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Supplementation of Palmitic and Omega-3 Fatty Acid Concentrate Flushing During Late Gestation Enhances Ewe and Lamb Performance

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ABSTRACT

Nutrient adequacy during late gestation and early lactation in ewes is critical for reproductive success, metabolic adaptation, and neonatal vitality. This study aimed to evaluate the effects of flushing concentrates containing different fatty acid sources administered during late gestation on reproductive performance, colostrum composition, neonatal behavior, and blood metabolites of ewes and lambs. Fifteen ewes at the fourth month of gestation (35.70 ± 5.32 kg) were assigned to a randomized block design based on body weight to three treatments: R1 (palm oil; rich in palmitic acid, saturated fatty acids), R2 (lemuru fish oil; rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); unsaturated fatty acids (UFA), and R3 (soybean oil; rich in linoleic and α-linolenic acids, UFA). The flushing diets were isocaloric and isonitrogenous, with equivalent omega-3 levels in treatments R2 and R3, and were provided during 2–3 weeks of late gestation and 2 weeks of early lactation. Data were analyzed using ANOVA, followed by Duncan's test. The fat source of each diet did not significantly affect litter size, birth weight, sex ratio, and colostrum composition. Colostrum yield was affected by treatments ($p<0.05$), with the highest udder volume in R1 and exhibited higher IgG levels (10%) in R2. The mean plasma concentrations of glucose and cholesterol in ewes were within normal ranges, indicating sufficient metabolic adaptation. Lambs from ewes fed the UFA diet stood earlier ($p<0.05$), but suckling time was not affected. In conclusion, flushing concentrate supported normal reproductive performance, metabolic status, and neonatal vitality in ewes. The lack of significant differences among fatty acid sources suggests that UFA did not confer additional benefits, likely due to ruminal biohydrogenation.

***Keywords:** colostrum; fatty acid; flushing;lamb performance; reproductive performance*

INTRODUCTION

The productivity of small ruminants, particularly of local ewes, is primarily determined by the reproductive efficiency and quality of lambs. Local sheep breeds are characterized by high prolificacy and lambing rates, non-seasonal breeding, and high adaptability (Ibrahim *et al.*, 2023). However, prolificacy is often limited by nutritional deficiencies during late gestation and early lactation (Chaves *et al.*, 2024), which can disrupt reproduction and productivity (Astuti *et al.*, 2020). Nutritional management through flushing improves reproductive performance (Yildirir *et al.*, 2022).

Dietary supplementation with 5% lemuru fish oil increases embryo numbers in Etawah goats (Astuti *et al.*, 2020). Similarly, Nurlatifah *et al.* (2024) reported that the addition of 6% lemuru fish oil enhanced steroidogenesis, ovulation, conception rate, and prolificacy in ewes. Flushing during the late gestation period supplied sufficient nutrients for both ewes and lambs when energy demands increased up to 1.5 times (NRC, 2007) as feed intake capacity declined (Plante-Dubé *et al.*, 2025).

Fat functions as an energy source, increasing the energy supply without reducing dry matter intake, and regulates reproductive hormones such as progesterone and estradiol, which are essential for gestation and fetal development (Pereira *et al.*, 2022). Palm oil provides SFA, mainly palmitic acid (C16:0), which serves as a stable energy substrate supporting maternal and fetal requirements during late gestation. However, excessive SFA may alter lipid metabolism and reduce membrane fluidity, potentially affecting placental nutrient transfer (Rosa-Velazquez *et al.*, 2021).

Lemuru fish oil is rich in long-chain n−3 PUFA, particularly eicosapentaenoic acid (EPA; C20:5 n−3) and docosahexaenoic acid (DHA; C22:6 n−3), which modulate steroidogenic enzymes and prostaglandin synthesis, thereby improving ovulation, embryo survival, and anti-inflammatory balance (Nurlatifah *et al.*, 2024). Although dietary UFA are susceptible to ruminal biohydrogenation, a proportion of long-chain PUFA and their biohydrogenation intermediates may escape the rumen and exert systemic biological effects (Kyriakaki *et al.*, 2023), including improved membrane fluidity and maternal–fetal nutrient transfer, thereby promoting better lamb survival (Nurlatifah *et al.*, 2022). Soybean oil contains linoleic (C18:2 n−6) and α-linolenic acids (C18:3 n−3), which serve as precursors for the biosynthesis of long-chain PUFA and, despite partial biohydrogenation in the rumen, contribute to reproductive performance, colostrum yield, and udder development in ewes, and to neonatal thermoregulation, which ultimately supports pre-weaning lamb growth (Macías-Cruz *et al.*, 2017).

Additionally, vitamin D enhances calcium absorption and modulates steroid hormone synthesis (Fleet, 2022), improves colostral IgG concentrations and lamb birth weights (Kobeisy *et al.*, 2021). Although extensive studies have been conducted on macronutrient supplementation, such as carbohydrates and fats, in small ruminants (Khotijah *et al.*, 2017; Pujiawati *et al.*, 2018; Astuti *et al.*, 2020; Nugroho *et al.*, 2021; Nurlatifah *et al.*, 2024), studies examining the combined effects of fatty acid profiles and vitamin D on reproductive performance, maternal metabolism, colostrum quality, and lamb performance are still limited.

