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Colostrum Quality of Ewe Fed Flushing Diet Containing EPA and DHA Associated with Lamb Performance

A. Nurlatifah[a], L. Khotijah[a], R. I. Arifiantini[b], M. S. Maidin[c], & D. A. Astuti[a,*]
[a]Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University
[b]Department of Veterinary Clinics, Reproduction, and Pathology, Faculty of Veterinary Medicine, IPB University
Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia
[c]Department of Biology, Faculty of Science, Universiti Putra Malaysia
Selangor, 43400, Malaysia
*Corresponding author: dewiaa@apps.ipb.ac.id
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ABSTRACT

This study evaluated the effect of a flushing diet containing docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) from lemuru fish oil in ewe ration on colostrum quality and lamb performance. Twenty Garut ewes were divided into four treatments of flushing concentrate: control concentrate (P1), flushing concentrate containing 6% palm oil (P2), flushing concentrate containing 3% lemuru oil and 3% palm oil (P3), flushing concentrate containing 6% lemuru oil (P4). The animal consumed Napier grass and concentrate with a diet ratio of 30%:70% based on dry matter. The experimental ewes were fed flushing rations two weeks before and two weeks after mating continued with 2 weeks before and two weeks after lambing. The ewes and their lambs were put together until weaning time with around two months. The parameters observed in ewes were nutrient consumption and their colostrum qualities. Meanwhile, the lamb parameters measured were nutrient consumption, lamb blood metabolites at birth, and lamb performance. The experimental design used a randomized blocked design (RBD). Analysis of variance (ANOVA) followed by Duncan test was used to analyze data. The results showed that treatment did not significantly affect dry matter consumption in ewe and lamb, ewe's colostrum quality, and lamb's blood metabolites. However, the treatment significantly improved (p<0.05) average daily gain and weaning weights of lambs. In conclusion, the flushing ration did not affect the quality of colostrum produced. Feeding of experimental ewes 2 weeks before and 2 weeks after mating continued with 2 weeks before and 2 weeks after parturition with flushing rations of 6% lemuru oil containing EPA and DHA can produce twin lambs with good growth performances such as daily gain and weaning weight similar to the control, which has single litter size.

***Keywords:** colostrum; flushing diet; lemuru oil; lamb performance*

INTRODUCTION

Nutrition during pregnancy is an important aspect that can be modified because it affects lambs' viability and body composition at birth (Peñagaricano *et al.*, 2014). The development and improvement of reproductive performance during pregnancy have to be planned before pregnancy. The nutritional status of the maternal animal before mating significantly affects fertility and oocyte quality (Grazul-bilska *et al.*, 2012). Additionally, the nutrient status of the mother at the beginning of pregnancy significantly affects implantation, embryo resistance, placental development, and the development and organogenesis of the fetus (Roberti *et al.*, 2018).

Energy demand increases at the end of pregnancy because energy and protein supplies are generally needed for lactation. Providing sufficient nutrients for the ewe and fetus during pregnancy can increase birth weight, implicating better lamb growth until the weaning period (Behrendt *et al.*, 2019). Feeding management during pregnancy affects the present productivity and the next generation's productivity (Schoonmaker & Eastridge, 2013).

Nutrient consumption of ewe during pregnancy and lactation is associated with lamb mortality. The growth rates of lambs in ewes fed with a good quality ration are higher than those of lambs born to ewes fed with insufficient nutrients during pregnancy (Sen *et al.*, 2015). Lamb birth weight significantly affects the resilience of the lamb's performance during the pre-weaning period, which is essential to increase immunity in lambs and promote lamb growth. Besides that, fulfilling nutrient requirements during lactation is essential due to its effect on milk quality. Lamb growth rate is positively correlated with the amount and quality of milk produced by the maternal ewe (Rosales Nieto *et al.*, 2018).

Feeding ewes for six weeks before and four weeks after lambing with fish oil containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) could reduce the weight loss of ewes during lactation and increase milk protein content (Capper *et al.,* 2007). However, supplementation of ration with DHA alga derivate during late pregnancy and early lactation has no effect on body weight, serum, and hormone metabolism but increases the colostrum IgG concentration and increases growth and development of the offspring at the beginning of lactation (Keithly *et al.,* 2011).

