Blood hematology profile at postpartum in Ettawa grade does fed with different fatty acid flushing diets during the late gestation period and different litter sizes

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Abstract. Physiological stress occurs in does immediately after parturition. This study evaluated the blood hematology profile at postpartum in Ettawa grade does fed with different fatty acid flushing diets during the late gestation period and different litter sizes (LS). A total of 15 Ettawa grade does with a gestational age of 4 months were used in this study during the flushing period. The flushing ration was supplemented with a 2.8% fatty acid level in lauric acid (T1), α-linolenic acid (T2) and α-linolenic acid (T3). A factorial randomized complete design 3 x 2 in which the first factor was fatty acids and the second factor was litter sizes (LS1 and LS2) was used to measure blood hematology. Leukocyte level was higher in LS2 compared to LS1 ($P<0.05$), but it was not affected by fatty acids. Even though the lymphocyte level was highest in T3 and LS2 ($P<0.05$), it was still in the normal range. Monocyte and neutrophil were lower in T3 compared to T1 ($P<0.05$), but it was similar to T2. In conclusion, the results suggest that flushing in the late gestation period with supplementation of 2.8% α-linolenic acid improved the immune system at postpartum on the does giving birth to twins 2.

Keywords: α-linolenic acid, hematology, immunoglobulin G.

1 Introduction

Doe at the late gestation requires high energy because fetal growth occurs very rapidly. According to Abd-Allah [1] in the late gestation period, 80% of fetal growth occurs, it causes an increased need for feed intake to support the development of the fetus. The does metabolic burden increases with rapid fetal development, causing oxidative stress [2]. Oxidative stress worsens the does systemic inflammatory response to gestation. Oxidative stress is exacerbated by bleeding and leakage of fluids caused by the birth process. It will have an impact on blood hematology which is one indicator of the condition of livestock. Aquino *et al*. [3] state that ruminant mothers experience physiological stress and nutritional imbalances immediately after parturition, under these conditions they must still produce milk to feed her offspring. The ability of does can give birth to more than one child, the does may experience higher physiological stress.

One effort in the fulfillment of livestock nutrients, especially in the critical phase is with flushing management. So far, different energy sources, including carbohydrate and fat sources, have been used as supplements in the flushing ration. Besides, fat plays a role in increasing energy density in feed to reduce post-partum negative energy balance. Fat supplementation, especially polyunsaturated fatty acid (PUFA), can reduce oxidative stress from parturition [4]. Linolenic acid (omega-3) is a precursor for the formation of series-3 eicosanoids, including prostaglandins [5]. Eicosanoids are molecular signals associated with several functions in the body, including inflammation. Series 3 prostaglandins are anti-inflammatory compared to series two prostaglandins synthesized from arachidonic acid [5]. Linoleic acid (omega-6) is the precursor of arachidonic acid.

This study evaluated the blood hematology profile at postpartum in Ettawa grade does fed with different fatty acid flushing diets during the late gestation period and different litter sizes.

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2 Materials and Methods

A total of 15 Ettawa grade does with a gestational age of 4 months were used in this study during the flushing period. The treatment with a flushing period conducted from 4th-month gestation until 2 weeks after parturition. The animals were fed *Pennisetum purpureum* and concentrate on the ratio of 30:70 of ration dry matter. Concentrate consisted of soybean meal, corn gluten feed (CGF), cassava flour, solid cassava waste, CaCO3, premix, salt, and oil supplemented. The ration treatments were: T1= add 6.1% coconut oil (contain 2.8% lauric acid), T2 = add 4.7% sunflower oil (contain 2.8% linoleic acid) and T3 = add 5.2% flaxseed oil (contain 2.8% α-linolenic acid). The rations were formulated iso-nitrogen (crude protein, CP=15%) and iso-energy (total digestible nutrient, TDN=75%). A factorial randomized complete design 3 x 2 in which the first factor was fatty acids (lauric, linoleic, and α-linolenic acid) and the second factor was litter sizes (LS1 and LS2) was used to measure blood hematology profile. Blood samples were collected from does within 2 hours after parturition. Each sample was put into glass tubes containing EDTA. Measurement of hematological values includes hemoglobin used Sahli method, hematocrit used the microhematocrit method; erythrocyte, and leukocyte used Neubauer improved cell counting chamber. The level of differential leukocyte cells was obtained by Giemsa stain. Data were analyzed using a two-way analysis of variance followed by post hoc analysis using the Duncan test by the SAS program.

3 Results and Discussion

Levels of hematocrit, hemoglobin, and erythrocyte were not a significant difference in fatty acids treatments and litter sizes (P>0.05) (Table 1). These values for all of these treatments were within the normal range [6]. The mean of leukocyte was higher in LS2 compared to LS1 (P<0.05), but it was similar in fatty acid treatments (P>0.05) (Table 1). This condition was suspected to be higher stress in does with LS2 compared to does with LS1. Although the levels of leukocytes were similar in fatty acid treatments, leukocyte in the does with α-linolenic acid treatment tends to be lower than other treatments due to the role of α-linolenic acid in the anti-inflammatory process. Otherwise, the treatment with linoleic acid and LS2 were closer to the standard upper threshold [6].

Table 1. Effect of different fatty acid flushing diets during the late gestation period and different litter sizes on hematology value at postpartum in Ettawa grade does.