Therefore, this study aimed to evaluate flushing concentrates enriched with distinct fatty acids sources palm oil (palmitic acid; SFA), lemuru fish oil (EPA and DHA; animal UFA), and soybean oil (linoleic and α-linolenic acids; plant UFA) with vitamin D supplementation to achieve a potential synergistic role in optimizing reproductive performance, maternal metabolism, colostrum quality, and lamb performance in local ewes.

MATERIALS AND METHODS

Ethical Approval

All animals used in the experiment had their research procedures comply with ethical standards for animal welfare and care. This study was reviewed and approved by the Animal Care and Use Committee of the National Agency for Research and Innovation (BRIN), Indonesia (ethical approval number 136/KE.02/SK/07/2025).

Animals and Management

A feeding trial was conducted for six months at the Experimental Farm, and blood analyses were conducted at the Laboratory of Meat and Draught Animal Nutrition, Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, Indonesia. Fifteen pregnant local ewes at 4 months of gestation (average body weight 35.70±5.32 kg) were used in this study. The ewes were housed in individual pens (1.5 m × 1 m) equipped with feeders and waterers. The experiment was conducted over six months, from late gestation (four months of gestation) to 30 days postpartum. A total of 11 liveborn lambs (7 females and 4 males), with an average birth weight of 2.58±0.57 kg, were reared with their ewes without feed supplementation. Environmental temperature and relative humidity were monitored daily, with values from 24.54–32.44 °C and 59%–89%, respectively.

Experimental Diets

The ewes fed a diet consisting of elephant grass (*Pennisetum purpureum*) and concentrate (basal and flushing) at 3.5% of body weight, with a forage to concentrate ratio of 30:70 (dry matter basis); water was provided *ad libitum*. Ewes were fed twice daily at 07:00 and 13:00, elephant grass and concentrate were offered simultaneously at each feeding. The daily feed intake (DMI: g $h^{-1}day^{-1}$) was calculated as the difference between the feed offered and refusals collected the following morning, expressed as dry matter.

All feed ingredients, including forage and concentrate components, were obtained from local commercial suppliers in Bogor, Indonesia. Lemuru fish oil was obtained from the byproducts of lemurus (*Sardinella lemuru*) from Muncar, East Java, Indonesia. Palm and soybean oils were purchased from commercial sources. All oils were stored in airtight containers and incorporated into the concentrate according to the experimental treatment formulations.

The basal ration contained soybean meal, corn, cassava byproducts, copra meal, molasses, premix, salt, and no added oil. During the flushing period (the final 2–3 weeks of gestation and the first 2 weeks after parturition), ewes were assigned to three isocaloric and isonitrogenous concentrate treatments: R1, consisted of palm oil (rich in palmitic acid; SFA); R2 consisted of lemuru fish oil (rich in EPA and DHA; animal UFA); and R3 consisted of soybean oil (rich in linoleic and α-linolenic acids; plant UFA). Lemuru fish oil (R2) was added at half the inclusion level of R1 and R3 to achieve a comparable total omega-3 fatty acid content between fish and soybean oil unsaturated fat sources. In all flushing rations, vitamin D was supplemented at the same level across all flushing treatments, with a two-fold increase compared with the basal diet. The ingredients and nutrient compositions are presented in Tables 1 and 2, respectively. Flushing diets were provided during late gestation and were continued through early lactation for 2–3 weeks. Outside this period, the ewes received a basal concentrate (Figure 1).

Reproductive Performance

Reproductive performance was evaluated based on litter size, birth weight, and the sex ratio of the lambs. According to Somanjaya *et al.* (2022), the litter size was recorded as the total number of lambs born per ewe. The birth weight was measured in kilograms within 24 h of birth, and the sex ratio was calculated as the proportion of males to females.

Table 1. Ingredient composition of basal and flushing concentrate diets (%)

Ingredients	Treatments			
	Basal	R1	R2	R3
Soybean meal	12.88	22.50	22.50	22.50
Corn	32.94	15.71	15.71	15.71
Cassava byproduct	30.38	26.29	26.29	26.29
Copra meal	12.88	14.29	14.29	14.29
Pollard	0	6.78	9.78	6.78
Molasses	6.15	5.43	5.43	5.43
$CaCO_3$	1.08	0.50	0.50	0.50
Premix*	1.01	0.88	0.88	0.88
DCP	1.08	0.50	0.50	0.50
Salt	1.54	1.00	1.00	1.00
Lemuru fish oil	0	0.00	3.00	0.00
Soybean oil	0	0.00	0.00	6.00
Palm oil	0	6.00	0.00	0.00
Vitamin D	0.06	0.12	0.12	0.12

Note: R1 = palm oil–based concentrate (rich in palmitic acid; saturated fatty acids); R2 = lemuru fish oil–based concentrate (rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); animal unsaturated fatty acids (UFA)), and R3 = soybean oil–based concentrate (rich in linoleic and α-linolenic acids; plant UFA). CaCO3, calcium carbonate; DCP, dicalcium phosphate. *Each kg of concentrate contained vitamin A (3,000,000 IU), vitamin E (500 mg), B-complex vitamins, minerals, amino acids, probiotics, antioxidants, and additives.

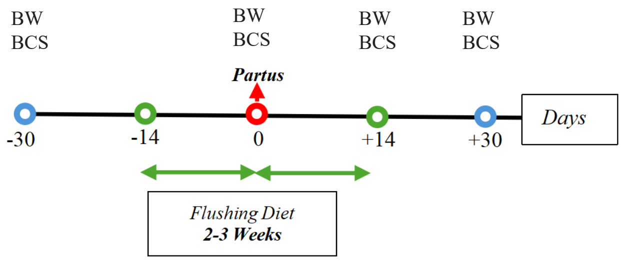


Figure 1. Flushing program timeline of ewes during late gestation and early lactation. Note: BW, body weight; BCS, body condition score.