One of the local feed ingredients affordable and with high contents of DHA and EPA is lemuru fish oil. Lemuru fish oil contains omega-3 polyunsaturated fatty acids (PUFA), namely, eicosapentaenoic acid 13.70% (EPA) and docosahexaenoic acid (DHA) at the level of 22.47% from total fatty acid. Omega 3 in 5% flaxseed oil fed to ewes can increase twin-type lamb born (Kia & Safdar, 2015). The effects of EPA and DHA supplementation on the reproductive performances of ewes are still debated. A review by Altomonte *et al*. (2018) showed a decrease in milk quality as indicated by the decreased milk fat and protein contents with microalgae in ruminant nutrition.

In contrast, an increase in milk fat content of sheep fed DHA and EPA by using 4.5% fish oil was also reported by Caper *et al.* (2007). However, supplementation of long-chain fatty acid (FA) at the end of pregnancy can also affect FA profile of enterocytes and increase absorption in the small intestine (Garcia *et al.,* 2014). Therefore, the increase in colostrum quality is expected to affect the high nutrition consumed by the lambs so that their growth performances will increase. The effects of DHA and EPA supplementation in the ration of ewes during pre and after lambing have not been recognized with a definite effect on lamb performance. Therefore, this study aimed to evaluate the effect of a flushing diet containing EPA and DHA in the ewes on the quality of the colostrum, blood metabolite, and lamb performance.

MATERIALS AND METHODS

This research was conducted for eight months in the Laboratory of Nutrition for draught animals, Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University. Animal Care and Use Committee of IPB University approved the current study with No 119-2018 IPB University.

Table 1. Compositions of flushing concentrate (100% dry matter basis)

Feed ingredients	Treatments			
	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
$CaCO_3$	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

Note: P1= control concentrate, P2= flushing concentrate with 6% palm oil, P3= flushing concentrate with 3% palm oil and 3% lemuru oil, P4= flushing concentrate with 6% lemuru oil.

Twenty primiparous Garut ewes, 12–14 months of age with an average body weight of 28.92±4.94 kg and lambs born to experimental ewes were used in this experiment and were divided into four treatments. Each treatment consisted of five ewes. Twenty-two lambs were divided into four treatments following the treatment group of maternal ewes. Each lamb with an average birth weight (BW) of 1.88±0.68 kg was observed for two months from birth to two months of age. Ewes and their lambs were kept in individual cages and allowed to suckle from birth until weaning at two months old.

Ration

The experimental ewes were fed a total mixed ration at 3.5% body weight with a ratio between Napier grass: a concentrate of 30:70 based on dry matter (Table 1). EPA and DHA contents in the flushing concentrate were adjusted to the needs of linolenic acid in the ration reported by Pudelkewicz *et al*. (1968), which was 0.5%. The EPA and DHA levels of P3 flushing ration were 0.47%, while the P4 flushing rations were 0.94% (Table 2).

Treatments with flushing concentrate containing different oil sources for ewes were as follows: P1 was control concentrate; P2 was flushing concentrate containing 6% palm oil (flushing diet without being enriched with EPA and DHA); P3 was flushing concentrate containing 3% palm oil and 3% lemuru oil (flushing diet enriched with EPA and DHA according to maintenance); and P4 was flushing concentrate containing 6% lemuru oil (flushing diet enriched with EPA and DHA two times of maintenance).

Flushing concentrate was given to the experimental ewes twice, starting from two weeks before mating until two weeks after mating. Flushing concentrate was also given two weeks before parturition until two weeks after parturition. Other than that, animals were fed with a control ration during pregnancy and lactation.

Table 2. Nutrient content of flushing concentrate and grass dry matter basis (%)

Nutrient	Treatments				*Penisetum purpureum*
	P1	P2	P3	P4	
Dry matter	82.93	83.67	83.60	84.00	22.54
Crude protein	14.39	17.5	17.87	17.49	9.01
Crude fat	1.22	7.84	7.61	7.42	2.11
Crude fiber	9.92	9.47	9.12	9.25	32.57
NFE	63.33	54.15	54.22	54.44	45.03
TDN	71.34	73.16	73.24	72.93	49.48
EPA and DHA	**-**	**-**	**0.67**	**1.34**	-
Palmitic acid	**-**	**2.61**	**1.30**	**-**	-
Ca	0.73	0.82	0.82	0.82	0.46
P	0.65	0.56	0.56	0.56	1.10

