Research Article

Effects of Lemuru Fish Oil and Palm Oil Flushing on Intake, Rumen Fermentation, and Apparent Digestibility in Gestating Garut Ewes

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Abstract | Flush feeding is nutritional strategy design to enhance nutrient quality of diet in short-term period during critical reproductive phase. This study evaluated effect of a flushing diets with lemuru fish oil, palm oil, and their combination on nutrient digestibility and growth performance response in gestating Garut ewes. Twenty ewes were randomly assigned to four idetary treatments; C = control diet PO = palm oil enriched + flushing diet; PFO = lemuru fish and palm oil + flushing diet; FO = Lemuru fish oil enriched + flushing diet. Flushing was applied for two weeks before and after mating, and resume two weeks prior to parturition through two weeks post partum. Outside that period, all ewes were fed with control diet. Feeding consumption and ADG were recorded during gestation. Fermentability and digestibility were observed during flushing period. The results showed that there was no difference (P>0.05) in dry matter intake among all treatments, although fat intake was significantly greater in PO compared to other treatment (P<0.05). Dry matter digestibility and organic matter digestibility were significantly improved in PO group (82.61%) compared to C (76.62%). Protein and fat digestibility also improved significantly (P<0.05) in oil-suplemented group compared to the control group. However, rumen fermentability, blood metabolites, body conditioning score (BCS), average daily gain (ADG), and feed efficiency (FE) were not signifficantly affected by oil supplementation. Inclusion of up to 4.2% palm oil, lemuru fish oil, or their combination in flushing diets improved apparent nutrient digestibility without impairing rumen fermentation or maternal performance, indicating a safe and efficient metabolic adaptation. Although the short-term maternal growth responses were not significant, these findings provide valuable insight for optimizing dietary energy strategies in future applications.

Keywords | Flushing diet, Digestibility, Rumen fermentation, Gestation, Performance, Garut ewes

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INTRODUCTION

The fulfilment of energy intake in the ewes gestation period is a crucial factor (Banchero *et al*., 2015). The gestating ewe requires higher feed nutrients in order to supply the energy and nutrient demands of embryo development, number of embryos conceived, colostrum production, and post-partum milk production (NRC, 2007). However, the pattern of animal farms developed in Indonesia relies on traditional systems and has a tendency to offer feed with inadequate nutrition, leading to a general reduction in livestock performance (Gootwine, 2020). The low quality of forage and concentrate that farmers gives leads to low nutrient uptake in the body of livestock, this imbalance of nutrient intakes continuously will cause obstruction and low nutrient intake to the fetus of the ewe which results in an increased probability of abnormal fetus growth (Meakin *et al*., 2022). Prolonged nutrient deficiency in gestation ewes will impact on increased the risk of prenatal morbidities and mortality (Gootwine, 2020). The maternal body mobilizes its own fat for fetal growth and development when suffering from continuous energy balance (Opsomer, 2015). This condition leads to negative energy balance (NEB), which disrupts the homeostasis of lipid metabolism in maternal liver (Esposito *et al*., 2014). In addition, undernutrition in the gestation ewes will affect fetal metabolism and development also the quality of health and performance of future offspring (Xue *et al*., 2019). The nutritional status of ewe is essential to ensure fetal viability and maintenance of the reproductive function of ewes, especially during the gestation period.

Various energy sources such as oil can be included in the feed ration to prevent maternal malnutrition and promote the growth of the fetus (Astuti *et al*., 2019; Hafez *et al*., 2015; Mahla *et al*., 2017). Oil in feed flushing may provide a natural and practical alternative to address problems of reproductivity and low productivity in ewes, as the treatment does not have to be continuous over a long period of time but only at specific times and for specific durations during the reproductive period. The flushing procedure, where nutrient levels are raised beyond the standard daily requirements for a period of 2-3 weeks prior to mating, also increases ovulation rates and fertility (Nurlatifah *et al*., 2024). Feeding oil as a flushing feed is worthwhile owing to the high energy content of oil, which is up to 2.25 times as much as that of carbohydrates (Haidar *et al*., 2016). In addition, it also improves the desired fatty acid that plays a role in health and reproduction such as polyunsaturated fatty acid (PUFA) from fish oil (Astuti *et al*., 2019; Hafez *et al*., 2015; Mahla *et al*., 2017; Nurlatifah *et al*., 2023). Due to the high biohydrogenation that PUFA undergoes in the rumen as a result of the metabolic processes of rumen microorganisms, the passage of PUFA to the duodenum is hindered, resulting in ewes naturally ingesting low levels of PUFA (Cal-Pereyra *et al*., 2023).

