis control program is the individual metabolism status of the dairy cow. Hypocalcaemia will affect the reduction of the transfer of calcium into the sarcoplasmic reticulum. This causes a decrease in all muscle contraction, including the teat sphincter muscle. The teat sphincter is responsible for the closing of the teat hole after milking and suckling. Reduction of this closure will cause increase risk of mastitis [2]. One of the factors that affects the condition of the teat sphincter is the presence of calcium levels in the body. Figure 3a and 3b shows the average calcium level in the blood of healthy cows and cows suffering from subclinical mastitis with various severity levels.

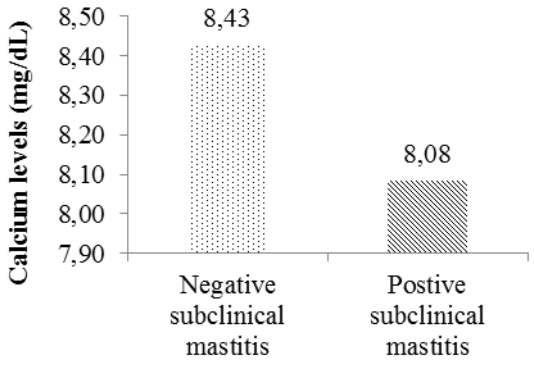

Figure 3a: Status of blood calcium levels of healthy dairy cows and dairy cows suffering from subclinical mastitis

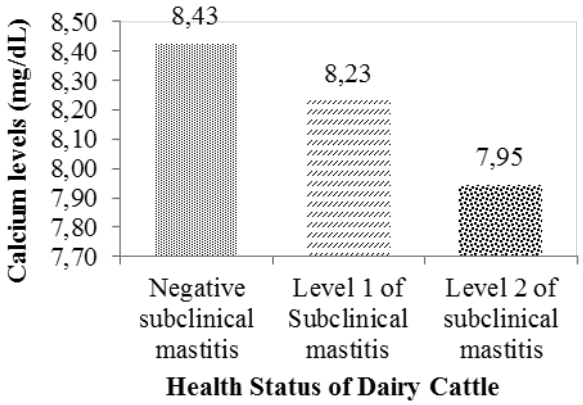

Figure 3b: Calcium level of FH crossbreed dairy cows in various severity levels of subclinical mastitis

Results of blood calcium status of health dairy cows and positive subclinical mastitis with various severity levels show no significant differences ($P>0.05$). Cows with positive subclinical mastitis has an average calcium level bellow normal and is categorized as having subclinical hypocalcaemia. Figure 3b clarifies that dairy cows with subclinical mastitis with severity level of positive 2, shows that the average calcium level are far lower than the other groups (7.95 mg/dL). Dairy cows suffering from subclinical mastitis severity level 2, have an average SCC > 1 000 000 cell/ml, pathogenic bacteria are found, and more than 2 teats with subclinical mastitis. Figure

3a and 3b shows the correlation between subclinical mastitis and low concentration of blood calcium or rather associated with subclinical hypocalcaemia.

Study by [12] shows the relation between strength and rate of intestinal smooth muscle in line with blood calcium concentration. Teat sphincter is composed of smooth muscles, contraction of the smooth muscles will cause the teat hole to close. Clinical or subclinical hypocalcaemia can reduce the strength and contraction rate of the smooth muscle which will interfere with teat hole closure. After milking, the teat hole will open very wide and widen even more in cows with high milk production. Hypocalcaemia sufferers tend to lie down because they are not able to support their body weight, due to weakness of body muscle contraction. With the teat hole being opened and cows tend to lie down, this will increase the possibility of bacteria entering through the teat hole which then becomes the base process of mastitis incidence. While neutrophil and lymphocyte of cows suffering from hypocalcaemia will experience a decline in its immune function [13]. Therefore clinical or subclinical hypocalcaemia increases the risk of subclinical mastitis. Some studies suggest that the risk of mastitis increases 8 times in cows suffering from hypocalcaemia. Hypocalcaemia also triggers stress in dairy cows. An increase of cortisol levels of 3-4 times will occur in dairy cows entering the initiation of parturition. It is found that cortisol increase 5-7 times in cows suffering from subclinical hypocalcaemia when in partus, meanwhile in cows suffering from milk fever it is found that cortisol increases 10-15 times more [14]. High levels of cortisol will cause immunosuppression in cows on its periparturient period and it is thought to occur 1-2 weeks before partus [15].