Note: Result of the Nutrition and Feed Technology Laboratory, IPB University (2019). P1= control concentrate, P2= flushing concentrate with 6% palm oil, P3= flushing concentrate with 3% palm oil and 3% lemuru oil, P4= flushing concentrate with 6% lemuru oil. TDN= total digestible nutrient; calculation results Wardeh (1981) TDN (total digestible nutrient)= 2.6407 + (0.6964 x %CP) + (1.2159 x %Fat) – (0.1043 x %fiber) + (0.9194 x %NFE). NFE= nitrogen free extract, EPA= eicosapentaenoic acid, DHA = Decosehaxanoic acid.

Measurement of Nutrient Consumption and Colostrum Quality of the Ewe

The nutrient consumption of experimental ewes during late pregnancy was measured from 14 d before to 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 in a day. After getting the amount of feed consumed, daily nutritional consumption was calculated by multiplying feed consumed by each feed's chemical content.

The quality of colostrum was measured by collecting 10 mL of colostrum samples that were milked 24 h after lambing. The samples were placed inside a cool box and taken to the laboratory for quality testing. The milk qualities measured were milk fat, protein, lactose, density, and solid nonfat (SNF). Each sample was measured in duplicate using an automatic ultrasonic milk analyzer (Lactoscan SLP Milk Analyzer). Milk sample was mixed gently 5-6 times to homogenize and avoid air enclosure in the milk sample, then 5 mL of milk were put in the sample holder. The starting button was activated, the analyzer sucked the milk, the measurements were conducted, and the results were shown on a digital display. Before measuring, the lactoscan was calibrated for sheep milk (Vasquezz *et al.,* 2015).

Measurement of Nutrient Consumption, Performance, and Blood metabolite of Experimental Lambs

Nutrient consumptions of experimental lambs were measured up to the age of 62 days. The ration given was a control ration (P1) with a concentrate: forage ratio of 70:30 as much as 3.5% BW by dry matter basis. Nutrient consumption of experimental ewes during late pregnancy was measured from 14 d before to 14 d after parturition, and the animals were given P1, P2, P3, and P4 rations. Daily nutrient consumption of concentrate and forage were calculated by removing residual feed from the offered one in a day. After getting the amount of feed consumed, daily nutrient consumption was calculated by multiplying the amount of feed consumed by each chemical content of the feed.

Birth weights of experimental lambs were measured within 24 hours after birth. After that, each experimental lamb was weighed once a week to determine the average daily gain (ADG), and the weaning weight was measured on weaning on the 56th day after birth. The body condition score (BCS) of each experimental lamb was measured by observing and touching the spine (backbone), loin, and hips (rump) to evaluate fat deposits (reserves) at the time of weighing the lamb on the 56th day after birth. The range of BCS is 1.0–5.0, starting from thin (score 1.0) to fat (5.0), according to Kenyon *et al*. (2014).

Blood samples were taken from the jugular vein of lamb using a 1-mL syringe (Andrade *et al.,* 2019) for measuring blood metabolites on the 7th day of age. Analysis of total blood glucose was conducted using Glucose KIT (Cat No. 112191, Greiner, AU), triglycerides KIT (Cat No. 116392, Greiner, AU), and total cholesterol KIT (Cat No. 101592, Greiner, AU). Enzymatic KIT techniques and spectrophotometer at 546 nm (Genesys 10S UV-Vis, USA) were used to measure blood metabolites. After getting the absorbance values, the blood cholesterol, glucose, and triglycerides concentrations were calculated using the following formulas:

Cholesterol = 200 x [(Sample absorbance/Standard absorbance) mg dL^{-1}]

Glucose = 100 x [(Sample absorbance/Standard absorbance) mg dL^{-1}]

Triglycerides = 200 x [(Sample absorbance/Standard absorbance) mg dL^{-1}]

Experimental Design and Data Analysis

This study used a randomized blocked design (RBD) with four treatments of flushing concentrate, each treatment with five replications of ewes. Grouping of ewes was conducted based on body weight, while lamb was grouped based on the type of birth. The data obtained were tested statistically by Analysis of Variance (ANOVA) (Durakovic, 2017), then followed by Duncan test using Statistical Analysis System (SAS) ver 19.0.1.