A feasible alternative to modify the amount of PUFA absorbed in the intestines, considering the importance of PUFA for reproduction and animal health, would be to add a source of PUFA itself, such as fish oil, to the feed (Mahla *et al*., 2017). Rich in omega-3 acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), fish oil's omega-3 content is one of its essential components. These DHA and EPA are especially biological agents. They may influence physiological processes and digestion by promoting the transcription of lipolytic genes and minimizing the transcription of lipogenic genes, potentially increasing the utilization of fatty acids for energy, which generates more energy than other metabolizable nutrients (Coleman *et al*., 2018). Lemuru fish oil is a fish waste of industrial processing, which is still rich in EPA and DHA those which are essential for small ruminants, making it suitable for use in flushing feeds while also increasing the value of the waste itself (Kosasih *et al*., 2021). However, the fishy odor of lemuru oil requires an adjustment in the feeding ratio to avoid interfering with the ratio of the total feed intake.

Reports on the fermentability and digestibility of fish oil as a source of PUFA in gestation ewe varies. Some studies report a reduction in digestibility rate as a result of a diet with high PUFA in the oil (Alshdaifat *et al*., 2023). Another work reported an opposite result (Nur-Atikah *et al*., 2018). Meanwhile, another source conveys that combining two oils such as canola as a source of PUFA and palm oil as a source of saturated fatty acid (SFA) can reduce fermentability but does not have a decisive effect on the nutrient intake and digestibility rate in goats (Adeyemi *et al*., 2016), These research results form the basis for the current study. This inconsistency has been influences by the type of oil and the fatty acid content (Candyrine *et al*., 2019). Because highly unsaturated oils can depress cellulolytic activity and fiber digestion, we reasoned that blending fish oil (n-3 PUFA) with palm oil (SFA) could moderate unsaturation-related microbial stress while retaining energy density and supplying long-chain n-3. Thus, we expected the PFO blend to better maintain rumen fermentation than fish oil alone while still improving apparent fat and overall nutrient digestibility. In addition, digestibility also correlates with gestation status. Change in rumen fermentability or digestibility will be correlated with ewe growth rate during gestation and reproductive success (Rahbar *et al*., 2014). Classically, flushing denotes brief energy supplementation before mating to promote ovulation. Here, we use an operational definition short-window, oil-enriched supplementation at two checkpoints: peri-mating (−14 to +14 d) and late-gestation/early-postpartum (−14 d pre-partum to +14 d postpartum). This design tests whether lipid inclusion can improve apparent digestibility while maintaining rumen fermentation under distinct physiological demands. The inclusion of the late-gestation/early-postpartum period aimed to evaluate whether short-term lipid supplementation could enhance digestive efficiency during the most metabolically demanding stage, when nutrient partitioning shifts toward fetal growth and colostrum synthesis. This second window was selected to test if the same mechanism improving fermentability during peri-mating could also mitigate the negative energy balance in periparturient ewes. While reproductive improvements are the classical endpoint of flushing, this study focused on the digestive and fermentative mechanisms that underlie such outcomes, providing a physiological basis for subsequent reproductive studies. Because the supplementation windows were brief and the study was not powered for downstream productive endpoints, we did not expect detectable changes in maternal BW, BCS, ADG, or FE, and reproductive/offspring/lactational outcomes were outside the scope of this study.

MATERIALS AND METHODS

Feeding protocol and supplementation windows

The basal ration consisted of elephant grass (*Pennisetum purpureum*) and concentrate in a 30:70 ratio on a dry-matter (DM) basis and was offered at 3.5% of body weight (DM basis). The control diet was formulated to meet NRC (2007) requirements for twin-bearing ewes. All treatment diets were iso-nitrogenous and iso-energetic relative to the control and contained soybean meal, wheat pollard, dried cassava, lemuru fish oil, palm oil, molasses, $CaCO_3$, premix, and NaCl (Table 1). Four dietary treatments were evaluated: control (C; no added oil), palm oil (PO; 4.2% palm oil of total diet DM), blend (PFO; 2.1% palm oil + 2.1% lemuru fish oil of total diet DM), and fish oil (FO; 4.2% lemuru fish oil of total diet DM).

