Effects of Different Milk Somatic Cell Count Level on Plasma Total Protein, Albumin, and Globulin in Transition Dairy Cows

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ABSTRACT
This study tried to confirm whether there is an effect of milk somatic cell count (SCC) levels on total protein, albumin, and globulin levels in plasma of dairy cows. A total of 18 Friesian Holstein cows in transition were included in this study, divided into three groups based on their milk SCC levels, namely low (<160,000 cells/mL), moderate (160,000-400,000 cells/mL), and high (> 400,000 cells/mL). The variables measured were levels of total protein and other protein derivatives, namely albumin and globulin in the blood plasma of dairy cows. The results showed that cows with low milk SCC had a significantly higher total protein content ($p<0.05$) compared to cows with high milk SCC (109.67 g/L vs 85.15 g/L). This difference was also followed by plasma globulin and plasma albumin, although the difference was not significant ($p>0.05$). In conclusion, there is a relationship between the amount of SCC in milk and plasma protein levels in transition dairy cows.

Keywords: *Albumin; Dairy cows; Globulin; Somatic cell counts; Total protein*

1. INTRODUCTION

One of the vital periods of dairy cows production is in between -3 wks to +3 wks relative to calving, which is known as the transition period [1–3]. In this period, there is physiological changes from a gestational non-lactating to a non-gestational lactating phase. The dairy cows in the transition period experience sudden changes in metabolic and immune functions, because of behavioural and hormonal changes around parturition, which in the end also causing the decrease of dry matter intake (DMI) in this period [4]. These changes impact the energy balance of the cows, which in turn may causing an elevated incidence of diseases, such as ketosis, displaced abomasum, milk fever, retained placenta, metritis, lameness, and clinical mastitis [5].

In addition of the “stressful” transition period, somatic cell count (SCC) in milk can indicates the level of or intramammary infections (IMI) [6]. The SCC elevation is known to be associated with the occurrence of either subclinical or clinical mastitis, although it is not the only indicator [7]. SCC level reflects the susceptibility of IMI [8]. Increased SCC level might increase the susceptibility of IMI, hence resulting a higher risk of mastitis occurrence [9].

The adaptation mechanisms of dairy cows to cope with stressors caused by either transition period or IMI (reflected by SCC level) might reflected in plasma protein and its derivates (albumin and globulin), as a biomarker for protein transport/mobilization from or to specific organ or tissues [10]. Plasma total protein and its derivates might elevated due to the presence of pathogenic or non-pathogenic stressors [11]. Albumin, specifically, is a component of protein that categorized into negative acute phase proteins (-APP) that also can be observed via blood plasma. Albumin is also known to be associated with liver functionality [12]. This study, therefore, tried to confirm whether there are any relationships between SCC level and plasma total protein including its derivates in transition dairy cows.

2. MATERIALS AND METHODS

2.1. Animals and Experimental Design

The Ethical Committee of Universitas Padjadjaran, Bandung, Indonesia, had approved the protocol of this experiment with registration number: 0718070998. A total of 18 Holstein Friesian Cows with body weight (400±30 kg) were randomly selected from West Java Regional Dairy Cows Breeding and Artificial Insemination Development Institute (BPPIB-TSP), Bunikasih, Cianjur, West Java, Indonesia. Cows were fed ad libitum forage and concentrates individually (fed twice daily). The ratio of forage:concentrate is 60:40, while the forage consists of napier grass and *Indigofera zollingeriana* with the ratio of 45:15. Offered feed (forages and concentrates) and feed refusal per individual cow were weighed daily. Feed intake were measured by subtracting offered feed intake and feed refusal per individual cow. Drinking water was provided ad libitum. The transition diet is formulated with a similar protein and energy content among groups (CP 16.57% and total digestible nutrients or TDN 59.52% (Table 1). The individual milk yield (morning and evening milking) were recorded daily.

Analysis of feed ingredients is carried out at Ruminant Nutrition and Feed Chemistry Laboratory (Universitas Padjadjaran, West Java, Indonesia) using proximate analysis. Milk SCC levels were determined based on milk sample at week one of lactation period (transitional period), using a LactoScan SCC Analyzer (Milkotronic, Bulgaria) in a veterinary Laboratory of Animal Hospital, Lembang, Bandung, West Java, Indonesia.

