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Haematological Profile of Dairy Cattle Fed with A Diet Supplemented with Depolarized Katuk Leaf (*Sauropus androgynus*)

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Abstract. This study was aimed to assess the health status of dairy cows after consuming depolarized katuk leaves (*Sauroupus androgynus*) as a feed additive by measuring their hematological profile. Nine Friesian-Holstein cows were divided into three groups based on their diet: complete feed (control), complete feed plus depolarized katuk leaf powder (P1; 100 g per day), and complete feed plus depolarized katuk leaf pellet (P2; 100 g per day). Supplementation of depolarizing katuk leaves in the diet started two weeks before parturition until three months of lactation. Blood sampling was carried out during the 10th week of lactation. The analysis of hematological profiles includes total erythrocytes, hematocrit, hemoglobin, total leukocytes, stress index (neutrophil-lymphocyte ratio), total platelets, and total protein. There were no significant differences in total erythrocytes, hematocrit, total leukocytes, stress index, total platelets, and total protein in groups P1 and P2 compared to controls. The hemoglobin levels of dairy cows in groups P1 and P2 were significantly lower ($P<0.05$) compared to controls, although these levels were still within normal limits. In conclusion, there was no negative impact of depolarizing katuk leaf supplementation in the ratio on the health status of lactating dairy cows based on the hematological profile.

1 Introduction

Dairy farming is an important livestock subsector in Indonesia because of its significant contribution to enhancing economic development, rural livelihood, and meeting people's need for animal protein [1]. During 2016 to 2021 the consumption of dairy products is significantly increasing. However, milk production is relatively stagnant, and the importation of dairy products is increasing every year [2]. Several strategies have been implemented to increase the national population of dairy cows and fresh milk production. In addition to increasing the population of dairy cattle, improving feed management is necessary to improve the production of fresh milk. Feed additives have improved nutrient utilization, health parameter, and animal performance [3].

Katuk leaf (*Sauroupus androgynus*) has been traditionally consumed as food and increased breast milk production in nursing mothers. Katuk leaf has been intensively studied since 1993 to understand its mode of action, active compounds, and the possibility of side effects [4]. Katuk leaf is able to increase the production of milk production in dairy cattle [5,6]. The removal of polar compounds in the katuk leaf (depolarized katuk leaf) can suppress the side effects but still can increase milk production to 40% in the first two months of lactation [7].

However, high milk production also has consequences on aspects of feed nutritional intake and health aspects related to the high metabolic rate during lactation. There is a 2 to 6 folds increase in blood flow in the mammary gland starting 2 to 3 days before parturition. During lactation, the mammary gland secretory cells utilize 80% of blood metabolites for milk synthesis [8]. The negative energy balance in the early lactation period may lead to an increased risk of metabolic disorder, lameness, and mastitis [9]. There will be a possibility that increased milk production by supplementation of depolarized katuk leaf can affect the health status of lactating dairy cows. The health status can be evaluated by the hematologic and biochemical profile of blood [10]. Hematological profile within normal physiological limits reflects a good health status and adaptability to internal and external adverse environmental conditions [9,11,12]. Here we evaluate the health status of dairy cows after consuming depolarized katuk leaves as feed additives by measuring their hematological profile.

2 Material and methods

2.1 Animals and management

A total of nine, on average 255 days of pregnancy and an average body weight of 737.44±60.48 kg, 2^{nd} to 5^{th}-lactation-old, Friesian Holstein cows were randomly assigned to three groups of seven animals each. This study

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was conducted in the commercial dairy farm of PT Great Giant Livestock, Terbanggi Besar, Lampung Tengah Regency, Lampung Province, Indonesia, at an altitude of 50 meters above sea level (m.a.s.l). The animals were housed in a normal shaded house with average temperature, relative humidity, and temperature-humidity index (THI): 26.6±2.04ºC, 84.2 ± 10%, and 77.9±2.05, respectively. The diet was fed twice daily in an equal portion with a total of 10% body weight, fulfills the nutritional requirement by NRC, and used *Pennisetum purpureum* as basal feed [13].

2.2 Experimental design

Table 1. Chemical composition of transition diet (before parturition)

Nutrient	Group		
	Control	P1	P2
Dry Matter (%)	100	100	100
Ash (%)	11.35	10.20	14.60
Crude Protein (%)	17.15	18.04	16.54
Crude Fiber (%)	21.60	21.23	21.43
Extract Ether (%)	1.79	1.20	2.35
Nitrogen Free Extract (%)	48.11	49.33	45.08
Calcium (%)	0.69	1.14	0.70
Phosphor (%)	0.55	0.62	0.48
Gross Energy (kal/gram)	4,099	4,012	4,004

This study used twenty-one cows that were divided into three groups based on their diet: complete feed (control), complete feed plus depolarizing katuk leaf powder (P1; 100 g per day), and complete feed plus depolarizing katuk leaf pellet (P2; 100 g per day). Supplementation of depolarizing katuk leaves in the diet started two weeks before parturition until three months of lactation. The depolarized katuk leaf pellet or powder was homogenized in the complete feed every morning before the feeding time. The chemical compositions of the diet are shown in Table 1 and Table 2.

