Immunity and Behaviour of Lambs Born from Ewes Fed a Flushing Diet Containing EPA and DHA

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ABSTRAK

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Nutrisi berperan penting dalam daya tahan tubuh dan status imunitas anak domba dari periode neonatal hingga sapih. Penelitian ini bertujuan untuk mengevaluasi efek konsentrat *flushing* mengandung *docosahexaenoic acid* (DHA) and *eicosapentaenoic acid* (EPA) asal minyak lemuru pada imunitas dan daya tahan tubuh anak domba. Dua puluh delapan anak domba neonatal dibagi kedalam empat perlakuan bergantung pada pakan yang dikonsumsi oleh induknya. Empat perlakuan meliputi : konsentrat kontrol (P1), konsentrat *flushing* mengandung 6% minyak sawit (P2), konsentrat *flushing* mengandung 3% minyak sawit dan 3 % minyak lemuru (P3), dan konsentrat flushing mengandung 6% minyak lemuru (P4). Domba diberi rumput gajah dan konsentrat dengan rasio 30:70% berdasarkan bahan kering. Induk dan anak domba ditempatkan pada kandang yang sama hingga usia sapih 2 bulan. Parameter yang diamati pada induk domba meliputi immunoglobulin G (Ig G) kolostrum. Parameter yang diamati pada anak domba adalah Ig G darah. Tingkah laku anak neonatal, respon fisiologis, hematologi saat dilahirkan dan saat sapih. Hasil menunjukkan anak dari domba yang diberi konsentrat P4, P3, P2 nyata (P<0,05) lebih singkat waktu berdirinya. Leukosit P1 saat sapih, nyata (P<0,05) lebih tinggi dari P2 dan P3. Leukosit P1 sama tinggi dengan P4. Perlakuan tidak berpengaruh nyata (P>0,05) pada Ig G dan respon fisiologis. Penelitian ini menyimpulkan, konsentrat *flushing* 6% minyak lemuru mengandung DHA dan EPA dua kali kebutuhan yang diberikan pada induk domba dapat mempercepat waktu berdiri pertama anak domba.

Kata Kunci: *Flushing*, Imunitas, Immunoglobulin, Minyak Lemuru

ABSTRACT

Nurlatifah A, Khotijah L, Arifiantini RI, Maidin MS, Astuti DA, Herdis. 2023. Immunity and survival rate of Lamb born from ewe fed flushing diet containing EPA and DHA. JITV 28(3):159-168. DOI:http://dx.doi.org/10.14334/jitv.v28.i3. 3110.

Nutrition can influence lamb survival and the immune status during the neonatal phase until weaning. This study aimed to investigate the effect of flushing of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) from Lemuru fish oil in the Ewe's diet on lamb immunity and survival. Twenty-eight neonatal lambs were divided into four treatments according to Ewe's diet. Four treatments: control concentrate (P1), flushing concentrate with 6% palm oil (P2), flushing concentrate with 3% lemuru oil and 3% palm oil (P3), and flushing concentrate with 6% lemuru oil (P4). The Ewe were fed Napier grass and concentrated in a 30:70% ratio based on dry matter. Ewe and their lambs remained together until weaning at about two months of age. The parameter observed in ewes was colostrum Immunoglobulin G (IgG). The parameters in the Lamb are blood Ig G, neonatal behavior of the Lamb, physiological response, and hematology of the Lamb at birth and weaning. The results showed that feeding P4, P3, and P2 to the Ewe resulted in a shorter latency to stand-in lamb (P<0.05). P1 has higher leukocytes (P<0.05) during weaning than P2 and P3. P1 has the same leukocyte as P4. Treatment has a non-significant effect (P>0.05) on Ig G and physiological response. In conclusion, administering 6% Lemuru oil containing EPA and DHA twice can shorten the latency to stand in newborn lambs.

Key Words: Flushing, Immunity, Immunoglobulin, Lamb Survival, Lemuru Oil

INTRODUCTION

Feeding the Ewe has a significant impact on preventing lamb mortality. Ideally, maternal immunity should be transferred to the Lamb in utero. However, placental barriers in ruminants may not allow IgG transmission (Ke et al. 2021). Therefore, the Immune status of the Lamb depends on passive transfer from colostrum content. Diet affects colostrum composition (Banchero et al. 2015) and lamb behavior (Ahmadzadeh et al. 2020). Shorter latency to rise and suckle is very important for lamb survival. It is associated with colostrum consumption to maintain thermoregulation, transfer passive immune media from the dam, and strengthen the lamb-maternal relationship (Dwyer et al., 2016).