Table 1: Nutrient composition treatment diet based on dry weight.

Nutrient contents	C	PO	PFO	FO	Penisetum purpureum
	%				
Dry matter	82.93	83.67	83.60	84.00	22.54
Crude protein	14.39	17.50	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
Nitrogen free extract	63.33	54.15	54.22	54.44	45.03
Total digestible nutrient (TDN) [a]	71.34	73.16	73.24	72.93	49.48

C= control Diet; PO= flushing diet using palm oil; PFO= flushing diet using mix lemuru fish and palm oil ; FO= flushing diet using Lemuru fish oil. aTDN= total digestible nutrient; calculated by Wardeh formula (1981).

Short-window supplementation (hereafter, "flushing") was applied during two prespecified periods: (i) peri-mating (−14 to +14 d relative to mating) and (ii) late-gestation/early postpartum (−14 d relative to the expected lambing date to +14 d postpartum). Outside these windows, all ewes received the control diet. Apparent digestibility and rumen-fermentation measurements were scheduled within the supplementation windows, whereas feed intake and ADG were recorded across gestation.

Nutrient consumption

The daily nutrient consumption of concentrate and forage was calculated by subtracting the feed residue data from the feed fed each day. After determining the amount of feed consumed, daily nutrient consumption was calculated by multiplying the feed consumed by the chemical content of each feed.

Rumen fluid sampling

Rumen fluids were collected during the first flushing period before mating, on the 14th day of flushing rations. Rumen fluids were taken 4 hours after feeding through a stomach tube using a vacuum pump. Approximately 100 ml of rumen fluids were collected, pH measured, saturated with HgCl2, and centrifuged to separate the supernatant and precipitate, The supernatant was analyzed for the total content of VFA and NH3. The protozoan population was counted according to the method of Ogimoto and Imai (1981).

Nutrient digestibility (%)

Digestibility at the beginning and end of gestation was measured using the total collection method. Total feces were collected 7 days before the end of the flushing period in early and late gestation. Digestibility was calculated by calculating the percentage difference between the total nutrients consumed and the total nutrients in the feces.

Average daily gain (ADG) and feed efficiency (FE)

ADG is the difference between the final weight and the previous weight. Body weight was measured once a week with a specific scale. FE is calculated from the ADG divided by dry matter consumption.

Body condition score (BCS)

Assessment of the body condition of livestock was done by observing and touching the backbone, loin, and rump to see fat deposits by four people with experience in assessing BCS. The BCS ranges from 1.0 – 5.0 and ranges from very thin (score 1.0) to very obese (5.0) (Russel *et al*., 1969).

Blood sampling

Blood samples were collected from the jugular vein of the lamb using a 3 mL syringe. 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 a spectrophotometer at 546 nm (Genesys 10S UV-Vis, USA) were used to measure blood metabolites.

Experimental design and data analysis

Randomized block design (RBD) with four diets (C, PO, PFO, FO) and n= 5 ewes per treatment (total n= 20). Ewes were blocked by initial body weight into three strata (24.0–26.5, 26.6–28.5, 28.6–30.0 kg) and randomized within block. Primary endpoints were rumen-fermentation indices (total VFA, NH_3, protozoa) and apparent digestibility (DM, OM, CP, EE); secondary endpoints were intake and maternal performance (BW, BCS, ADG, FE). Reproductive, offspring, and lactational outcomes were not collected. Data were analyzed with mixed models: treatment fixed, block random; for outcomes measured in multiple supplementation ("flushing") windows, ewe (nested in block) was a random intercept and window a within-subject factor; single-window endpoints used ANOVA with Duncan's test where appropriate. We report P-values with standardized effect sizes (partial η^2; Hedges' g) and 95% CIs; non-significant results are interpreted as "no clear difference detected within this design." Analyses were performed in SAS (ver. 19.0.1).