RESULTS

Feed Intakes of Ewes During Late Pregnancy

The types of flushing diets fed to experimental ewes had no significant effect ($p>0.05$) on dry matter, crude protein, crude fiber, and total digestible nutrient (TDN) consumptions during late pregnancy. However, the treatment had a significant effect ($p<0.05$) on crude fat consumption during late pregnancy. Nutrient consumptions of experimental ewes fed flushing diet during pregnancy are presented in Table 3.

Colostrum Contents of Ewes Fed Flushing Diets

The treatment of experimental ewes with different flushing rations did not affect ($p>0.05$) the total solid nonfat, density, lactose, fat, and protein contents of colostrum of experimental ewes 24 hours after lambing. The nutrient compositions of colostrum produced by experimental ewes fed with different flushing rations 24 hours after lambing are presented in Table 4.

Lamb Blood Metabolites

Treatments of experimental ewes with different flushing rations 2 weeks before lambing and 2 weeks after lambing did not have a significant effect ($p>0.05$) on blood glucose, cholesterol, and triglyceride concentrations of experimental lambs seven days after parturition (Table 5).

Nutrient Consumptions of Experimental Lambs

Flushing diets were given to experimental ewes 2 weeks before lambing and 2 weeks after lambing had no significant effect ($p>0.05$) on the nutrient consumption of experimental lambs in the weaning period. The nutrient

Table 3. Nutrient consumption of ewe during late pregnancy ($g^{-1}h^{-1}d^{-1}$)

Variables	Treatments			
	P1	P2	P3	P4
Dry matter	1225.3 ± 352.87	1177.5 ± 159.96	931.24 ± 164.25	1008.9 ± 170.99
Crude protein	161.91 ± 43.59	184.05 ± 24.17	149.27 ± 26.88	151.92 ± 25.47
Crude fat	17.33 ± 5.50^{c}	77.46 ± 10.01^{a}	60.22 ± 11.01^{b}	59.50 ± 9.98^{b}
Crude fiber	182.21 ± 65.83	171.40 ± 26.64	130.30 ± 23.17	160.78 ± 29.82
Total digestible nutrient	815.56 ± 222.52	800.06 ± 106.25	636.07 ± 113.53	667.94 ± 112.24

Notes: P1= control concentrate, P2= flushing concentrate with 6% palm oil, P3= flushing concentrate with 3% palm oil and 3% lemuru oil, P4= flushing concentrate with 6% lemuru oil. Means in the same row with different superscripts differ significantly (p<0.05).

Table 4. Colostrum contents of ewes flushing with concentrate containing DHA and EPA fatty acids from lemuru oil

Variables	Treatments			
	P1	P2	P3	P4
Fat (%)	8.87 ± 5.41	10.83 ± 5.44	13.35 ± 8.93	8.14 ± 4.18
Solid nonfat (%)	20.65 ± 0.77	14.56 ± 2.15	17.04 ± 20.03	16.38 ± 5.42
Density (kg L^{-1})	1.074 ± 0.14	1.048 ± 0.03	1.056 ± 0.06	1.056 ± 0.22
Lactose (%)	9.31 ± 0.35	6.80 ± 1.30	7.70 ± 9.04	7.39 ± 2.44
Protein (%)	9.80 ± 0.35	6.90 ± 1.00	8.08 ± 9.50	7.77 ± 2.58

Notes: P1= control concentrate, P2= flushing concentrate with 6% palm oil, P3= flushing concentrate with 3% palm oil and 3% lemuru oil, P4= flushing concentrate with 6% lemuru oil. EPA= eicosapentaenoic acid, DHA = Decosehaxanoic acid.

Table 5. Lamb blood metabolites at birth (mg dL^{-1})

Variables	Treatments				Normal[1]
	P1	P2	P3	P4	
Glucose	88.83 ± 13.15	78.93 ± 7.93	80.33 ± 17.74	71.30 ± 9.72	45–81
Triglyceride	68.14 ± 42.31	75.18 ± 17.01	69.46 ± 18.21	94.47 ± 51.03	6–200
Cholesterol	91.56 ± 28.04	78.15 ± 26.66	82.44 ± 16.71	81.91 ± 32.55	44–90

Notes: P1= control concentrate, P2= flushing concentrate with 6% palm oil, P3= flushing concentrate with 3% palm oil and 3% lemuru oil, P4= flushing concentrate with 6% lemuru oil. [1]Peter *et al*. (2002).

consumptions of experimental lambs at weaning day are presented in Table 6.