Several studies have been conducted to improve lamb survival. Polyunsaturated fatty acid (PUFA) in the ratio of ewes during lactation could improve milk quality for lamb survival. Ewes fed PUFA from a fish meal-enriched diet had eicosapentaenoic acid and docosahexaenoic acid in colostrum and milk compared to those fed the control diet (Coleman et al. 2018). Flushing ration with PUFA-rich fish oil increases the preovulatory follicle and ovulation rate with an increase in the kidding rate (Mahla et al. 2017).

Long-chain fatty acids Polyunsaturated fatty acids (PUFAs), such as arachidonic acid (C20: 4 (n-6)) and docosahexaenoic acid (C22: 6 (n3)), are fatty acids required for tissue development, synaptic transmission, and influence brain development. Neonatal behavior can be influenced by PUFA intake in Ewe's diet, especially docosahexaenoic acid (DHA3); 20: 06 (n-3) (Roque-Jiménez et al. 2021). The survival rate of newborn lambs can be increased by adding DHA from algae to the ratio of ewes (Pickard et al. 2008). In addition, diets high in EPA and DHA increase the concentration of EPA and DHA fatty acids in muscle tissue in growing lambs (Ferreira et al. 2014). In addition, feeding DHA and EPA during gestation may be a strategy for maintaining the immune system during gestation and for immune system development in lambs (Roque-Jiménez et al. 2021; Veshkini et al. 2020)

Lemuru fish oil is a by-product containing n-3 polyunsaturated fatty acids (PUFA) 13.70% EPA and 22.47% DHA. However, DHA and EPA effect in the ewe ration in late gestation and the lambing period has not been recognized with an apparent effect on lamb survival and immunity. Therefore, this study aimed to investigate the effects of a flushing diet in the ration of ewes on colostrum quality (Ig G), neonatal behavior, physiological response, and hematology.

MATERIALS AND METHODS

This study was conducted in the Laboratory of Nutrition for draught animals, Department of Nutrition and feed technology, Faculty of Animal Science IPB University. Approval for the current study was by the Animal Care and Use Committee (ACUC) at IPB University No. 119-2018 IPB

Primiparous ewes and lambs born from each Ewe were used for this experiment and divided into four treatments. Each treatment consisted of five ewes. Twenty-eight lambs were divided into four treatments depending on the Ewe. Each Lamb was examined from birth to two months of age. The ewes and their Lamb were kept in individual cages and allowed to suckle from birth until weaning at two months of age.

Ration

The experimental ewes were fed a total mixed ration at 3.5% body weight with a forage-to-concentrate ratio of 30:70 on a dry matter basis. Treatments with a flushing concentrate with different oil sources for ewes are as follows: control (P1), flushing concentrate with 6% palm oil (P2), flushing concentrate with 3% lemuru oil and 3% palm oil (P3), and flushing concentrate with 6% lemuru oil (P4). The nutrient content in P1 was adjusted to meet the nutrient requirements of a pregnant ewe according to NRC (2007), while P2, P3, and P4 are flushing concentrates with isoprotein and isoenergy. The EPA and DHA contents in the flushing concentrate are adjusted to the requirement for linolenic acid in the ration given by Pudelkewicz et al. (1968), which is 0.5%. The EPA and DHA levels of P3 flushing rations were 0.47%, while the P4 ration was 0.94%. The flushing concentrate was administered to the ewes twice, two weeks before, two weeks after mating, and two weeks before and two weeks after parturition; outside this period, the ewes received the control ration.

Measurement in the ewe

The nutrient consumption of Ewe after lambing was measured from lambing day until 14 d after parturition; the animals were given P1, P2, P3, and P4. Daily nutritional consumption of concentrate and forage was calculated by removing residual feed from the offered one daily. After getting the amount of feed consumed, multiply by each feed's chemical content.

Colostrum Immunoglobulin G (IgG) content was measured from colostrum collected 24 hours after lambing. ELISA and KIT measured the colostral IgG concentrations for ovine immunoglobulin G (Bioenzy Catalog no: BZ-08199100-EB).