RESULTS

Nutrient consumption

The data showed that there was no significant difference on DM intake of ewes neither in the treatment nor control group. However, the effect of treatment on the specific nutrient consumption during gestation is shown in Table 2. In the first month of gestation, the treatment group showed significant differences (P<0.05) compared to the control group in protein intake parameter with treatment PO (154.02± 22.86 g/h/d) and PFO (154.02± 22.86 g/h/d) had the highest values. However, from the second month to the end of gestation, protein consumption was not significantly different between all treatments (P>0.05). In early and late gestation, crude fat intake was greater (P<0.05) in PO compared with PFO and FO (70.46 ± 6.52 g/h/d vs 63.84 ± 10.32 g/h/d vs 64.82 ± 8.57 g/h/d). Fat intake was lowest (P<0.05) in the control (16.38 ± 5.64 g/h/d). Flushing treatment had no significant effect (P>0.05) on intake of crude fiber and nitrogen free extract from the beginning until the end of gestation.

Table 2: Effect palm and lemuru fish oil on nutrient consumption.

Total consumption (g/h/d)	Gestation phase	Treatment			
		C	PO	PFO	FO
Dry Matter	1st month	821.63± 109.03	977.79± 140.27	937.09± 168.59	861.54± 137.24
	2nd month	889.75± 125.30	967.90± 114.08	921.32± 172.73	890.05± 160.25
	3rd month	928.25±191.40	979.99±115.85	879.57±99.17	911.48±175.00
	4th month	1009.17± 175.42	1124.55± 116.76	1004.50± 124.19	1028.92± 174.68
	5th month	1212.68± 352.23	1177.80 ±159.51	931.63± 164.96	1008.99 ±170.45
Crude Protein	1st month	105.04± 10.53 c	154.02± 22.86 a	145.18± 27.30 a	130.32± 21.23 b
	2nd month	126.73±23.57	131.89±17.21	118.36±12.53	120.25±17.34
	3rd month	120.29±24.17	127.33±14.92	114.15±13.21	117.62±23.71
	4th month	146.32±17.38	153.97±19.05	137.85±14.63	139.95±25.52
	5th month	161.52± 43.67	180.02± 19.44	149.89± 35.40	152.88± 26.93
Crude Fat	1st month	16.46± 1.75 c	57.66± 7.58 a	53.15± 12.94 a	49.70 ± 12.67 b
	2nd month	14.49±2.86	15.03±1.87	13.43±1.47	13.76±1.81
	3rd month	13.52±2.90	14.21±1.70	12.78±1.45	13.36±2.38
	4th month	16.07±2.41	16.81±2.58	15.28±1.84	15.39±2.40
	5th month	16.38 ± 5.64c	70.46 ± 6.52 a	63.84 ± 10.32 b	64.82 ± 8.57 b
Crude Fiber	1st month	141.59±24.50	146.42±26.05	139.96±17.58	133.57±18.40
	2nd month	160.75±33.27	166.35±19.25	148.06±17.18	152.84±18.83
	3rd month	148.01±33.58	154.82±18.96	139.52±16.99	147.43±23.85
	4th month	172.65±31.37	179.77±32.42	165.36±22.45	165.59±23.44
	5th month	182.21±65.83	171.40±26.64	130.30±23.17	160.78±29.82
Nitrogen Free Extract (NFE)	1st month	557.35±93.33	614.48±11.82	589.64±63.85	527.58±55.10
	2nd month	572.70±107.22	595.82±77.20	534.44±56.73	543.51±77.66
	3rd month	542.67±109.72	574.05±67.42	514.80±59.20	531.18±105.81
	4th month	658.59±80.01	692.63±87.62	621.03±66.70	630.01±113.07
	5th month	716.95±99.01	713.97±82.42	687.14±86.39	622.01±88.02

C = control Diet; PO = flushing diet using palm oil; PFO = flushing diet using mix lemuru fish and palm oil; FO = flushing diet using lemuru fish oil. a - c: Means in the same row with different letters are differ significantly (p<0.05). Between-group differences in protein and fat intake occurred only during the supplementation windows (months 1 and 5) when diets differed by design; during months 2–4, all ewes received the control diet and intakes were similar.

Table 3: Rumen fermentability of ewe fed palm and lemuru fish oil.