Parameters	Litter size	T1	T2	T3	average	Reference Value*
Hematocrit (%)	LS1	26.00±1.00	27.00±4.58	28.00±4.24	**26.25±2.60**	22-38
	LS2	27.00	25.00	27.25±2.63	**27.67±2.73**	
	average	**26.25±0.96**	**26.50±3.87**	**27.50±2.81**		
Hemoglobin (g/dL)	LS1	8.53±0.50	9.73±2.00	9.40±0.57	**9.25±1.26**	8-12
	LS2	9.4	9.60	10.05±1.30	**9.80±1.10**	
	average	**8.75±0.60**	**9.70±1.64**	**9.83±1.09**		
Erythrocyte ($x10^6/mm^3$)	LS1	10.50±1.03	11.70±1.14	7.68±1.69	**10.92±2.33**	8-18
	LS2	13.68	10.92	13.08±3.92	**11.92±3.44**	
	average	**11.29±1.80**	**11.51±1.01**	**11.28±4.19**		
Leukocyte ($x 10^3/mm^3$)	LS1	10.35±1.72	10.72±1.05	9.47±6.75	**10.27±2.82**^{B}	3-13
	LS2	16.70	20.85	11.69±4.90	**14.05±5.43**^{A}	
	average	**11.94±3.47**	**13.25±5.14**	**10.95±4.98**		

T1: 2.8% lauric acid; T2: 2.8 linoleic acid; T3: 2.8% α-linolenic acid. Different superscripts in the same column mean significantly different (P<0.05). *Feldman et al. [6].

Our results showed that the levels of lymphocytes were affected by fatty acid profiles and litter size (P<0.05). The level of lymphocytes in the treatment with α-linolenic acid was higher compared to other treatments, and it was higher in LS2 compared to LS1 (P<0.05). The high level of lymphocytes in does with α-linolenic acid treatment because the average litter size tends to be higher compared to other treatments (data not shown). The result is in agreement with another study by Rusmana et al. [8]. However, Rosa et. al. [9] found a decrease in the counted number of lymphocytes in the intestinal mucosa of Wistar rats with omega-3 supplementation sourced from flaxseed oil and fish oil. Lymphocytes produce antibodies in response to antigens carried by macrophages. The levels of lymphocytes for all of these treatments were within the normal range [6].

The statistical results show the levels of monocyte, neutrophil, eosinophil, and basophil were not affected by litter size, but monocyte and neutrophil were affected by fatty acids. Monocytes of does that were treated with α-linolenic acid are lower compared to does that were treated with lauric acid, but it is similar from treatment with linoleic acid. The high level of monocytes can indicate phagocytic inflammation, such as chronic bacterial infection, excessive pain, suppurative inflammation, and stress [10]. According to Thawat et al. [7], monocyte levels were highest at the beginning of the postpartum. Monocytes, along with neutrophils, produce high amounts of reactive oxygen species (ROS), thus

contributing to oxidative stress [10]. The level of neutrophils in treatment with linolenic acid was significantly lower than in treatment with linoleic acid but it did not differ from the treatment of lauric acid.

Table 2. Effect of different fatty acid flushing diets during the late gestation period and different litter sizes on leukocyte differential count at postpartum in Ettawa grade does.

Parameters	Litter size	T1	T2	T3	average	Reference Value*
		----------(%)----------				
Lymphocyte	LS1	51.30±2.94	49.67±3.74	55.58±1.31	**51.76±3.56^{B}**	50-70
	LS2	49.14	54.65	58.99±1.71	**56.15±4.51^{A}**	
	average	**50.76±2.64^{b}**	**50.92±3.94^{b}**	**57.62±2.26^{a}**		
Monocyte	LS1	3.48±0.72	3.17±0.88	2.54±0.76	**3.13±0.78**	0-4
	LS2	5.17	2.06	2.02±0.03	**2.66±1.41**	
	average	**3.91±0.89^{a}**	**2.90±0.91^{ab}**	**2.23±0.48^{b}**		
Neutrophil	LS1	39.91±4.09	43.28±3.24	35.31±0.98	**40.40±4.06**	30-48
	LS2	39.66	38.14	35.85±2.82	**36.47±2.44**	
	average	**39.85±3.34^{ab}**	**41.99±3.69^{a}**	**35.64±2.07^{b}**		
Eosinophil	LS1	4.50±2.11	3.16±0.38	4.19±1.69	**3.92±1.46**	1-8
	LS2	5.17	4.12	3.25±0.67	**3.81±0.97**	
	average	**4.67±1.75**	**3.40±0.57**	**3.62±1.10**		
Basophil	LS1	0.80±0.23	0.72±0.00	0.88±0.16	**0.79±0.15**	0-1
	LS2	0.86	1.03	0.89±0.18	**0.91±0.14**	
	average	**0.82±0.19**	**0.80±0.15**	**0.89±0.15**		

T1: 2.8% lauric acid; T2: 2.8 linoleic acid; T3: 2.8% α-linolenic acid. Different superscripts in the same line or column means significantly different (P<0.05). *[6].

The level of eosinophil and basophils is similar in all treatments [6].

The statistical results show the levels of monocyte, neutrophil, eosinophil, and basophil were not affected by litter size, but monocyte and neutrophil were affected by fatty acids. Monocytes of does that were treated with α-linolenic acid are lower compared to does that were treated with lauric acid, but it is similar from treatment with linoleic acid. The high level of monocytes can indicate phagocytic inflammation, such as chronic bacterial infection, excessive pain, suppurative inflammation, and stress [10]. According to Thawat et al. [7], monocyte levels were highest at the beginning of the postpartum. Monocytes, along with neutrophils, produce high amounts of reactive oxygen species (ROS), thus contributing to oxidative stress [10]. The level of neutrophils in treatment with linolenic acid was significantly lower than in treatment with linoleic acid, but it did not differ from the treatment of lauric acid. The level of eosinophil and basophils is similar in all treatments [6].

4 Conclusion

In conclusion, the results suggest that flushing in the late gestation period with supplementation of 2.8% α-linolenic acid improved the immune system at postpartum on the does giving birth to twins 2.

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