Measurement in the lamb

Plasma immunoglobulin G (IgG) measured from blood samples with anticoagulant was collected from the jugular vein of lambs after seven days. ELISA and KIT measured plasma IgG concentrations for ovine immunoglobulin G (Bioenzy Catalog no: BZ-08199100-EB).

Observation of Lamb's Behavior began at birth and ended at successful emergence. The observation was recorded with a digital camera. The time and frequency

Table 1 Composition and nutrient content flushing concentrate (100% dry matter basis)*

Feed Ingredients	Treatment			
	P1	P2	P3	P4
	---------------------------%--------------------------------			
Soybean meal	17.14	28.57	28.57	28.57
Pollard	43.57	29.26	29.26	29.26
Dried cassava	30.00	26.86	26.86	26.86
Lemuru oil	-	-	3	6
Palm oil	-	6	3	-
Molasses	7.14	7.14	7.14	7.14
CaCO3	0.71	0.71	0.71	0.71
Premix	0.71	0.71	0.71	0.71
NaCl	0.71	0.71	0.71	0.71
Nutrient content	----------------------------%-------------------------------			
Crude protein	14.39	17.50	17.87	17.49
Crude fat	1.22	7.84	7.61	7.42
Crude fiber	9.92	9.47	9.12	9.25
NFE	63.33	54.15	54.22	54.44
TDN	71.34	73.16	73.24	72.93
EPA and DHA	-	-	0.67	1.34
Palmitic Acid	-	2.61	1.30	-
Ca	0.73	0.82	0.82	0.82
P	0.65	0.56	0.56	0.56

*Result of Nutrition and Feed Technology Laboratory, IPB Univesity (2019) P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. TDN= total digestible nutrient; calculation results Wardeh (1981) TDN= 2.6407 + (0.6964 x %CP) + (1.2159 x %Fat) – (0.1043 x %fiber) + (0.9194 x %NFE)

of observed lamb behavior are: a. Latency to standing (minutes) is the time it takes an infant from birth to standard standing b. Latency to suck (minutes) is the time it takes for an infant to go from birth to successfully sucking milk (Fonsêca et al. 2014)

Physiological responses such as rectal temperature, respiratory rate, and heart rate are measured from days 50 to 65. Measurements are taken three times a day at 07.00 a.m., 12.00 p.m., and 05.00 p.m. The condition of the cage in the morning is 24.13±0.55℃ and relative humidity (RH) 90±5.19%; during the day, the temperature is 30.50 ±0.36℃ and RH 65.55±6.42%, and in the afternoon, the temperature is 29.13±1.67 ℃ and RH 71±8.18%.

Blood hematology was measured at 7 and 56 days of age. Blood samples were collected from a jugular vein in the morning with a 1 mL syringe and collected in a sterile tube containing EDTA anticoagulants. Blood hematology parameters such as hematocrit, hemoglobin, total erythrocytes, and total leukocytes were analyzed according to standard procedures (Zhu et al. 2017).

Experimental design and data analysis

A completely randomized design of experiments (CRD) was used for the study. The data obtained were statistically tested (ANOVA), followed by the Duncan test using SAS ver 19.0.1.

RESULTS AND DISCUSSION

During the flushing period after lambing, treatment did not affect the total dry matter consumption, crude fiber consumption, Nitrogen Free Extract consumption (NFE), and Total Digestible Nutrients (TDN). Crude protein and fat consumption were significantly highest in treatment P2 and lowest in P1. P3 and P4 were the same with treatment P2. A significant difference in fat and protein consumption occurs due to the different fat and protein content in flushing concentrate compared to the control. Higher fat and protein consumption aligns with a higher tendency of total dry matter. Dry matter consumption, protein consumption, and TDN in this study fulfilled the nutrient needed for Ewe in the early lactating period with single or twin lambs. Based on NRC (2007), the nutritional requirements for lactating Ewe with BW of 40 kg was 720-930 g/day TDN and 156-224 g/day crude protein.