Lambs Performances

Birth and weaning weights. Feeding flushing diet to the experimental ewes 2 weeks before and 2 weeks after lambing significantly affected (p<0.05) the weaning weight of the experimental lambs but did not significantly affect (p>0.05) the lamb BW. Lamb BWs and weaning weights of experimental lambs are presented in Table 7.

ADG and BCS at weaning age. Feeding flushing diet to the experimental ewes 2 weeks before lambing and 2 weeks after lambing significantly affected (p<0.05) lamb body weight gain but did not affect (p>0.05) BCS of the lamb at weaning age. The highest ADG increase was found in control, and the lowest was in P3. There were no differences in ADGs between P2, P4, and control as well as between P2, P4, and P3. The weaning weights of experimental lambs in control (P1) and in P2 group were higher (p<0.05) than in P3 group, but there were no differences between P3 and P4 groups as well as between P4, P1, and P2 groups. ADG and BCS of experimental lambs at weaning age are presented in Table 8.

DISCUSSION

Nutrient Consumptions of Ewes During Late Pregnancy

During late pregnancy, dry matter consumption, protein consumption, and TDN consumption in this study fulfilled the nutrient requirements of pregnant ewes having twin embryos. Based on NRC (2007), the nutritional requirements for pregnant ewe with BW of 20 kg was 670 g^{-1}day for TDN and 103 g^{-1}day for crude protein. A significant difference in fat consumption was observed because the fat contents of flushing concentrate (P2, P3, and P4) were 7.5% higher than the control diet with a fat content of 1.2%. Higher fat consumption means higher energy consumption by ewes for preparing parturition and avoiding negative energy balance after lambing. Fat consumption in the control treatment was 17 $g^{-1}h^{-1}d^{-1}$ equivalent with 153 kcal, while the fat consumption of ewes fed P2, P3, and P4 ranged from 59.5 to 77.46 $g^{-1}h^{-1}d^{-1}$, which was equivalent to the potential energy of 535-697 kcal. Fat contains 9 kcal/g and has potential energy higher than protein and carbohydrate (Stinson *et al.*, 2018). Therefore, fat supplementation or a proper nutrition program three weeks before parturition may improve energy balance (Jolazadeh *et al.*, 2019).

Table 6. Nutrient consumption of lamb (2 months age) born to ewes fed with flushing concentrate

Variables	Treatments			
	P1	P2	P3	P4
Dry matter ($g^{-1}h^{-1}d^{-1}$)	206.82 ± 9.35	246.42 ± 39.77	247.12 ± 48.29	262.16 ± 32.29
Crude protein ($g^{-1}h^{-1}d^{-1}$)	28.40 ± 2.26	33.18 ± 4.31	32.62 ± 6.05	34.58 ± 4.91
Crude fat ($g^{-1}h^{-1}d^{-1}$)	4.33 ± 0.11	5.21 ± 0.94	5.29 ± 1.06	5.61 ± 0.63
Crude fiber ($g^{-1}h^{-1}d^{-1}$)	15.85 ± 3.04	21.46 ± 8.15	24.08 ± 6.36	25.62 ± 0.80
Total digestible nutrient ($g^{-1}h^{-1}d^{-1}$)	149.00 ± 8.48	176.00 ± 26.10	175.20 ± 33.37	185.60 ± 24.20
Ca ($g^{-1}h^{-1}d^{-1}$)	1.44 ± 0.11	1.67 ± 0.22	1.65 ± 0.30	1.75 ± 0.24
P ($g^{-1}h^{-1}d^{-1}$)	1.46 ± 0.07	1.79 ± 0.38	1.85 ± 0.39	1.97 ± 0.19
DM consumption (% body weight)	3.30 ± 1.48	2.30 ± 0.65	3.25 ± 0.81	2.60 ± 0.57

Notes: P1= control concentrate, P2= flushing concentrate with 6% palm oil, P3= flushing concentrate with 3% palm oil and 3% lemuru oil, P4= flushing concentrate with 6% lemuru oil.