Variable	Treatment			
	C	PO	PFO	FO
Total VFA (mM)	80.43±10.33	73.58±6.98	80.13±12.79	69.34±23.79
NH_3 (mM)	10.97±4.84	12.48±7.05	11.85±3.84	13.58±3.76
Rumen pH	6.54±0.32	6.86±0.21	6.82±0.29	6.60±0.21
Protozoa population (log CFU /mL),	5.42 ± 0.56	5.39±0.46	5.48±0.59	5.23±0.20
Bacterial population (log cell /mL)	5.51±0.43	5.18±0.15	4.71±0.21	5.39±1.29

C= control Diet; PO= flushing diet using palm oil; PFO= flushing diet using mix lemuru fish and palm oil ; FO = flushing diet using lemuru fish oil.

Table 4: Nutrient digestibility of ewe fed palm and lemuru fih oil in early and late gestation.

Digestibility (%)	Gestation phase	Treatment			
		C	PO	PFO	FO
Dry matter	Early	73.48±2.58	80.99±2.63	78.67±1.05	77.02±8.35
	Late	76.62 ± 3.24 b	82.61 ± 1.86 a	80.02 ± 3.44 ab	79.74 ± 4.60 ab
Organic matter	Early	85.06±1.23	89.86±4.25	85.22±6.06	86.06±4.85
	Late	79.43±2.69 b	85.41±1.52 a	82.93±2.95 ab	82.72±4.08 ab
Crude protein	Early	76.89±2.61b	86.54±2.11 a	83.83±1.52 a	83.03±3.79 a
	Late	81.82 ± 3.46	85.72 ± 1.96	85.56 ± 3.15	81.92 ± 5.18
Crude fat	Early	57.76±7.71 b	93.94±1.35 a	93.54±0.22 a	92.12±2.17 a
	Late	88.61 ± 1.66 b	95.71 ± 0.65 a	95.64 ± 0.54 a	94.75 ± 2.45 a
Crude fiber	Early	68.86±2.97	75.85±3.33	73.16±1.61	74.27±11.70
	Late	47.2 ± 13.69	65.49 ± 5.94	59.46 ± 11.22	63.12 ± 10.47
NFE	Early	79.75 ±1.79	83.04±2.40	80.80±0.80	78.37±8.60
	Late	83.46 ± 2.09	87.92 ± 1.07	85.08 ± 2.17	85.81 ± 2.92

C = control Diet; PO = flushing diet using palm oil; PFO = flushing diet using mix lemuru fish and palm oil; FO = flushing diet using lemuru fish oil. a - b: Means in the same row with different letters are differ significantly (p<0.05).

Rumen fermentability

There was no significant effect of treatments (P>0.05) on rumen fermentability, including total VFA, NH3, protozoan population, and bacterial population. The results showed that total VFA ranged from 69.34-80.43 mM. The total protozoan population ranged from 5.23 to 5.48 log CFU m/L in this experiment, both in the control or flushing with oil treatment. The rumen fermentability data for the ewes are presented in Table 3.

Nutrient digestibility

Dry matter and organic matter digestibility were not significantly different (P>0.05) during early gestation. However, it was significantly different on late gestation, flushing treatment showed a significant result (P<0.05) on dry matter and organic matter di1gestibility. Nutrient digestibility data are presented in Table 4. Dry matter and organic matter digestibility in treatment groups were significantly higher (P<0.05) than the control group with PO (82.61 ± 1.86 %) treatment as the highest values. The crude protein values in the treatment group also were significantly different in early gestation (P<0.05) but not at late gestation. Crude protein in treatment PO, PFO, and FO was significantly higher approximately 8% compared to control (P>0.05; 86.54±2.11 %; 83.83±1.52 %;83.03±3.79 vs 76.89±2.61 %, respectively). Flushing treatment significantly increases fat digestibility by approximately 7-36% compared to control both in the early and late phase of gestation (P>0.05; control 57,76% vs. flushing 93,94±1,35% in early gestation and 88.61 vs. 95.71 for control and oil respectively in late gestation). Treatment did not significantly (P>0.05) affect crude fiber and NFE digestibility in early and late gestation. The nutrient digestibility of ewes during early and late gestation is presented in Table 4.

Blood metabolite

The result indicated that the treatment had no significant effect (P>0.05) on all blood metabolite parameters of ewe during early and late gestation which is shown on Figure 1.

Growth performance

The average daily gain (ADG) and feed efficiency (FE) of treated ewes were showed not significantly different

(P>0.05) during gestation.