Treatment had no effect (P>0.05) on the concentration of immunoglobulin (IgG) in the colostrum and blood plasma of the lambs. Immunoglobulin data from ewe colostrum and Lamb blood plasma are shown in Table 2. Immunoglobulin G (IgG) is an anti-infective component in blood, colostrum, and milk that contain glycoproteins that dissipate environmental pathogens by binding or encapsulating barriers (Balan et al. 2019). In this study, a high-fat concentrate containing DHA and EPA had no significant effect on immunoglobulin in the colostrum of ewes. This result aligns with another study that reported that feeding EPA and DHA has no significant effect on lambs' colostral IgG and serum IgG concentration (Moreno-Indias et al. 2020). Colostrum synthesis depends on the diet consumed by Ewe during gestation (Banchero et al. 2015). However, the transfer of immunoglobulin did not influence the diet. High-energy feeding harms IgG concentration. An increase in prolactin regulates IgG transfer from blood to colostrum. In contrast, another study reported that feeding positively affects colostrum production and IgG transfer because high nutrient consumption increases colostrum production, affecting the total amount of IgG absorbed by the Lamb. No significant effect means that high-fat content in flushing ration does not decrease IgG concentration in the colostrum. In this study, immunoglobulin concentration ranged from 0.05mg/mL to 0.014 mg/mL. Colostrum is collected 24 hours after lambing. In agreement with another report, low IgG concentration states that colostral IgG concentration decreases rapidly with time (approximately 3.3 mg/mL/h) and drops to zero mg/mL after 24 hours post-lambing (Hinde & Woodhouse 2019).

There is no significant difference in IgG concentration in lamb plasma, as immunoglobulin concentration in colostrum is the same in all treatments. Transfer of Immunoglobulin from Ewe to Lamb by passive transfer. Usually, immunity from the Ewe is transferred in utero to the fetus, born with body immunity. However, in ruminants, there are placental barriers. Therefore, the transfer of immunoglobulin from Ewe to Lamb depends on colostrum (Ke et al. 2021). In this study, immunoglobulin concentration in lambs at 7 days of age is 0.42-0.67 mg/mL. This concentration may lead to increased abomasal secretion and proteolytic activity of intestinal cells and reduce the efficiency of IgG absorption after birth. The ruminant small intestine cell can absorb IgG and effectively transfer it to the blood only 24 hours after birth (Alves et al. 2015).

The behavior of newborn lambs in terms of survival and latency to stand was significantly (P<0.05) faster in treatments P2, P3, and P4 than in controls (P1). Treatment had no significant effect (P>0.05) on latency to suckling (Table 3). Latency to stand, or the time required for a lamb to stand after birth, is related to the survival and growth rate of the Lamb. The shorter time to stand, the greater the chance for the Lamb to avoid cold stress (Kenyon et al. 2019). Latency to standing is significantly shorter in lambs from ewes-fed flushing diets compared to controls. This result is consistent with the report by (Pickard et al. 2008) that adding DHA and EPA to the ration of ewes at the end of gestation can increase survival and influence neonatal behavior.

Neonatal behavior can be influenced by birth weight, sex, litter size, and nutritional status of the dam. Single-born lambs stand up faster than twins (da Porciuncula et al. 2021). This study shows that Lamb from sheep treated with a flushing diet tends to be born in the twin type but requires a shorter latency to stand up than control animals, which tend to be born singles; this is likely due to the fact that the behavior of newborn lambs from sheep fed with the flushing diet is influenced by the added length of the PUFA chain in the sheep diet, especially DHA and EPA (Gulliver et al. 2012). EPA and DHA are essential fatty acids for brain tissue development, synaptic transmission, and retinal development (Duttaroy & Basak 2020). DHA supplementation during pregnancy may positively affect brain cell membrane development, including the cerebellum, which controls locomotor development (Pickard et al. 2008).

Shorter latency to stand in Lamb from Ewe gave lemuru oil as a DHA and EPA source in line with another study on the Lamb (Encinias et al. 2004). The researchers reported an increase in lamb survival fed with higher fat compared to low-fat diets. Adding fat can increase brown adipose tissue's (BAT) thermogenic capacity responsible for nonshivering thermoregulation. Right after birth is a critical time for a newborn lamb because it is associated with heat loss to maintain homeotherm before digested colostrum. The newborn Lamb must metabolize energy reserves in brown fat tissue and increase muscular activity by shivering (Plush et al. 2016).