3.4. The Effect of Blood Calcium Level and Parity towards Udder Health and the Relations between Subclinical Mastitis Status with Blood Calcium Level towards Milk Production

Based on the foregoing discussion it is clear that subclinical hypocalcaemia occurs to dairy cows on the first to sixth lactation, with different amount of milk production and udder health status in the center of dairy farms in West Java Province. To see more clearly the effect of calcium levels from a dairy cow towards subclinical mastitis incidence and a decrease in milk production, therefore further data processing is carried out, hereinafter presented in Figures 4a and 4b

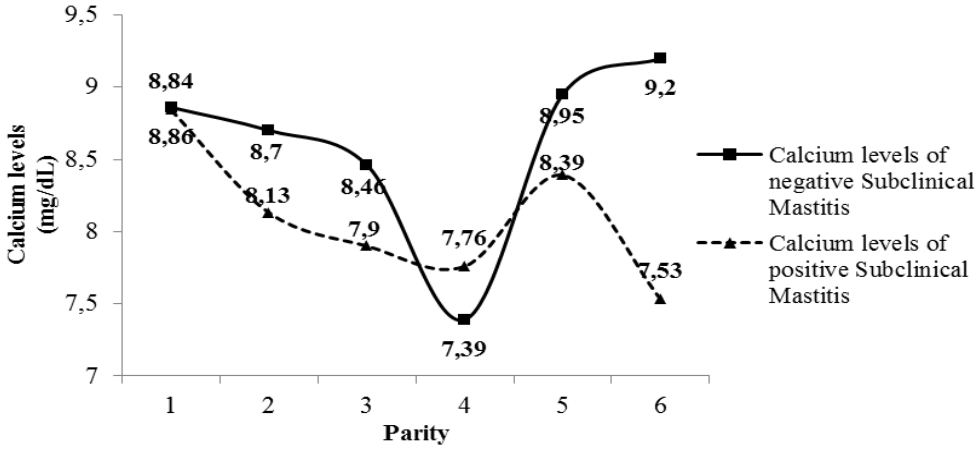

Figure 4a: Correlation between parities and blood calcium level towards udder health of dairy cow

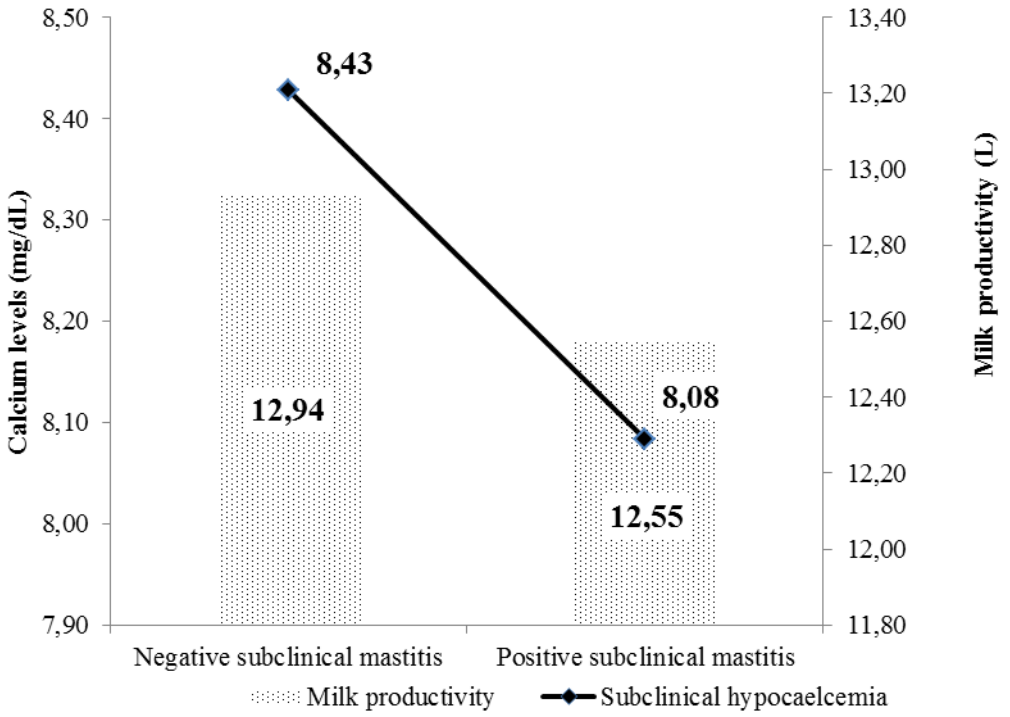

Figure 4b: Correlation between subclinical mastitis status and blood calcium level towards milk production

Dairy cows that tested positive for subclinical mastitis has a lower calcium level compared to healthy cows, that are negative subclinical mastitis. In the forth parity both healthy and subclinical mastitis suffering cows calcium level are the lowest compared to the other parities. Blood calcium levels shown in Figure 4a are in line with the results in Figure 2. When milk production is high, mineral levels in the a cows blood usually decreases. In general dairy cows reaches peak lactation at the age of 6-8 years or on the 4th until 6th lactation, and thereafter decline [16]. Maximum milk production of FH cow in its origin is achieved in the 5th lactation, whereas in tropical areas it can be faster, that is the 3rd lactation [17].