Table 1. Nutrient content of the experimental diet

Nutrient Fraction	Content
Dry Matter (DM)	53.50
Ash (%)	13.03
Crude Protein (%)	16.57
Crude Fat (%)	5.15
Crude Fiber (%)	21.45
TDN (%)*	59.52
Ca (%)	0.61
Zn (mg/kg DM)	82.92
Se (mg/kg DM)	0.43

*TDN = Total Digestible Nutrient

2.2. Blood Sampling

Blood samples were taken at week one after calving. Blood samples were collected via coccygeal vein from each cow into blood sample tubes (vacutainer) with a purple cap containing Ethylenediaminetetraacetic acid (EDTA) anti-coagulant agent (BD Vacutainer, Portsmouth, UK) for measurement of plasma NEFA and NAbs. Vacutainer tubes containing blood samples were then centrifuged in 1500 rpm for 15 minutes at 4°C. After the centrifugation, the plasma aliquots were transferred into 1.5 mL PCR Tubes (Eppendorf, Hamburg, Germany) and stored frozen at -20 °C till analysis.

2.3. Plasma Total Protein, Albumin, and Globulin Determination

The plasma total protein was determined using the Biuret methods [13]. Plasma albumin was determined using bromcresol green (BCG) method [14]. While globulin was determined by manual calculation by subtracting plasma total protein with plasma albumin concentration [15,16].

2.4. Statistical Analysis

Data were analysed using analysis of variance (ANOVA) in GLM procedure in SAS Statistics (Ver. 9.4, Cary, NC, USA) to determine the effects of different milk SCC levels to production performance (milk yield and feed intake) and plasma protein contents (plasma total protein, albumin, and globulin concentration). Cows were divided into three groups based on their milk SCC levels, namely low (SCC<160,000 cells/mL, n=7), moderate (SCC 160,000-400,000 cells/mL, n=7), and high (SCC>400,000 cells/mL, n=4). The data were expressed as least square means (LSM) of the respective parameter with the pooled standard error of means (SEM). The difference is considered significant at $p<0.05$.

3. RESULTS AND DISCUSSION

In this study, the milk yield was ranging from 13.44 to 16.25 kg/head/day and the feed intake was ranging from 40.05 to 46.78 kg/head/day, although the difference was not significant ($p>0.05$; Table 2). Cows with a higher SCC in milk had 2-3 kg/head/day more milk yield compared with moderate or low SCC group (16.25 vs 13.44 vs 14.15 kg/head/day, respectively). Furthermore, cows with a higher SCC in milk had a lower feed intake compared with moderate or low SCC (40.05 vs 46.78 vs 43.77 kg/head/day, respectively). The milk yield in this study was similar with earlier study in tropical area [17], with a range of 13.44 to 16.25 kg/head/day, even though the feed intake was higher compared with mentioned earlier study, suggesting a less efficient milk production.

Regarding the plasma total protein, higher concentration was found in low SCC level compared to moderate or high SCC level in this study (109.67 vs 96.11 or 85.15 g/L, respectively; $p\leq0.05$; Table 2; Figure 1). The plasma protein concentration in high SCC level in this study is in agreement with the normal value of plasma total protein which ranged in 82±6.1 g/L [11]. While cows in low or moderate SCC level exceed the reference values. Plasma total protein might reflect the amount of protein mobilization or utilization in the animal to cope with metabolism changes, especially with the hepatic energy synthesis [18,19].

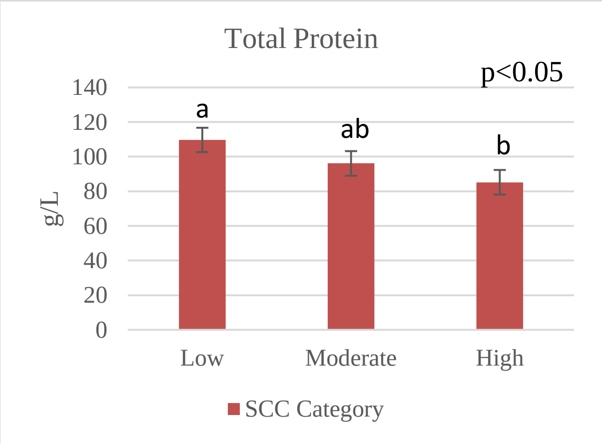

Figure 1. Plasma total protein (g/l) in different milk SCC level in dairy cows. Different letter between bars represents significant differences at $p<0.05$.

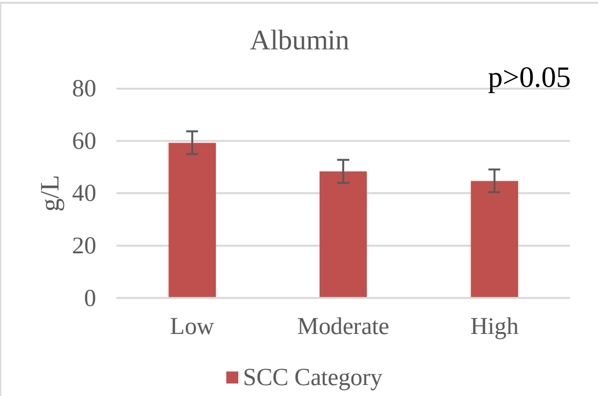

Figure 2. Plasma albumin (g/l) in different milk SCC level in dairy cows.