Lamb is born with fewer energy reserves, so it is crucial to find udders and consume milk as quickly as possible (Agenbag et al. 2021). Latency to suckle or the time needed for a lamb right after birth to suckle to its dam in this study was not significant. Latency to suckle

Table 2. Nutrient consumption of Ewe after lambing

Parameters Consumption	Treatment			
	P1	P2	P3	P4
Dry matter (g/h/d)	1180.53±286.05	1276.30±154.56	1131.02±310.49	946.39±143.17
Crude protein ((g/h/d)	152.29±35.05[b]	218.83±27.32[a]	192.46±57.56[ab]	156.82±20.27[ab]
Crude fat ((g/h/d)	18.58±4.94[c]	92.75±11.77[a]	77.47±23.87[b]	62.72±7.72[ab]
Crude fiber ((g/h/d)	170.82±47.05	195.99±23.73	175.38±43.56	148.50±28.45
NFE ((g/h/d)	715.97±170.18	769.03±93.60	676.41±189.08	686.07±139.41
TDN ((g/h/d)	811.72±193.50	1002.40±122.70	877.32±249.48	727.70±102.86

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4 flushing concentrate 6% lemuru oil. [a,b,c], different superscript letters on the same line are significantly different (P<0.05)

Table 2. Effect of flushing concentrate containing EPA and DHA into immunoglobulin in colostrum and blood plasma of lambs

Parameters	Treatment			
	P1	P2	P3	P4
IgG in Colostrum (mg/mL)	0.06±0.07	0.05±0.06	0.05±0.04	0.14±0.22
IgG in Lamb's blood plasma (mg/ML)	0.67±0.44	0.65±0.41	0.42±0.29	0.53±0.43

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. [a,b,c], different superscript letters on the same line are significantly different (P<0.05)

Table 3. Lamb behavior born from Ewe fed a flushing diet

Parameters	Treatment			
	P1	P2	P3	P4
Latency to stand (minute)	51.99±58.60[b]	21.78±18.63[a]	15.16±16.36[a]	7.33±6.27[a]
Latency to suckle (minute)	82.13±53.54	78.27±44.43	43.20±35.96	35.57±31.55

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. [a,b,c], different superscript letters on the same line are significantly different (P<0.05)

is also related to the time of successful standing. Latency to suckle tends to be shorter in Lamb from Ewe fed a flushing diet. Shorter latency to stand means that it can provide nutrients, especially to maintain thermoregulation, passive immune media transfer from the parent, and strengthen the relationship between Lamb and Ewe that can affect the survival rate (Dwyer et al. 2016).

Treatments did not significantly affect (P>0.05) lambs' rectal temperature, heart rate, and breath frequency. Physiological responses to Lamb are presented in Table 4. Maintaining normal physiological responses is important because it can support the body's thermoregulation under normal circumstances(Mota-Rojas et al. 2021). The average heart rate of lambs in the morning, day, and afternoon during the study was still within the average heart rate range in sheep (Seixas et al. 2021). The usual range of heart rate is 70-80 times per minute. The result in this study obtained lower than those reported by (Astuti et al. 2019; Fazio et al. 2016) at the age of 30 days; the average heart rate frequency was 150 per times minute. This difference might be due to the age at which physiological response data collection was started in Lamb, aged 50 days. There is a tendency to decrease the heart rate frequency daily compared to the Lamb at birth (Fazio et al. 2016).

The results of the respiration rate in this study are the same as the report of Astuti et al. (2019) and Fazio et al. (2016) in goats and sheep. In Lamb, respiratory frequency is higher than in adult sheep because Lamb has a more significant proportion of body surface area per body weight than adult sheep. Therefore the respiratory rate is higher (Koether et al. 2015). The respiratory rate of a lamb tends to decrease over time. The decrease in respiratory rate from first birth to 30 days ranges from 20 times per minute^{-1} (Fazio et al. 2016).

The rectal temperature of the lamb range within the normal range reported by Seixas et al. (2021) states that the normal range of average rectal temperature is 38.3-39.9ºC. Normal rectal temperature indicates that the Lamb is in good health. The rectal temperatures in this study are consistent with those reported in Lamb (Astuti et al. 2019; Fazio et al. 2016). The temperature of lambs aged 1-30 days ranges from 39.1 to 39.8℃ under normal conditions. The rectal temperature of a newborn lamb fluctuates until 30 days of age, probably due to two factors: Thermoregulation in Lamb is not very specific, or the mechanism of thermoregulation is still under development (Aleksiev 2009). In this study, the normal physiological response of Lamb suggests that the ewe diet treatment is still able to maintain the thermoregulation of the body in good condition, even though the temperature conditions during the daytime environment may cause increased stress.