Colostrum Yield and Quality

Colostrum yield was measured manually by estimating the udder volume (mL) using Archimedes' principle of water displacement with graduated containers, following the modified method of Nurlatifah *et al.* (2022). Colostrum quality was evaluated based on Immunoglobulin G (IgG) concentration using a Brix refractometer (Kerbl® Buchbach, Germany). Colostrum nutrients such as protein, fat, and lactose were determined using a Lactoscan SLP® ultrasonic milk analyzer (Permana *et al.*, 2024).

Blood Profile of Ewes and Lambs

Blood samples were collected to evaluate the hematological profile and plasma metabolites of the ewes and lambs. In ewes, approximately 3 mL of blood was drawn from the jugular vein using sterile disposable syringes in the fourth month of gestation and at 24 h postpartum and was transferred into EDTA tubes. In lambs, blood samples were collected using sterile 1 mL disposable syringes at 7 days of age.

Table 2. Nutrient composition of concentrate and forage (% dry matter basis)

Ingredients	Treatments				
	Grass	Basal	R1	R2	R3
Dry matter (%)	17.59	86.19	86.77	86.36	87.17
Ash (%)	0.94	12.32	7.06	7.41	8.37
Crude protein (%)	10.94	15.74	22.79	24.24	24.23
Ether extract (%)	1.68	2.27	6.42	2.78	6.51
Crude fiber (%)	26.52	13.83	8.11	7.87	8.40
Nitrogen free extract (%)	51.82	55.84	55.62	57.70	52.49
TDN (%)	60.11	66.27	76.61	75.14	74.82
Vitamin D (IU/kg)	–	300	600	600	600
Palmitic acid (%)	–	-	1.65[1]	0.39[2]	0.71[3]
α-linolenic acid (%)	-	-	0.02[1]	0.01[2]	0.46[3]
EPA (%)	–	-	-	0.18[2]	-
DHA (%)		-	-	0.27[2]	-
Total omega-3 (%)	–	-	0.02[1]	0.46[2]	0.46[3]

Note: Results obtained from the Center for Biological Resources and Biotechnology Research, IPB University (2025). R1 = palm oil–based concentrate (rich in palmitic acid; saturated fatty acids); R2 = lemuru fish oil–based concentrate (rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); animal unsaturated fatty acids (UFA)), and R3 = soybean oil–based concentrate (rich in linoleic and α-linolenic acids; plant UFA). TDN: total digestible nutrients, calculated according to Wardeh (1981). EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid. Fatty acid composition was derived from the respective oil components used in each diet formulation. [1] Nurlatifah (2020), [2] Haryati (2024), [3] Feng *et al.*, (2015).

Erythrocyte and leukocyte counts were determined using a Neubauer hemocytometer with Hayem's and Turk's solutions. Diluted samples were examined under a light microscope (10× ocular, 40× objective), and cell numbers were calculated using standard formulas, expressed as $\times 10^6$ cells mm^{-3} for erythrocytes and $\times 10^3$ cells mm^{-3} for erythrocytes and leukocytes, respectively. Hemoglobin (Hb) concentration was measured using the Sahli method, and the hematocrit (or packed cell volume [PCV]) was measured using the microhematocrit after centrifugation at 5000 rpm for 3 min.

Plasma glucose and cholesterol concentrations were analyzed using enzymatic methods using commercial kits (cod. 10260 and 101592) from Human Diagnostics, Germany. The absorbance was measured using a Genesys 10S UV–Vis spectrophotometer at 500 nm, and the concentrations were calculated using the following formulas:

Glucose (mg dL^{-1})= [(sample absorbance/standard absorbance)] × 100

Cholesterol (mg dL^{-1})= [(sample absorbance/standard absorbance)] × 200

Neonatal Behavior

Neonatal behavior was observed for the first two hours after birth, in accordance with Dwyer *et al.* (2003): (1) standing time was defined as the length of time (minutes) from the completed delivery of the lamb until the ability to stand on four legs for a minimum of 5 s; and (2) suckling time was defined as the length of time (min) from the completed delivery of the lamb until it successfully attached to the teat and suckled correctly for a minimum of 5 s.

Lamb Morphometric Measurements

Lamb morphometrics were measured to evaluate skeletal growth and body size. The parameters recorded by Rinaldi *et al.* (2024) included body length, height, and girth (Figure 2). Measurements were collected within 24 h after birth and at 30 days of age using a measuring tape with an accuracy of 0.1 cm.

Experimental Design and Statistical Analysis

A randomized block design (RBD) was used with three treatments and five replicates (blocks). The ewes were grouped according to body weight. Data were analyzed using ANOVA and Duncan's multiple range test was used to test for statistically significant differences. Neonatal behavior was analyzed using a General Linear Model with IBM SPSS Statistics v.25.

RESULTS

Feed Intake

The average daily intake of the ewes during the feeding trial is shown in Table 3. The type of flushing diet did not significantly affect ($p>0.05$) dry matter intake (DMI); however, DMI increased by an average of 8.88% across treatments (Table 3).