Table 2. Chemical composition of lactation diet (before parturition).

Nutrient	Group		
	Control	P1	P2
Dry Matter (%)	100	100	100
Ash (%)	13.66	12.50	12.37
Crude Protein (%)	18.50	17.63	18.26
Crude Fiber (%)	24.73	11.61	13.77
Extract Ether (%)	8.76	8.42	9.09
Nitrogen Free Extract (%)	34.35	49.84	46.51
Calcium (%)	0.89	0.93	1.03
Phosphor (%)	0.61	0.56	0.62
Gross Energy (kal/gram)	4,042	4,002	3,996

2.3 Data collection and analysis

On the 10th week of lactation, blood samples were taken from three cows per group in the morning. Three ml of blood samples were collected from the coccygeal vein by venipuncture with a 21-gauge needle into Ethylenediaminetetraacetic acid (EDTA)-coated tube, and the samples were immediately placed in an ice bath until hematological analysis. The hematological analysis was conducted in Balai Veteriner Lampung, Bandar Lampung City, Lampung Province, Indonesia. The blood samples were analyzed for hemoglobin, hematocrit, erythrocyte count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leucocyte count (WBC), leucocyte differential (lymphocyte, monocyte, neutrophil, eosinophil, and basophil), platelet count, and total plasma protein.

The data obtained were analysed using GraphPad Prism Version 9.0. Significant statistical differences were calculated by one-way ANOVA followed by Dunnett's test. $P < 0.05$ was considered statistically significant.

3 Result and discussion

3.1 Erythrocyte count

There was no significant difference in the erythrocyte count between groups consuming depolarized katuk leaves (P1 and P2) and the control group **(Fig. 1)**. Supplementation of katuk leaves in other animals' feed, such as rats [14], rabbits [15] and buffaloes [16] can significantly increase the erythrocyte count. The range of erythrocyte count of cows in this study is still in the reference interval for cattle [17] with interval 4,590,000 – 7,110,000 numbers/µl and an average 6,3 ± 0,7 x 10^6/ µl. This erythrocyte count is relatively lower than the

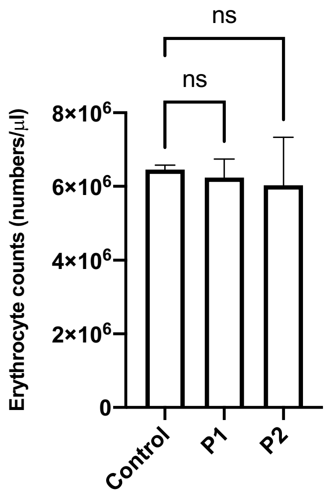

Fig. 1. Erythrocyte counts in lactating dairy cows after consuming depolarized katuk leaves (P1 and P2) at the 10th week of lactation (n=3, mean ± SD). Statistical significance was calculated using one way ANOVA, followed by Dunnett's test, ns: not significant.

previous study by Suprayogi et al [12]. It might be caused by different altitudes of study area; the previous study area was Pangalengan highland with altitude of 1,000-1,400 m.a.s.l. Another study by Mariana et al. [18] showed that the erythrocyte count of dairy cows would increase as the increase of altitude.

3.2 Haemoglobin, haematocrit, and erythrocyte index

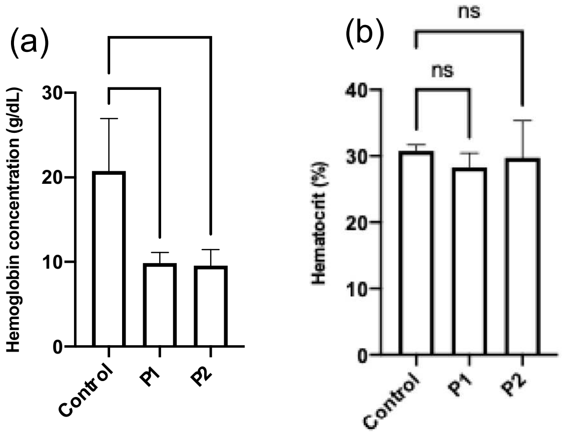

Fig. 2. **(a)** Hemoglobin concentration and **(b)** hematocrit in lactating dairy cows after consuming depolarized katuk leaves (P1 and P2) at the 10th week of lactation (n=3, mean ± SD). Statistical significance was calculated using one way ANOVA, followed by Dunnett's test, ns: not significant, *: p > 0.05 vs control

The supplementation of katuk leaves powder (P1) and pellet (P2) significantly reduced the hemoglobin concentration than the control group (P<0.05) **(Fig. 2a)**. Esievo and Moore [19] reported that the serum iron and iron binding capacity decreased in the early lactation until the mid-lactation which cause the hemoglobin concentration to remain low until four months after lactation. The higher milk production in group P1 and P2 might lower the serum iron and the iron-binding capacity then decrease the hemoglobin concentration. The lower hemoglobin concentration causes a significantly lower quantity and amount of hemoglobin (MCH and MCHC, respectively) in a single erythrocyte. However, the lower hemoglobin concentration is still in the physiological interval range [17].