Dairy cows suffering from subclinical mastitis have an average milk production and calcium level lower than healthy cows (Figure 4b). This study also shows that there is a correlation between udder health status with calcium level towards milk production. A healthy cow have a calcium level close to normal (8.43 mg/dL) and average milk production of 12.94 L/day, where as a cow suffering from subclinical mastitis have a 8.08 mg/dL calcium level (subclinical hypocalcaemia) with milk production of 12.55 L/day. According to Figure 1, dairy cows in West Java have an average blood calcium level of 8.21 mg/dL and is bellow the normal limit (8.5-10 mg/L). Most of the dairy cows in West Java suffer from subclinical hypocalcaemia (Figure 2). [18] announced that macro mineral deficiency can cause a decrease in the production and quality of milk. The range of milk production of dairy cows that are kept in the tropics, including Indonesia amounting to 7-15 liters/cow/day [19]. According to studies that have been conducted in concentrated areas of dairy farms in West Java, shows the average milk production per cow per day are, West Java as much as of 13.93 liters/cow/day [19]; Kunak Bogor as much as 9.78 ± 3.56 liters/cow/day [20]; Ujung Berung Bandung as much as 13.62 ± 3.96 liters/cow/day [21]; and KPSBU Lembang as much as 13.3 ±3.64 liters/cow/day [22].

There are a few things that causes hypocalcaemia, among others: hypoparatirodism, vitamin D deficiency, kidney disease and hypomagnesaemia [23;2]. Calcium plays an important role in regulating the physiological and biochemical processes that include neuromuscular excitability, blood coagulation, secretion process, membrane integrity as well as the transport of plasma membrane, enzyme reaction, release of hormones and neurotransmitters and intercellular activity of a number of hormones [24]. The main source of calcium needed for the body are from feed. These minerals are absorbed in the intestine from the surface of the mucosal cells that are formed specifically from collection of microvilli then enters the cytoplasma of the intentinal cells [25]. Calcium helps maintain contraction through a process that is mediated by troponin and tropomyosin. Nerve impulses at the neuromuscular junction will be delivered directly to each sarcomere. Normal sarcoplasmic reticulum membrane is not permeable towards calcium but has a transmembrane calcium – ATPase that will pump calcium into the sarcoplasmic reticulum to maintain calcium concentration. Through nerve impulses, sarcoplasmic reticulum becomes permeable to calcium. As a result, calcium diffuses towards the interior miofibril, and internal calcium concentration will increase. The increase of concentration of calcium is enough to trigger a conformational change in troponin and tropomyosin, therefore muscle contraction occurs. When nerve stimulation ends, sarcoplasmic reticulum membranes becomes impermeable to calcium, so that calcium in the miofibril will be pumped out towards the sarcoplasmic reticulum , therefore muscle relaxation will return.

Regulation of blood calcium level are conducted by several organs that interacts together, that is liver, parathyroid glands, kidney and bone of the cow. Cow receive vitamin D_3 form the diet or through vitamin D_3 synthesis in the skin under the influence of ultra violet rays from the sun. In the liver tissue is where vitamin D_3 first time undergo activation to turn into 25 hydroxy D_3 [=25(OH)D_3]. Decreased blood calcium levels will stimulate the release of parathyroid hormone that is found in the parathyroid glands. This hormone has the ability to stimulate the release of calcium and phosphor from the bone. In the kidney, metabolite of vitamin D_3 [=25(OH)D_3] which is synthesized in the liver becomes a very active form up to 1,25 dihydroxy vitamin D3 [1,25 (OH) 2D3]. Compound 1,25(OH) $_2$D3 is responsible for the absorption of calcium from the bone and particularly the gastrointestinal tract, where the small intestine is the main source of calcium during birth, because mobilization of calcium from the bone require a long time, that is 10-14 days (Payne 1989). This condition is essential, because the muscles of the small intestine is very sensitive to low calcium levels which can decrease the activity of the small intestine. Low levels of calcium will decrease rumen motility thereby reducing the intake of nutrition and subsequently the decrease of intestinal activity will reduce calcium absorption from the gastrointestinal tract.

The role of calcium as a micro-mineral is very important in the body, so cows that lose calcium during milking process have to immediately replace the lost calcium. The inability of cows in response to such needs causes blood calcium concentration to drop and causes disturbances in the role of calcium function including muscle contraction

In general blood calcium concentration of cows suffering from subclinical hypocalcaemia is under 8 mg/dL. Implication of the decline of the function of calcium has a broad impact towards the immune system and other disease in cows on their periparturient period. Study by [26] on dairy cows in KUD Karang Ploso Malang, also shows the same thing. Parturient hypocalcaemia in cows in KUD Karang Ploso Malang increases the risk of

dystocia incidence by 7.8; placental retention 2.6; metritis 4.1 and lameness as big as 6.6 times compared to cows that are not experiencing parturient hypocalcaemia.

4. Conclusion

Most FH crossbreed dairy cows from variety of lactation age, that are being cared for in concentrated areas of small dairy farms in West Java Provence suffers from subclinical hypocalcaemia. Subclinical mastitis cows have a low calcium level under the normal limit, with milk production lower compared to healthy cows, therefore subclinical mastitis can be one of the predisposing factor from subclinical mastitis.

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