Body Weight Changes of Ewes

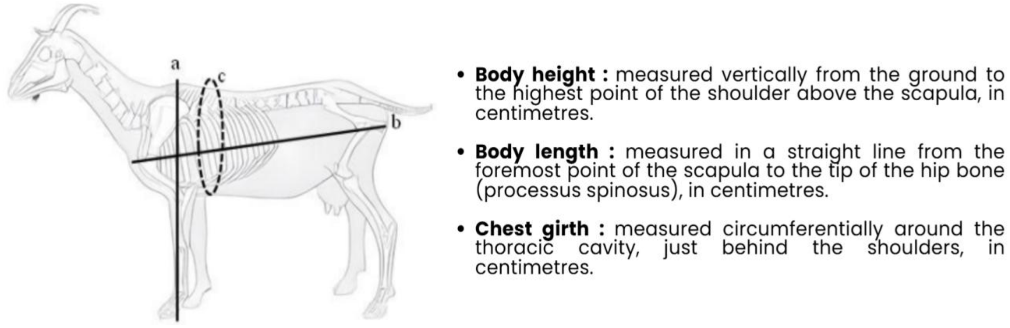
The type of flushing diet did not significantly affect ($p>0.05$) ewe body weight during late gestation or early lactation (Table 3). All ewes experienced physiological weight loss of approximately 16.7%-19.1% from late gestation to parturition, followed by gradual recovery up to 30 days postpartum.

Reproductive Performance

The type of flushing diet had no significant effect ($p>0.05$) on litter size, litter birth weight, or individual birth weight of the lambs (Table 4). The litter size ranged 1-2 lambs per ewe, with average individual birth weights of 1.75–2.17 kg and a mean total litter birth weight of 3.33±1.41 kg, which were within the normal range for local sheep.

Udder Volume and Colostrum Quality

Udder volume was significantly affected ($p<0.05$) by the type of oil source in the flushing diet (Table 4). The mean udder volume was 453.56-539.56 mL. The average composition of colostrum was not significantly affected ($p>0.05$) by treatment, whereas colostral IgG



- **Body height :** measured vertically from the ground to the highest point of the shoulder above the scapula, in centimetres.
- **Body length :** measured in a straight line from the foremost point of the scapula to the tip of the hip bone (processus spinosus), in centimetres.
- **Chest girth :** measured circumferentially around the thoracic cavity, just behind the shoulders, in centimetres.

Figure 2. Method used for morphometric measurements. a, Body height; b, Body length; c, chest girth. Source: Rinaldi *et al.* (2024).

Table 3. Feed intake and body weight of local ewes fed flushing concentrates containing different fatty acid sources during late gestation and early lactation

Variables	Treatments		
	R1	R2	R3
Feed intake (g h-1 d-1)			
4 months of gestation	795.86±84.61	868.64±72.07	872.99±30.41
Parturition	848.72±46.50	828.01±40.67	883.78±61.69
14 days postpartum	880.17±49.90	814.98±59.33	905.21±97.09
30 days postpartum	938.40±93.76	911.87±90.67	934.44±66.62
Body weight (kg)			
4 months of gestation	36.78±6.31	35.84±9.08	35.50±6.41
Parturition	30.63±2.10	29.00±6.31	29.22±5.69
14 days postpartum	28.67±1.70	31.51±5.35	29.50±3.30
30 days postpartum	28.89±3.67	31.18±3.70	30.15±5.80

Note: R1 = palm oil–based concentrate (rich in palmitic acid; saturated fatty acids); R2 = lemuru fish oil–based concentrate (rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); animal unsaturated fatty acids (UFA)), and R3 = soybean oil–based concentrate (rich in linoleic and α-linolenic acids; plant UFA).

concentrations ranged from 20.04% to 24.20% Brix, indicating adequate passive immune transfer across treatments.

The average composition of colostrum was as follows: fat 9.19±2.56%, protein 6.96±2.09%, solid not fat (SNF) 14.70±4.43%, lactose 6.61±1.98%, and density 49.9 ± 18.85 g L^{-1}, which were all within the normal range reported for healthy ewes. These findings suggest that variations in fatty acid profiles among the treatments did not alter colostrum yield, indicating sufficient nutrient availability for normal mammary metabolism and colostrogenesis.

Blood Hematology of Ewes

The flushing diets showed no significant differences (p>0.05) in red blood cell (RBC) counts, hemoglobin (Hb) levels, and white blood cell (WBC) counts among treatments, both during the 4 months of gestation and at parturition (Table 5). The mean values across treatments were 7.96±1.64 × 10^6 mm-3 for RBC, 10.99±1.27 g dL-1 for Hb, 28.73±3.64% for PCV, and 8.94±1.79 × 10^3 mm-3 for WBC, all of which were within normal physiological ranges. However, PCV differed significantly (p<0.05), with slightly higher values in palm oil (R1) compared to the other groups.

Blood Metabolites of Ewes

The flushing diets had no significant effect (p>0.05) on plasma glucose or cholesterol concentrations in ewes during the 4 months of gestation and parturition (Table 5). Average glucose levels were 59.16 mg dL-1; 54.70 mg dL-1, cholesterol 53.83 mg dL-1; 84.68 mg dL-1. Glucose levels tended to decline during parturition, reflecting increased fetal energy utilization and lactation. Cholesterol levels increased during parturition, indicating the mobilization of steroid hormone synthesis and milk production.