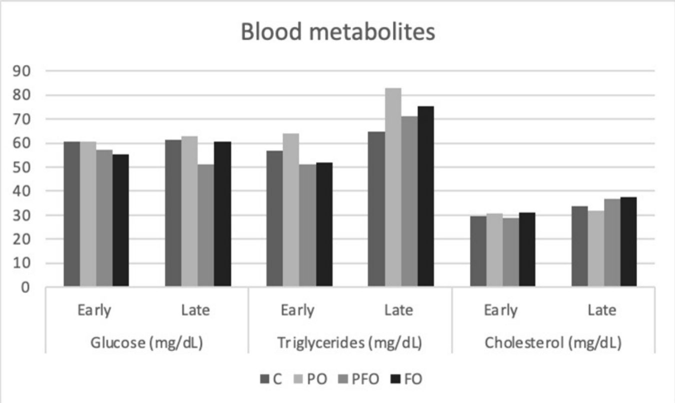

Figure 1: Blood metabolite concentrations (glucose, triglycerides, cholesterol) in ewes during early and late gestation across dietary treatments. No significant differences were observed among treatments (P > 0.05).

Table 5: Average daily gain (ADG) and feed efficiency (FE) of ewe fed palm and lemuru fish oil during gestation.

Parameters	Treatment			
	C	PO	PFO	FO
ADG (g/h/d)	132.82± 16.16	143.90± 48.54	103.98± 15.71	124.14± 32.69
FE (%)	14.52± 5.32	15.36± 4.04	12.34± 1.00	12.87± 2.75

C = control Diet; PO = flushing diet using palm oil; PFO = flushing diet using mix lemuru fish and palm oil; FO = flushing diet using lemuru fish oil. Means in the same row with different superscripts differ significantly (P<0.05).

Body score condition (BCS)

Body score condition (BCS) during gestation was not affected by feeding palm oil and lemuru fish oil (P>0.05). However, there is a tendency for the BCS score to increase with increasing gestational age. The BCS data of ewes during gestation are shown Table 6.

Table 6: Body condition score (BCS) of ewes fed palm and lemuru fish oil during gestation.

Variables	Gestational age	Treatment			
		C	PO	PFO	FO
BCS	1st month	2.94±0.41	3.09±0.39	3.05±0.27	2.84±0.23
	2nd month	2.80±0.31	2.85±0.35	2.95±0.29	2.85±0.38
	3rd month	3.20±0.20	3.05±0.44	3.20±0.20	3.00±0.30
	4th month	3.06±0.31	3.02±0.34	3.26±0.18	3.04±0.21
	5th month	3.05±0.32	3.17±0.21	3.44±0.33	3.30±0.44

C= control Diet; PO= flushing diet using palm oil; PFO= flushing diet using mix lemuru fish and palm oil ; FO= flushing diet using lemuru fish oil.

DISCUSSION

Nutrition intake during the gestation period is a critical role in ensuring suffficient energy for maternal maintanance, weight gain and reproductive success (Kenyon *et al*., 2014; Nurlatifah *et al*., 2024). In this study, average dry matter intake monthly during gestation did not differ signifficantly among treatment. This result was consistent with previous results using canola oil, castor oil, and sunflower oil (Maia *et al*., 2012) and essential oil (Malekkhahi *et al*., 2015) in ewes. However, this study disagreed with previous studies and showed that reduction in DMI was due to low palatability (De la Fuente-Vazquez *et al*., 2014; Mirzaei-Alamouti *et al*., 2021) and higher energy density (Ferreira *et al*., 2014). The results indicate that the use of lemuru fish oil or the increase of the energy density of the diet did not affect palatability and the amount of DMI. This finding was in line with results of previous research that adding 10% of fish oil did not make a significant difference to DM intake. This clarified that a decrease in DMI would only occur when oil addition exceeded 30% due to changes in the feed palatability (Hernández-García *et al*., 2017). Total DMI showed a tendency to increase with the growing gestational period. This increased DMI was related to the requires higher nutrient intake for fetal development (Gallo and Tedeschi, 2020). The total DMI in this study has fulfilled total dry matter intake needed by ewes with twin lambs recommended by NRC (2007) which is 670 g/h/d. From a terminology standpoint, we use "flushing" operationally to denote short-window supplementation. While the classical usage refers to pre-mating, we included late-gestation and early postpartum to capture digestive and fermentation responses under the distinct physiological demands of late pregnancy and the immediate postpartum period. This framing avoids implying reproductive or lactational benefits beyond the endpoints measured here.