Table 7. Lambs birth weights and weaning weights

Variables	Born type	Treatments			
		P1	P2	P3	P4
Birth weight (kg)	Single	2.65 (n= 1)	3.64 (n= 1)	-	-
	Duplet	3.25 ± 0.183 (n= 2)	2.36 (n= 1)	2.33 ± 0.62 (n= 4)	2.52 ± 0.37 (n= 4)
	Triplet	1.67 (n= 1)	1.94 ± 0.31 (n= 4)	1.93 ± 0.28 (n= 3)	1.96 (n= 1)
	Average	2.70 ± 0.75 (n= 4)	2.29 ± 0.71 (n= 6)	2.16 ± 0.50 (n= 7)	2.41 ± 0.41 (n= 5)
Weaning weight (kg)	Single	10.81 (n= 1)	12.86 (n= 1)	-	-
	Duplet	12.84 ± 0.03 (n= 2)	12.79 (n= 1)	10.74 ± 0.68 (n= 4)	9.84 ± 1.11 (n= 4)
	Triplet	9.67 (n= 1)	10.08 ± 0.92 (n= 4)	6.34 ± 0.76 (n= 3)	12.07 (n= 1)
	Average	11.54 ± 1.57^{a} (n= 4)	10.99 ± 1.58^{a} (n= 6)	8.85 ± 2.44^{b} (n= 7)	10.28 ± 1.38^{ab} (n= 5)

Notes: P1= control concentrate, P2= flushing concentrate with 6% palm oil, P3= flushing concentrate with 3% palm oil and 3% lemuru oil, P4= flushing concentrate with 6% lemuru oil. Means in the same row with different superscripts differ significantly (p<0.05).

Table 8. Lambs average daily weight gains and body condition scores

Variables	Born type	Treatments			
		P1	P2	P3	P4
Average body weight gain ($g\ t^{-1}\ d^{-1}$)	Single	145.71 (n= 1)	164.64 (n= 1)	-	-
	Duplet	189.23 ± 13.68 (n= 2)	196.32 (n= 1)	153.62 ± 21.68 (n= 4)	153.62 ± 20.62 (n= 4)
	Triplet	142.7 (n= 1)	145.66 ± 18.08 (n= 4)	78.71 ± 16.74 (n= 3)	183.68 (n= 1)
	Average	164.67 ± 27.19^{a} (n= 4)	157.26 ± 24.89^{ab} (n= 6)	121.52 ± 43.95^{b} (n= 7)	137.64 ± 31.32^{ab} (n= 5)
Body condition scores	Single	3.25 (n= 1)	3 (n= 1)	-	-
	Duplet	3.12 ± 0.17 (n= 2)	3.25 (n= 1)	2.87 ± 0.07 (n= 4)	2.81 ± 0.12 (n= 4)
	Triplet	3.0 (n= 1)	2.97 ± 0.25 (n= 4)	2.80 ± 0.09 (n= 3)	3 (n= 1)
	Average	3.12 ± 0.14 (n= 4)	3.02 ± 0.22 (n= 6)	2.83 ± 0.08 (n= 7)	2.85 ± 0.13 (n= 5)

Notes: P1= control concentrate, P2= flushing concentrate with 6% palm oil, P3= flushing concentrate with 3% palm oil and 3% lemuru oil, P4= flushing concentrate with 6% lemuru oil.

Colostrum Content of Ewe Fed Flushing Diet

In this study, the range of colostrum fat contents was 8.14%–13.35%. This value was higher than the colostrum content of the other sheep, according to Nowak & Poindrone (2006). This achievement is due to lemuru oil and palm oil supplementation. This result agrees with the other reports using megalac and fish oil in sheep, which reached 6.8%–9.6% of milk fat (Caper *et al.*, 2007). High colostrum fat is good for lamb as a source of fatty acids, fat-soluble vitamins, cholesterol, triglycerides, and phospholipids (Pereira, 2014). Nutrient content in the diet affects mammary gland development, the onset of lactogenesis, colostrum production, and hormone formation due to its role in regulating and providing the nutrients required to produce milk (Banchero *et al.*, 2015). Colostrum content is essential for survival in newborn lambs and is produced just after parturition containing high nutrients, immune globulin, enzymes, hormones, growth factors, and neuroendocrine peptides (Mašek *et al.*, 2014).