Treatment given to the Ewe did not affect (P>0.05) hematocrit values, hemoglobin levels, erythrocytes count, or leukocyte count in the lambs' blood at birth. The hematological data of the seven-day-old lambs are shown in Table 5. Hematological measurements provide essential information about the health status. This study's total number of erythrocyte lamb ranged from 9.46 to 11.24×10^6 mm. The number of erythrocytes Lamb in this study is still in the normal range of $9\text{-}15 \times 10^6$ mm (Al-Jbory & Al-Samarai 2016). Erythrocytes or red blood cells bind and transport oxygen to all body tissues (Glenn & Armstrong 2019). Normal erythrocytes in this study mean that the Lamb born from an ewe with a flushing ration has a normal and healthy red blood cell.

The lamb blood's hemoglobin value (Hb) in this study ranged from 10.60 to 11.64 g/dL, within the normal range. Normal hemoglobin levels range from 8-11 g/dL (Rahman et al. 2018). Hemoglobin functions to bind oxygen in the blood consisting of porphyrin, Fe, glycine and methyl, propionyl, and vinyl side groups (Barupala et al. 2016). The average hemoglobin level in this study means that feeding the Ewe with flushing can maintain the hemoglobin content and the capacity to transport oxygen in the blood of born lambs at normal levels.

Hematocrit is the percentage of blood cells in the total blood volume (Watson & Maughan 2014). The mean hematocrit of Lamb in this study was in the normal range of 29.28% to 32.00%. The normal hematocrit in sheep is 23% to 37%% (Rahman et al. 2018); this indicates that flushing rations fed to the Ewe can improve the hematocrit level of the Lamb at birth under normal conditions.

Leukocytes are active units in the body's defense system and act against antigens or foreign substances entering the body, prevent infection and phagocytosis, and produce or distribute antibodies as part of the immune response—the low total leukocytes in lambs correlated with a high risk for disease attack (Etim 2015). Leukocyte counts in the study ranged from 4.85 to 6.51×10^3 mm. These results indicate that leukocytes are within the normal range. Normal leukocyte counts in sheep range from $4\text{-}12 \times 10^3$ mm (Al-Jbory & Al-Samarai, 2016). This result means that Lamb born from Ewe fed a flushing diet has excellent and healthy immunity.

Treatment had a significant effect (P<0.05) on the leukocytes of weaning Lamb. Leukocyte levels were highest in controls compared with P2 and P3. P4 treatment was not different from the control and P2 and P3. Treatment had no significant effect (P>0.05) on hematocrit levels, hemoglobin content, and erythrocyte counts in the blood of lambs. The hematological data of Lamb sat weaning age are shown in Table 6.

Treatment significantly affected the total leukocyte count in the weaned lambs' blood. Leukocytes are blood components that play a role in maintaining the body's immune system, which is tasked with destroying foreign

Table 4. The physiological response of the Lamb

Parameters	Measurement Time	Treatment			
		P1	P2	P3	P4
Heart rate (time per minute)	Morning	81.50±16.26	80.80±9.96	87.03±12.24	94.10±13.97
	Day	87.50±6.36	83.08±20.24	87.37±6.10	105.67±4.93
	Afternoon	63.50±10.60	77.73±15.37	84.00±11.75	91.20±10.32
Respiratory rate (times per minute)	Morning	51.16±0.23	53.50±7.38	56.13±7.74	48.06±5.88
	Day	56.50±7.77	58.11±5.37	55.20±5.54	63.26±6.19
	Afternoon	49.50±2.12	57.44±6.23	55.23±4.63	48.73±4.52
Rectal temperature (℃)	Morning	39.14±0.41	39.19±0.36	39.13±0.28	39.36±0.21
	Day	39.40±0.07	39.53±0.21	39.64±0.17	39.75±0.17
	Afternoon	38.97±0.45	39.33±0.27	39.41±0.07	39.52±0.22

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. [a,b,c], different superscript letters on the same line are significantly different (P<0.05)