Table 4. Feed intake and body weight of local ewes fed flushing concentrates containing different fatty acid sources during late gestation and early lactation

Variables	Treatments		
	R1	R2	R3
Reproductive performance			
Liter size	2.00±1.22	2.00±1.00	1.40±0.55
Litter birth weight (kg)	3.51±1.20	3.46±1.79	3.03±1.47
Birth weight (kg h-1)	1.75±0.98	1.89±0.46	2.17±0.83
Male : Female ratio	4:06	7:03	4:03
Udder volume and colostrum composition			
Udder volume (mL)	539.56±49.96^{a}	486.72±35.62^{ab}	453.56±39.35^{b}
Ig G (% Brix)	22.00±2.55	24.20±6.91	20.04±2.19
Fat (%)	9.27±2.88	9.14±2.76	9.14±2.63
SNF (%)	15.97±3.55	15.15±6.36	12.99±3.09
Density (%)	54.79±15.37	51.72±26.91	43.29±13.94
Lactose (%)	7.18±1.59	6.81±2.85	5.85±1.38
Salt (%)	1.17±0.27	1.12±0.49	0.95±0.24
Protein (%)	7.56±1.68	7.17±3.01	6.15±1.46

Note: R1 = palm oil–based concentrate (rich in palmitic acid; saturated fatty acids); R2 = lemuru fish oil–based concentrate (rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); animal unsaturated fatty acids (UFA)), and R3 = soybean oil–based concentrate (rich in linoleic and α-linolenic acids; plant UFA). Means in the same row with different superscripts are significantly different (p<0.05).

Table 5. Blood hematology and metabolite profiles of local ewes fed flushing concentrates containing different fatty acid sources during late gestation and parturition

Variables	Treatments					
	R1		R2		R3	
	4M	D0	4M	D0	4M	D0
Blood Hematology						
RBC (10^6 mm^{-3})	7.95±2.51	8.89±1.76	7.44±2.23	7.95±1.62	7.84±1.39	7.69±1.47
Hb (g dL^{-1})	10.56±1.21	11.96±2.10	10.44±1.20	11.20±1.18	11.34±0.52	10.44±0.96
PCV (%)	27.40±3.97ab	30.20±3.77	26.0±3.67b	27.80±2.86	31.20±3.83a	29.80±4.15
WBC (10^6 mm^{-3})	7.69±2.13	10.20±3.03	8.97±2.74	9.75±0.74	8.15±1.83	8.86±1.42
Blood Metabolite (mg dL^{-1})						
Glucose	57.60±2.62	51.88±1.64	59.96±1.85	54.82±3.03	59.91±2.39	57.40±4.08
Cholesterol	55.43±3.43	84.83±3.35	50.78±1.90	81.89±2.36	55.27±4.26	87.33±2.74

Note: 4M = 4 months gestation; D0 = parturition; RBC, red blood cell; Hb, hemoglobin; PCV, packed cell volume; WBC, white blood cell; 4M, 4 months last gestation; D-0, parturition; R1 = palm oil–based concentrate (rich in palmitic acid; saturated fatty acids); R2 = lemuru fish oil–based concentrate (rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); animal unsaturated fatty acids (UFA)), and R3 = soybean oil–based concentrate (rich in linoleic and α-linolenic acids; plant UFA). Means in the same row with different superscripts are significantly different (p<0.05).

Neonatal Behavior

The flushing diets significantly affected ($p<0.05$) the standing time of the lambs, whereas the suckling time was not influenced ($p>0.05$) (Table 6). Lambs born to ewes fed the soybean oil (R3) diet stood about 2.2 times faster than those fed palm oil (R1) and 1.25 times faster than those given fish oil (R2). The average suckling time was 40.30±8.20 min and did not differ among treatments.

Lamb Performance

Flushing diets did not have a significant effect ($p>0.05$) on either body weight or morphometrics of lambs at birth until the first week postpartum (Table 7). Across all treatments, lamb body weight increased by approximately 50% within the first week of life, indicating normal growth and adequate milk intake. Ewes receiving UFA (lemuru fish and soybean oils) produced lambs with slightly higher average weights, 4.05 ± 0.60 kg, and larger morphometric dimensions than those fed palm oil.

Blood Hematology of Lambs

The hematological values of lambs at 7 days postpartum were not significantly affected ($p>0.05$) by the maternal dietary treatments (Table 8). RBC and WBC counts, Hb, and PCV remained within normal physiological ranges, with averages of RBC 8.25 $\times 10^6$ mm^{-3}, Hb 10.58 g dL^{-1}, PCV 29.71%, and WBC 8.05 $\times 10^3$ mm^{-3}, indicating good health and adequate colostrum intake.

Blood Metabolites of Lambs

Plasma metabolite levels were not affected by the treatments ($p>0.05$) (Table 8). The mean concentration of glucose was 80.25 mg dL^{-1}, and that of cholesterol was 97.41 mg dL-1, respectively, reflecting stable energy balance and lipid metabolism across all groups. These results show that maternal dietary treatment did not adversely affect neonatal metabolic status.