Fat contents of colostrum in experimental ewes fed flushing rations were not significantly different from the control, meaning that DHA and EPA from lemuru fish oil did not reduce milk fat content. This result is different from Kittesa *et al*. (2001) & Capper *et al*. (2007), which reported a decrease in colostrum fat content in the dam supplemented with fish oil. In contrast, no decrease in fat content was also reported by Mattos *et al*. (2002) and Or-Rashid *et al*. (2010), and milk fat content did not decrease in dam supplemented with fish meal. Therefore, the decrease in milk fat can be caused by EPA and DHA that inhibit lipogenesis. However, this reduction occurs if the crude fat content in the ration is high (Mattos *et al.,* 2002).

Lactose in the colostrum functions as the primary energy source and is also associated with the osmotic potential of milk and total milk production (Caper *et al.,* 2007). This study showed that no difference existed in colostrum lactose content between treatments. Milk lactose content in this study ranged from 6.8% to 9.1%. The milk's lactose content is likely to increase continuously with the increase in the lamb ages. According to Sanchez *et al*. (2014), milk lactose increased 3% from birth to 60 days after parturition.

The colostrum lactose content found in the present experiment is also higher than that reported by Caper *et al*. (2007) that adding 5.2% megalac in the ration produced 3.1% milk lactose and supplementation of 4.5% fish oil produced 4.8% milk lactose. The high content of lactose in the colostrum of ewes given a flushing diet can support the optimal growth performance of lamb because high lactose is correlated with the availability of high energy for lamb growth and development.

The protein content of colostrum is not significantly different among the treatments. Colostrum protein in this study ranged from 6.9%–9.8%. These results agree with Or-Rasyid *et al.* (2010) report that fish meal supplementation to the ewe produces 8.13% protein content in colostrum, which continues to decrease with the increasing age of the lamb. Protein content in colostrum is important and available in sufficient quantities for lamb because it functions as a source of amino acids for developing body tissue, as well as passive immune transfer, antimicrobials, facilitating the absorption of nutrients, growth factors, hormones, and enzymes (Hernández-Castellano *et al.,* 2014). Milk protein consists of casein, β-lactoglobulin, α-lactoalbumin, immunoglobulins, serum albumin, lactoferrin, lactoperoxidase, lysozyme, protease-peptone, and transferrin. Each content has a specific function to support health performance (Pereira, 2014).

Solid nonfat in colostrum consists of nonfat dry matter, such as protein, lactose, minerals, and vitamins. The content of solid nonfat in this study was not significantly different, ranging from 14.56% to 20.25%. The results align with the report of Abd-Allah (2013) that high energy and high-protein rations more than the recommendation produce solid Nonfat (SNF) of colostrum in the range of 23.14 to 23.52%. High in SNF contents of colostrum means higher nutrients consumed by lamb. High production and colostrum quality can indirectly increase lamb survival by facilitating filial bonding and increasing lamb neonatal behavior (Agenbag *et al.,* 2021).

Lamb Blood Metabolites

Blood glucose concentrations of experimental lambs were not significantly different among the treatments indicating a reflection of the same sufficient energy status in all experimental lambs. Blood metabolites show the number of nutrients absorbed and circulated throughout the body via blood vessels. Blood glucose levels in this study are in the range of normal levels, same with data reported by Peter *et al*. (2002). Blood glucose concentrations obtained in the present study ranged from 78.93 to 88.83-mg dL^{-1}. This result is higher than that reported by Astuti *et al*. (2011) in growing Garut sheep fed with a basal diet with a blood glucose concentration of 37.50-mg dL^{-1}. This higher result is due to the high quality of colostrum consumed by the experimental lambs. Therefore, high blood glucose concentrations indicate that the lamb has sufficient energy demand. High glucose concentration in the blood in the present study is still in a normal condition that is needed to support the growth and development of lamb to provide optimal performance.

In this study, the blood cholesterol concentrations of experimental lambs were still in the standard range (Peter *et al.,* 2002). Blood cholesterol concentrations obtained in the present study ranged from 78.15 to 91.50-mg dL^{-1}. This result aligns with the result obtained in Garut sheep fed with a control diet having a blood cholesterol concentration of 79.79 mg dL^{-1} (Pujiawati *et al.,* 2018). Normal levels are good because cholesterol functions as a precursor for the synthesis and formation of steroid hormones, maintaining the cell membrane's integrity and forming bile acids in the liver to be used in digesting fats. Lamb blood metabolite will reach the highest cholesterol value on the 30th day and then decrease on days 60th, 90th, and 120th. This condition is related to the diet consumed by the lamb and the development of the rumen organ of lamb (Ashour *et al.,* 2015).