The higher protein intake at the early gestation in the flushing treatment (PO, PFO, FO) was expected due to the high protein content in the flushing treatment concentrate which reached 17% while the control was only 14%. There was no difference in the middle of gestation (2nd-4th month of gestation) since those fed the control diet (basal diet) during this period. At the end of gestation, the results did not differ in terms of protein intake, although during the period of flushing feed administered, there was a tendency to increase DMI in the control treatment, resulting in total protein consumed in the control treatment equal to the protein intake of the ewes treated with the flushing treatment. The low protein intake in FO was due to the tendency of lower dry matter in FO. Consumption of protein in the ewes is sufficient for the nutritional needs of the ewes during pregnancy based on the recommendations of the NRC (2007), which was 103 g/day. Consumption of crude protein is necessary to meet amino acid requirements for fetal muscle growth (Sandoval *et al*., 2020). High prenatal crude protein intake is also required to support milk production in post-partum (Ahmed *et al*., 2016). Differences in protein and fat intake were confined to the supplementation windows (months 1 and 5), aligning with the higher lipid/protein content of the flushing diets. During the intervening months, when all ewes consumed the control diet, intakes converged and no between-group differences were detected. Together with the time-linked improvements in apparent digestibility, these patterns indicate acute, diet-composition effects rather than carry-over/programming responses beyond the supplementation periods.

The higher amount of fat intake in early and late gestation also correlated with nutrient content during flushing treatment. The crude fat content in the flushing concentrate was about 7%, while it was only 2% in the control concentrate. The results of this study are in agreement with those of Maia *et al*., (2012) where sheep which fed with canola oil, sunflower oil, and castor oil had significantly increased the fat, crude fiber, and Beta-N intake. The high intake of crude fat is required to boost the body's fat intake for the development of reproductive hormones (Mirzaei-Alamouti *et al*., 2021).

Different from non-ruminants that utilized absorbed glucose as an energy source, ruminants more frequently used volatile fatty acids (VFAs) as an energy source obtained from the process of gluconeogenesis. This study showed that the use of 4.2% on DM basic of palm oil (PO), mixed palm and fish oil (PFO), and lemuru fish oil (FO) did not alter total VFA production; this result was associated with fish oil supplementation in cows in previous study (Darabighane *et al*., 2021) Similar results were reported in cattle fed 5% oil (Hristov *et al*., 2005). VFA was also not significantly different in sheep fed 10 g/kg fish oil and 20 g/kg sunflower seed oil compared to control (Toral *et al*., 2009). The result of this study was higher than other experiments where administration of 5% fish oil resulted in a total VFA of 47.5-54.07% (Szumacher-Strabel *et al*., 2002). The presence or absence of an effect on rumen VFA concentration from oil flushing was highly dependent on the type of oil and forage fed. Feeding equal amounts of oil in this study provides similar data and highlights that feeding fish oil below 10% did not disrupt the rumen ecosystem or affect the rumen fermentation process (Vargas *et al*., 2017). In contrast, feeding oil above 20% generally led to a decrease in fiber fermentation activity in the rumen, with the presence of high oil leading to changes in the ecosystem of rumen microbial activity by blocking the contact of microbial enzymes and feed substrate due to physical coating of high feed fat (Konno *et al*., 2020). Whereas PUFA administration can risk decreasing VFA production and reducing protozoan populations (Hristov *et al*., 2005). In this study, the provision of oil supplementation, palm oil, fish oil, and their mixture, was still at a reasonable level that was more concerned with increasing the efficiency of rumen fermentation and producing VFA that tended to be higher than causing a decrease in rumen activity and causing the potential appearance of metabolic diseases (Lopreiato *et al*., 2020).

The administration of lemuru fish oil and palm oil in this study did not change the total NH3 production. Total NH3 in the rumen was a reflection of the degradation activity of feed proteins and endogenous proteins by rumen microbes to fulfill the N balance in the livestock body (Kamra, 2005). NH3 is dissolved nitrogen found in the rumen fluid for the rumen bacteria to synthesize protein when the carbon chain is available (Bach *et al*., 2005). Inconsistencies in the results of oil administration in sheep rumen had been reported (Gómez-Cortés *et al*., 2008; Zhang *et al*., 2008) It was reported that sunflower seed oil tends to reduce ammonia production in cattle, but fish oil tended to increase it. However, there was no significant difference because of giving lemuru fish oil in a diet of 4.2% indicating that the feed treatment did not affect the activity of proteolytic bacteria in degrading protein to produce nitrogen. This was supported by the findings of the previous study (Toral *et al*., 2009) Experiments with fish oil and sunflower oil showed that N metabolism, ammonia formation, and VFA originating from amino acid deamination were not affected.