Table 6. Neonatal behavior of lambs born from ewes fed flushing concentrates containing different fatty acid sources (minutes)

Variables	Treatments		
	R1	R2	R3
Standing	31.33±7.37^{b}	25.0±7.55^{ab}	14.20±6.22^{a}
Suckling	39.33±12.42	47.0±23.43	41.60±9.02

Note: R1 = palm oil–based concentrate (rich in palmitic acid; saturated fatty acids); R2 = lemuru fish oil–based concentrate (rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); animal unsaturated fatty acids (UFA)), and R3 = soybean oil–based concentrate (rich in linoleic and α-linolenic acids; plant UFA). Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 7. Body weight and morphometric measurements of lambs born from ewes fed flushing concentrates containing different fatty acid sources

Variables	Treatments					
	R1		R2		R3	
	D+1	D+7	D+1	D+7	D+1	D+7
Body weight (kg)	2.97±0.42	3.57±1.15	2.25±0.54	4.50±0.48	2.55±0.6	3.93±0.78
Body length (cm)	24.63±0.35	28.70±1.25	23.10±2.60	28.60±1.21	22.44±3.78	26.52±2.32
Body height (cm)	34.33±2.40	36.10±2.80	33.47±3.71	34.70±2.82	31.64±2.85	36.07±3.44
Chest girth (cm)	35.83±2.31	36.37±3.07	32.77±3.21	38.97±1.38	31.76±4.59	38.23±3.45

Note: D+1 = 1 day postpartum; D+7 = 7 days postpartum; R1 = palm oil–based concentrate (rich in palmitic acid; saturated fatty acids); R2 = lemuru fish oil–based concentrate (rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); animal unsaturated fatty acids (UFA)), and R3 = soybean oil–based concentrate (rich in linoleic and α-linolenic acids; plant UFA).

Table 8. Blood hematology and metabolite profiles of lambs born from ewes fed flushing concentrates containing different fatty acid sources at 7 days postpartum

Variables	Treatments		
	R1	R2	R3
Blood hematology			
RBC (10^6 mm^{-3})	7.93±0.42	8.07±0.67	8.75±0.93
Hb (g dL-1)	10.73±0.64	10.73±0.12	10.28±0.93
PCV (%)	32.33±1.53	28.00±1.73	28.80±1.30
WBC (10^6 mm-3)	8.02±0.64	7.97±0.37	8.15±0.70
Blood metabolite (mg dL^{-1})			
Glucose	76.30±2.38	80.48±2.04	83.96±3.34
Cholesterol	99.19±3.45	99.16±2.34	93.89±3.26

Note: RBC, red blood cell; Hb, hemoglobin; PCV, packed cell volume; WBC, white blood cell; R1 = palm oil–based concentrate (rich in palmitic acid; saturated fatty acids); R2 = lemuru fish oil–based concentrate (rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); animal unsaturated fatty acids (UFA)), and R3 = soybean oil–based concentrate (rich in linoleic and α-linolenic acids; plant UFA).

DISCUSSION

Feed Intake

Feed consumption showed an overall increasing trend from 4 months of gestation to 30 days postpartum, with an average improvement of 8.88% across treatments. Although the flushing diet did not produce a significant difference in feed intake, all treatments supported a physiological increase in feed intake as the ewes transitioned to early lactation. This pattern is consistent with that reported by Chen *et al.* (2024), where DMI decreased before lambing and increased significantly after parturition, as the ewe transitioned from late gestation to lactation. This response is closely linked to fetal growth pressure and hormonal adjustments that temporarily reduce rumen capacity and appetite when near term, followed by a marked improvement in intake driven by increasing nutrient demands for colostrum production and milk secretion. Postpartum improvements also contribute to body weight recovery and reproductive performance in ewes, with rumen fatty acid biohydrogenation influencing these outcomes by altering lipid availability and metabolism. Biohydrogenation may convert dietary UFA into SFA forms, limiting duodenal flow but enabling energy provision that aids in DMI recovery and reduces the negative energy balance (Sammad *et al.*, 2022).

Body Weight

The body weight observed during late gestation and early lactation reflects physiological adaptation, with a 16.7%–19.1% reduction, within the normal range of 10%–20% reported for sheep (Cranston *et al.*, 2017). This reduction results from fetal placental expulsion and the mobilization of maternal energy reserves to support parturition and lactation (Pugh *et al.*, 2020). Although the differences were not statistically significant ($p>0.05$), ewes fed lemuru fish oil (R2) and soybean oil (R3) tended to recover their body weights more rapidly than those fed palm oil (R1).

This trend may be associated with differences in energy density and energy partitioning among dietary fat sources rather than the direct post-absorptive effects of UFA. Most dietary UFA undergo extensive ruminal biohydrogenation and, therefore, primarily function as energy sources, similar to SFA. Long-chain n−3 PUFA (EPA and DHA) from lemuru fish oil, as well as linoleic (n−6) and α-linolenic (n−3) acids from soybean oil, are hydrogenated mainly in the rumen; thus, their main contribution during early lactation is to increase dietary energy supply, whereas biohydrogenation intermediates and a small proportion of rumen-escaped PUFA may still modestly influence lipid metabolism and insulin sensitivity (Coleman *et al.*, 2018). R1, consisting of palm oil, rich in the SFA palmitic acid, supplies dense energy (Rosa-Velazquez *et al.*, 2021); however, during early lactation, when production demands are high, palmitic acid tends to partition energy toward milk synthesis and is associated with increased adipose tissue mobilization rather than replenishment of body reserves (Álvarez-Torres *et al.*, 2025). Combined dietary fat and vitamin D supplementation likely supports postpartum energy balance and body weight recovery mainly by improving calcium homeostasis and metabolic adaptation (Nisar *et al.*, 2024).