Blood triglyceride concentrations in this study ranged from 68.14 to 94.47-mg dL^{-1}. According to Peter *et al*. (2002), this value is normal. This finding aligns with the result reported by Ashour *et al*. (2015) that studies the growth and development of newborn lamb until weaning. Triglyceride level also agrees with the result reported in Garut sheep fed with a control diet (Astuti *et al.,* 2011). High blood triglyceride levels are essential in lamb because these molecules are used to store energy reserve.

Lamb Nutrient Consumption

Dry matter consumptions of experimental lambs were not significantly different, indicating that the addition of lemuru oil as a source of EPA and DHA in ewe's diet does not affect the ability of lamb to consume rations during weaning. Greenwood *et al*. (2010) explained a tendency to consume more nutrients in lamb born with light BW than heavy ones. However, this study showed no significant difference in dry matter consumption; this result aligns with the lamb BWs that do not differ between treatments and are not affected by weaning at eight weeks.

Treatment of nutrient supplementation given to the ewe during gestation does not affect the feed intake of lambs at weaning. This result agrees with Daniel *et al*. (2007) that post-weaning intake of lambs derived from ewe fed energy-restriction during pregnancy is not different from lamb in the control treatment.

Lamb Performance

Birth and weaning weight. Giving EPA and DHA in ewe 2 weeks before parturition did not affect lamb birth weight but affected the weaning weight. The nonsignificant difference in the birth weights of lambs in this study was almost the same as those reported by Pickard *et al*. (2008) and Keithly *et al*. (2011) that EPA DHA supplementation given to ewe during gestation did not affect the lamb birth weight.

Lamb birth weights in this study ranged from 2.16 to 2.67-kg. The birth weights of lambs in this study are almost the same as those reported by Khotijah *et al*. (2015), which provide flushing rations using sunflower oil. The same birth weights among treatments in the present study have a good potential of treatment with flushing ration since the ewes fed flushing ration tended to have larger litter size with similar BW. Ewes fed flushing rations containing EPA DHA in P3 P4 did not born in a single type, but in duplet and triplet types, and their BWs were the same as the control treatments tended to be single and a duplet born type.

Low weaning weights in P3 agree with data on the lower ADG in P3, and birth weights also tend to be lighter in P3 (Table 7). Low weaning weight in P3 is due to P3 having twin litter size compared to P1 and P2. This condition affected lamb BW at birth. This result shows that even though lamb in P4 is born in a twin type, the weaning weight can be the same as P1 and P2, which have lamb born in a single type.

ADG and BCS weaning age. The highest ADG was found in control because the litter size is less than in ewe fed flushing diet, and the lowest was in P3. The lowest ADG in P3 is likely related to the tendency for birth weight to be lighter than the other treatments. However, the result in ADG of single and twin type lambs in this study was higher than the result reported 141 g/head/day in a single type and 84 g/head/day in twin type (Fassah *et al.,* 2015), or in the single kid is 120 g/head/day (Astuti *et al.,* 2019).

BCS in weaning lamb is closer to describing nutrition sufficiency than bodyweight (Kenyon *et al.,* 2014). BCS in this study was not significantly different between treatments. BCS values ranged from 2.80 to 3.12. This result is higher than BCS on weaning lamb in a grazing area. BCS of single-type lamb was 1.56 and 1.53 in the twin type (Heimbach *et al.,* 2020). This BCS value approaches the ideal BCS value of 3 (Bragg *et al.,* 2021). BCS values < 2 and > 3 are associated with an imbalance metabolism in the body. Greenwood *et al*. (2010) explained differences in body fat characteristics influenced by the ewe's nutrients at the end of pregnancy. Lamb born to ewe given restricted nutrients during pregnancy had a higher proportion of body fat (34.6 *vs*. 27.4%) and lean muscle (65.8 *vs*. 71.9%) than controls.

CONCLUSION

The EPA and DHA from lemuru oil did not affect colostrum quality. Flushing rations of 6% lemuru oil containing EPA and DHA in the ewes can produce twin lambs with good growth performance such as daily gain and weaning weight same as the control, which has a single litter size.

CONFLICT OF INTEREST

We certify no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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