In addition, there is no significant difference in the hematocrit **(Fig. 2b)** and the average size of erythrocyte

Table 3. Erythrocyte index in lactating dairy cows after consuming depolarized katuk leaves (P1 and P2) at the 10th week of lactation

Group	MCV (fl)	MCH (pg)	MCHC (g/dl)
Control	47.6 ± 0.62^a	32 ± 9.44^a	64 ± 19.02^a
P1	45.3 ± 0.15^a	15.7 ± 0.76^b	33 ± 1.65^b
P2	49.5 ± 2.78^a	15.9 ± 0.46^b	30.6 ± 1.05^b

Statistical significance was calculated using one way ANOVA, followed by Dunnett's test. Different superscript at the same row indicate significant difference (P<0.05)

(MCV) between control and groups consuming katuk leaves (P1 and P2) **(Table 3)**. In contrast, katuk leaves and its ethanol extract increase the hemoglobin concentration and hematocrit in lactating buffaloes [16] and rats [14]. The different effects compared with [14] and [16] might be caused by different solvents. Fraction polar, non-polar, and semi-polar of katuk leaves has different physiological effect in lactating rats [4].

The hemoglobin concentration and hematocrit of cows in this study are relatively higher than in the previous study by Suprayogi et al [12]. The hemoglobin concentration and hematocrit of dairy cows raised on a low land significantly higher than that are raised in high land [18]. The higher temperature on low land stimulates an intense thermoregulation process and dehydration.

3.3 Total protein plasma

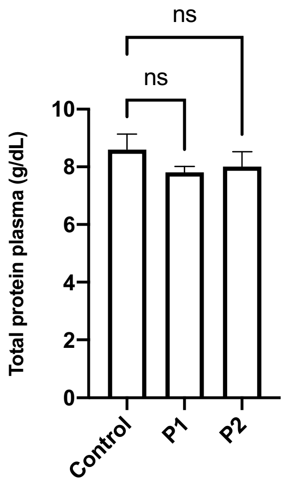

Fig. 3. Total protein plasma in lactating dairy cows after consuming depolarized katuk leaves (P1 and P2) at the 10th week of lactation (n=3, mean ± SD). Statistical significance was calculated using one way ANOVA, followed by Dunnett's test, ns: not significant.

Interestingly, depolarized katuk leaves did not change the level of total protein plasma even though they significantly increased milk production **(Fig. 3)**. The total protein plasma level will increase with the progress of lactation in lactating goats due to the catabolism of protein for milk synthesis [8]. This result suggests that depolarized katuk leaves did not disturb the balance between anabolism and catabolism of protein in the body.

3.4 Leukocyte count and stress index

There was no significant difference in the leukocyte count between groups consuming depolarized katuk leaves (P1 and P2) and the control group **(Fig. 4a)**. Supplementation katuk leaves also did not change leukocyte numbers in lactating buffalo [16] and rabbit [15]. The average leukocyte counts of dairy cows in this study is 3.63±1.83 x 10^3/µl. This numbers are lower than the reference interval [17] and other studies in lactating dairy cattle [12,18]. However, the percentage of all types of leukocytes is still in the reference interval.

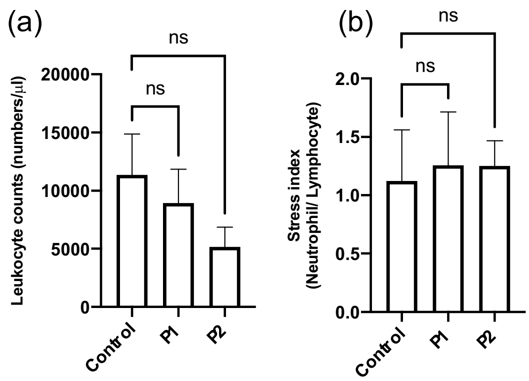

Fig. 4. (a) Leukocyte counts and **(b)** Stress index in lactating dairy cows after consuming depolarized katuk leaves (P1 and P2) at the 10^{th} week of lactation (n=3, mean ± SD). Statistical significance was calculated using one way ANOVA, followed by Dunnett's test, ns: not significant, *: $p > 0.05$ vs control

In addition, the neutrophil-lymphocyte ratio or stress index is not significantly different between the control group and groups consuming depolarized katuk leaves (P1 and P2) **(Fig. 4b)**. The interval of stress index in this study is 0.82–1.62 with an average 1.21±0.33. This result is still in the physiological stress index reported in lactating dairy cattle: 0.41–1.63 [12] and 1.13–1.59 [20]. Exposure to stress will increase the percentage of neutrophils and decrease the percentage of lymphocytes in the leukocyte population. Thus, stress will exacerbate the immune function mediated by lymphocytes.

4 Conclusion

There was no significant negative impact on the health status of lactating dairy cows after consuming depolarized katuk leaves, except a lower hemoglobin concentration within the physiological interval range. The nutritional adequacy should be considered in the application of depolarized katuk leave as feed additive in lactating dairy cows.

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