Table 5. Hematology of lamb blood at seven days of age

Parameters	Treatment			
	P1	P2	P3	P4
Erythrocytes (10^6 mm)	10.33±2.07	9.46±2.18	9.70±1.41	11.24±2.17
Hemoglobin (g/dL^{-1})	11.48±1.90	10.60±1.01	10.91±1.10	11.64±1.26
Hematocrit (%)	29.28±4.34	29.83±2.71	27.85±2.47	32.00±5.16
Leukocytes (10^3 mm)	6.51±2.11	4.85±1.41	4.93±1.19	5.56±2.22

P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. ^{a,b,} different superscript letters on the same line significantly different (P<0.05).

Table 6. Hematology of lamb blood at weaning age

Parameters	Treatment			
	P1	P2	P3	P4
Erythrocytes (10^6 mm)	8.41±0.07	8.83±1.94	8.47±0.44	9.73±0.95
Hemoglobin (g/dL)	12.85±0.63	11.56±2.40	11.82±0.74	12.88±0.62
Hematocrit (%)	32.50±2.12	31.00±1.89	31.02±2.36	32.80±1.64
Leukocytes (10^3 mm)	11.80±0.28^a	6.92±2.99^b	6.41±0.73^b	10.54±3.95^{ab}

Source: Peter et al. (2002)
P1= control concentrate, P2= flushing concentrate 6% palm oil, P3= flushing concentrate 3% palm oil 3% lemuru oil, P4= flushing concentrate 6% lemuru oil. ^{a,b,} different superscript letters on the same line significantly different (P<0.05).

bodies that are harmful to the body (Etim 2015). Although treatment P1 has higher leukocyte levels, the result is within the normal range, according to (Al-Jbory & Al-Samarai 2016).

Average leukocyte values in weaning sheep are 4-12 10^3 mm. Average leukocyte values can be interpreted as a sign that there are no non-specific disorders in the Lamb's body and its immune status is in order. The difference in leukocyte levels in the P1 treatment may be related to the type of birth control.

A high number of leukocytes in the P4 treatment indicates that although the type of birth in P4 is more likely to be twins with a lower birth weight than the control (Nurlatifah et al. 2022), it can still produce a lamb with the same total leukocytes as the control, this is consistent with the finding report that differences in the hematological profiles of lambs can be influenced by birth type and birth weight (Ashour et al. 2015).

Supplementation of 6% lemuru oil containing EPA and DHA twice from maintenance to the Ewe may produce a lamb with a good immune system at weaning age. The effect of n-3 PUFA on immunity is thought to be related to changes in the production of eicosanoids such as PGE2. PGE2 may inhibit lymphocyte proliferation. The decrease of PGE2 by EPA metabolism may increase lymphocyte proliferation. The tendency for leukocyte proliferation occurs with an increase in EPA and DHA (Peterson et al. 1998). Fish oil supplementation altered the composition of lymphocyte fatty acids, increasing ω 3-3/ω-6 from 0.18 to 0.62. Supplementation with DHA-rich fish oil results in a 40% increase in lymphocyte proliferative capacity, which depends on Concavallin A as determined by thymidine incorporation. DHA-rich fish oil also causes an increase in phagocytosis of neutrophils and monocyte (Gorjao et al. 2006).

The hematocrit, hemoglobin, total leukocytes, and total erythrocytes obtained in this study are within the range of average values, according to (Al-Jbory & Al-Samarai 2016; Rahman et al. 2018). Hematocrit values, hemoglobin, and erythrocytes are not significantly different, suggesting that feeding flushing feed to the Ewe can maintain normal hematology in lambs born to weaning. The number of hematocrits, hemoglobin, leukocytes, and erythrocytes increased significantly with the lambs' age, which agrees with the result of this study. There is improvement in total leukocyte, hemoglobin, and hematocrit values at the weaning age compared to the neonatal period (Antunović et al. 2012).

CONCLUSION

In conclusion, feeding 6% Lemuru oil containing EPA and DHA on ewe diet during early and late gestation until two weeks post-partum could improve the behavior of newborn lambs by shortening latency to stand from birth to normal standing. Feeding 6% lemuru fish oil showed no significant effects on lamb IgG levels but did alter the total leukocytes of Lamb at weaning. The mechanism of omega-3 effect on lamb immunity needs further investigation.

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