In previous study, it was reported that clinical ketotic cows have higher plasma total protein concentration compared to subclinical ketotic cows [20]. The higher concentration of plasma total protein in low SCC group might related to the protein mobilization due to activated immune system which upregulates the production of immunity-enhancing agents, including the immunoglobulins to cope with pathogenic infections, so that resulting a low SCC in milk. However, this result needs to be validated by further study.

Along with the trend in relationship of plasma total protein concentration with milk SCC level, we found that the plasma albumin concentration also declined as increasing milk SCC level, although the difference was not significant ($p>0.05$; Table 2; Figure 2).

All of the albumin levels in this study slightly exceed the reference values of 37±2.5 g/L[11], suggesting a high liver function [3]. In accordance with the elevation of plasma total protein, it seems that high concentration of plasma albumin in low SCC group in this study reflect the hepatic energy utilization [2,18] to cope with metabolic changes which might appear due to pathogenic infections. However, further study is needed to confirm this trend and whether these dynamics have an agreement with other metabolic shift biomarkers, including other types of acute phase proteins.

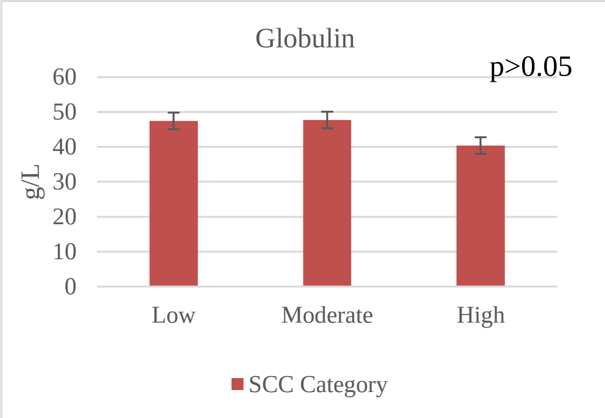

Figure 2. Plasma globulin (g/l) in different milk SCC level in dairy cows.

In terms of plasma globulin concentration, no significant difference was found in any SCC groups ($p>0.05$; Table 2; Figure 3). The range of plasma globulin in this study is in range of reference value of 45±6.6 g/dl [11], suggesting a normal metabolic processes. It was reported that the plasma globulin concentration elevated as a metabolic response due to the presence of stressors, especially the heat stress [11]. In this study, plasma globulin concentration difference of 7.5 g/dl was found between low and moderate SCC group and high SCC group (47.41 and 47.72 vs 40.42 g/dl), although the difference was not significant. It seems that the trends of globulin were in line with either plasma total protein and albumin in this study that suggesting a metabolic response from stressors such as pathogenic infection and inflammation which resulting a lower SCC level. However, this result needs to be validated with further study to assess globulin dynamics in different managerial or environmental condition.

4. CONCLUSION

In conclusion, milk SCC level in transition dairy cows was associated with the concentrations of plasma protein and its derivate. Higher plasma protein concentration seems reflecting a high protein mobilization from and to organs and tissues to cope with stressors, such as pathogenic infection and inflammation, resulting a low SCC level. However, further study needs to confirm the dynamics of plasma protein level in relationship with SCC in the different time points (different weeks relative to calving) so more comprehensive observation could be performed in transition dairy cows. Even though needs to be validated with further study, the result in this study

Table 2. Effects of milk somatic cell count levels on performance parameters and plasma total protein, albumin, and globulin.

Parameter	SCC Levels[1]			SEM	p-value	Significance[2]
	Low (n=7)	Moderate (n=7)	High (n=4)			
Performance						
Milk Yield (kg/head/day)	14.15	13.44	16.25	0.91	0.5	NS
Feed Intake (kg/head/day)	43.77	46.78	40.05	2.09	0.5	NS
Plasma protein						
Total Protein (g/L)	109.67[a]	96.11[ab]	85.15[b]	4.65	0.03	$p<0.05$
Albumin (g/L)	59.3	48.38	44.73	2.31	0.5	NS
Globulin (g/L)	47.41	47.72	40.42	3.57	0.2	NS

[1]SCC Levels:
Low: SCC<160,000 cells/mL
Moderate: SCC 160,000 - 400,000 cells/mL
High: SCC>400,000 cells/mL
[2]NS: Non-significant

seems promising to describe the effect of transition period in relation with SCC levels in dairy cows management.

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