Reproductive Performance

The flushing concentrate did not significantly influence the reproductive performance (Table 4), indicating that all treatments supported sufficient gestational performance. The litter size 1.4–2.0 observed in this study was slightly smaller but remained within the normal range, which is consistent with previous findings (Nurlatifah *et al.*, 2022). Likewise, the mean birth weight of 1.75–2.17 kg was comparable to the previously reported range of 2.16–2.70 kg, and the average litter birth weight of 3.33±1.41 kg fell within the normal range of 1.70–3.90 kg for local sheep breeds (Mesele & Hadgu, 2024). The lack of significant differences between palm oil (R1), lemuru fish oil (R2), and soybean oil (R3) diets was consistent with their primary roles as dietary energy sources following extensive ruminal biohydrogenation. UFA from R2 and R3 are largely hydrogenated to saturated forms in the rumen, resulting in comparable energy partitioning to support gestational requirements (Macías-Cruz *et al.*, 2017), with no differential effects on fetal development or litter traits. These findings indicate that the effectiveness of flushing is associated more with dietary energy supply than with the fatty acid profile, as ruminal biohydrogenation tends to equalize metabolic contributions, despite differences in unsaturated fatty acid content. Vitamin D supports calcium-mediated uterine contractility and promotes optimal reproductive performance (Nisar *et al.*, 2024).

Udder Volume and Quality of Colostrum

Average udder volume range 453.56-539.56 mL and colostral IgG 20.04%-24.20% Brix indicated adequate passive immune transfer. Palm oil (R1) resulted in a greater udder volume and higher SNF and protein levels, reflecting the role of palmitic acid (C16:0) in preferentially partitioning energy for milk synthesis (Álvarez-Torres *et al.*, 2025), as it serves as an efficient energy substrate for triglyceride synthesis and mammary energy metabolism (Bionaz *et al.*, 2020). Although UFA supplied by lemuru fish oil and soybean oil undergo ruminal biohydrogenation, they primarily function as an energy source, and differences in energy partitioning may still influence colostrum characteristics. Lemuru fish oil (R2) tended to increase IgG and protein concentrations despite its lower volume, which may reflect the effects of dietary energy supply and the contribution of biohydrogenation intermediates, or a small proportion of rumen escaping EPA and DHA to immune transfer (Nurlatifah *et al.*, 2022). Soybean oil (R3) produced a balanced colostrum composition, reflecting the moderate influence of linoleic and α-linolenic acids on mammary metabolism

(Özyürek *et al.*, 2020). Vitamin D improves calcium absorption and mammary gland function, thereby contributing to optimal collostrogenesis (Kobeisy *et al.*, 2021). Collectively, colostrum fat 9.19±2.56%, protein 6.96±2.09%, and IgG > 20% Brix in normal ranges (Kessler *et al.*, 2021), suggesting flushing diets supported mammary function and colostrum quality.

Blood Hematology of Ewes

The hematological parameters of the ewes remained within the normal ranges (Table 5). PCV was slightly lower in ewes supplemented with fish oil (R2), reflecting mild hemodilution mediated by EPA and DHA through the eicosanoid and nitric oxide pathways, all of which were 20%–43% within the normal range (Islam *et al.*, 2018). Mean RBC 7.96±1.64 ×10^6 mm^{-3}, Hb 10.99±1.27 g dL^{-1}, and WBC 8.94±1.79 ×10^3 mm^{-3} were unaffected ($p>0.05$) by treatments, indicating stable hematopoietic and immune functions. A slight increase in RBC and Hb in the palm oil treatment (R1) reflects erythropoiesis supported by palmitic acid (C16:0; SFA), which provides dense metabolic energy and stabilizes metabolic status (Álvarez-Torres *et al.*, 2025). Soybean oil (R3) suggests a balance between lipid metabolism and cellular homeostasis (Bionaz *et al.*, 2020). White blood cells (WBC) increase slightly at parturition, which is associated with parturient stress and uterine involution (Pugh *et al.*, 2020). The WBC count within the normal range is approximately 4-12 × 10^3 mm^{-3} (Santarosa *et al.*, 2022). Adequate vitamin D levels during gestation likely contribute to erythropoietin regulation and calcium-mediated immune function (Rillaerts *et al.*, 2025).

Blood Metabolites of Ewes

The blood metabolite profiles of the ewes were not significantly affected ($p>0.05$) by the flushing diets (Table 5). Mean plasma glucose 59.16 mg dL^{-1} (late gestation) and 54.70 mg dL^{-1} (parturition) and cholesterol 53.83 mg dL^{-1} (late gestation) and 84.68 mg dL^{-1} (parturition) remained within the normal range for ewes around 48.53–75.12 mg dL^{-1} (Samadi *et al.*, 2024), indicating stable metabolic adaptation during late gestation and parturition. Reductions in glucose levels near parturition in R1 and R2 reflect increased glucose uptake by the uterus and mammary tissue to support fetal and colostrum development (Chaves *et al.*, 2024). Ewes fed soybean oil (R3) maintained more stable glucose, consistent with the role of linoleic and α-linolenic acids enhancing insulin sensitivity and hepatic gluconeogenesis (Lima *et al.*, 2025).

Plasma cholesterol concentration declines slightly in late gestation and increases at parturition, reflecting hepatic lipid mobilization and steroidogenesis (Mohammadi *et al.*, 2016) required for parturition and lactation (Pesántez-Pacheco *et al.*, 2019). This pattern aligns with the findings of Nurlatifah *et al.* (2022), which showed cholesterol levels 78.15–91.50 mg dL^{-1} that subsequently increase postpartum as a response to increased hormone synthesis and milk production. An increase in cholesterol also increases vitamin D activity, which enhances calcium-mediated enzymatic reactions (Nisar *et al.*, 2024).

Neonatal Behavior

The standing behavior of lambs was significantly influenced ($p<0.05$) by the flushing concentrate, whereas the suckling behavior was unaffected (Table 6). Lambs from ewes fed unsaturated fat sources (R2 and R3) stood 1.3–2.2 times earlier than those from palm oil-fed ewes (R1), indicating improved neuromuscular coordination and vitality. The average standing time in this study (14-31 minutes) was shorter than the 80 minutes reported by Abdul-Rahman & Yaro (2010).

This improvement may reflect the maternal influence of long- and short-chain UFAs (EPA, DHA, linoleic, and α-linolenic acids), which enhance neuronal membrane fluidity and muscle development (Khotijah *et al.*, 2017). An adequate vitamin D status during late gestation may support calcium and skeletal muscle metabolism, contributing to a faster motor response (Dzik & Kaczor, 2019). The greater neonatal responsiveness in the UFA-fed groups was also in line with the balanced colostrum composition (Table 4), indicating improved maternal energy utilization and neonatal adaptability through coordinated metabolic and neurological mechanisms.

Lamb Body Weight and Morphometrics Measurement

Lamb birth weight and body weight at 7 days postpartum (Table 7) were comparable to those reported by Nurlatifah *et al.* (2022), who reported birth weights of 2.16–2.70 kg at birth and 8.85–11.54 kg at weaning in lambs from EPA and DHA supplemented ewes. Mean morphometric traits at birth were body length 25.57±2.85 cm, height 33.72±2.28 cm, and chest girth 35.13±3.10 cm, which were within the expected ranges for local lambs (Rinaldi *et al.*, 2024). Fish oil (R2) showed slightly greater body length and chest girth, reflecting the anabolic effects of long-chain n–3 PUFA on fetal growth and membrane integrity (Coleman *et al.*, 2018). Palm oil (R1), consisting mainly of palmitic acid (C16:0), provided dense energy but lower postpartum gain, whereas soybean oil (R3), rich in linoleic and α-linolenic acids, promoted steady growth. During late gestation, vitamin D enhances calcium absorption and promotes skeletal development (Nisar *et al.*, 2024).

Hematological Properties of Lambs

Hematological parameters of lambs at 7 days postpartum did not differ significantly ($p>0.05$) across treatments, remained within normal ranges for healthy lambs (RBC 8-18 ×10^6 mm^{-3}; Hb 8-12 g dL^{-1}; WBC 4-13 × 10^3 mm^{-3}) according to Khalil *et al.* (2022), and PCV 22%–38% (Sarmin *et al.*, 2022).

The slightly lower PCV and RBC counts observed following diets containing fish oil (R2) may reflect mild hemodilution due to plasma expansion mediated by

EPA- and DHA (Sharma *et al.,* 2017). Conversely, the supplement with palm oil (R1) supported erythropoiesis through its high energy density, whereas soybean oil (R3) maintained balanced hematological parameters via an improved membrane lipid composition (Bionaz *et al.,* 2020). Mild WBC elevation in groups R1 and R3 suggested normal neonatal leukocytosis due to immune activation following colostrum intake and IgG absorption (Table 4). The similarity in hematological parameters between lambs and their parental ewes (Table 5) indicates that the fatty acid source supports metabolic and immune development.

Blood Metabolites of Lamb

The mean plasma glucose concentration of 80.25 mg dL^{-1} and plasma cholesterol level of 97.41 mg dL^{-1} in lambs at 7 days postpartum were within the normal physiological ranges, indicating sufficient colostrum intake and stable metabolic function. The range in glucose (76.3-85.9 mg dL^{-1}) and cholesterol (88.5-104.2 mg dL^{-1}) levels was comparable to those reported by Souza *et al.* (2020), which suggests that saturated and unsaturated fat sources maintain neonatal homeostasis. In this study, diets containing lemuru fish oil (R2) tended to increase cholesterol concentrations, possibly by enhancing lipoprotein synthesis and hepatic lipid mobilization (Coleman *et al.,* 2018), whereas diets containing soybean oil (R3) were associated with relatively stable blood glucose levels, reflecting the limited impact of PUFA biohydrogenation on ruminal fermentation, thereby sustaining the production of gluconeogenic precursors such as propionate (Lima *et al.,* 2025). The diet enriched with palm oil (R1) provided enhanced energy levels but stimulated less lipid turnover. Diets supplemented with vitamin D improved calcium homeostasis, insulin secretion, and liver lipid metabolism (Nisar *et al.,* 2024), which contributed to the metabolic stability of newborn lambs.

CONCLUSION

Flushing ewes from late gestation to early lactation with diets containing concentrates enriched with different fatty acid sources combined with vitamin D did not significantly affect reproductive performance or colostrum properties in local ewe breeds, but was associated with subtle differences in metabolic adaptations. The absence of differential responses between the SFA and UFA-containing diets suggests that fatty acid types do not provide additional benefits, likely due to ruminal biohydrogenation, and that dietary vitamin D supplementation supports maternal metabolic adaptation and normal neonatal vitality.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest related to the writing and content of this manuscript.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this manuscript, the authors used artificial intelligence tools to assist with language editing, structural refinement, and readability improvement. After using this tool, the authors thoroughly reviewed and revised all content to ensure accuracy and integrity and took full responsibility for the final version of the manuscript.

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