Rumen pH in this study was still in normal conditions. The condition of neutral pH was considered ideal for rumen microbes to flourish. This neutral pH was particularly needed by cellulose bacteria to break down fibre (Holtzapple *et al*., 2022). The optimal pH range was 6.4-6.8 to be able to support the development of fiber-digesting bacterial activity (Khaing *et al*., 2016) The same results had also been reported in previous findings (Maia *et al*., 2012). This research showed that adding palm oil and lemuru fish oil has minimal effect on rumen cellulolytic processes of fiber and protein digestion and still maintains pH rumen in normal ranged (Nur Atikah *et al*., 2018). The absence of a significant difference indicated that the use of lemuru oil up to 6% in concentrate can maintain rumen conditions in ideal conditions.

The use of 4.2% fish oil and palm oil in the flushing feed did not change the total protozoa in the rumen. The total number of protozoa in this study was still within the normal range of 104-106 cells/mL (Kamra, 2005). A previous study found that the total number of protozoa may decrease when livestock consume oil. This was because the fatty acids was given will coat the cell membrane of protozoa which do not have lipolytic activity so the protozoa will die and their population will decrease (Tamminga and Doreau, 1991). However, the results indicated differences. Administering fish oil of up to 4.2% in the feed still maintained the total protozoa population. This was because the proportion of oil was at minimum so that it would mix well with other concentrate feed ingredients and this makes the oil not physically cover the rumen microbes and could be used by the host body (bypass) maximally as indicated by the increase in fat intake absorbed by the ewes during the treatment without significantly altering the rumen index parameters such as the VFA, NH3, and rumen pH. However, this still required a further in vitro study. These results supported a previous study that sheep fed with soybean oil, tallow, bergafat or fish oil, rapeseed oil, or linseed oil up to 6%, did not significantly affect rumen fermentability (Szumacher-Strabel *et al*., 2002).

In the early gestation period, the digestibility rates of dry matter and organic matter were not significantly different and this is in line with the initial DMI which was neither significantly different. Conversely, at the end of the gestation period, there was a difference in the rates of dry matter and organic matter digestibility. This was triggered by the high crude fat content in the flushing feed. Giving oil markedly increased the digestibility of dry matter in ewes (Candyrine *et al*., 2019) However, the opposite results were reported in effect of oil administration on digestibility in sheep (Patra and Yu, 2013). The inconsistency of these results could be caused by differences in the concentration of oil used in the feed, as well as the fatty acid content in the ingredients (Candyrine *et al*., 2019). However, the results of this study indicated an improvement in dry matter digestibility. This was probably because the use of 4.2% oil is still within the adaptable tolerance limit for rumen microbes. The addition of oil to the feed with the right amount (4-6% of DM basis) in this research had been found to provide an increase in feed digestion efficiency but without interfering with the rumen ecosystem, this is in accordance with the opinion in the previous study (Candyrine *et al*., 2019). This also agreed with the results of the total rumen microbial and protozoa populations as they were found not significantly different. The high concentration of nutrients in the form of energy contained in the oil may also affect the chances of more nutrients to be digested. Furthermore, the significant results found only at the end of gestation could be correlated with the gestational period. At the time of parturition, the animal produces progesterone and relaxin which prevent the muscles in the digestive tract from contractions and induce relaxation. The relaxation of the muscles in the digestive tract indicates a higher level of nutrient absorption. This is because the surface of the intestinal fibers tends to widen and allows for more optimum absorption (Meyer and Caton, 2016). The general enhancement trend of feed digestibility in these ewes was in line with previous studies (Bhatt *et al*., 2011; Ferreira *et al*., 2014). The larger gains in apparent digestibility observed in oil-supplemented groups are most consistent with diet-composition effects rather than endocrine programming. Increasing dietary EE (from ~2% to ~7%) raises EE digestibility and, via associative and arithmetic effects, can increase apparent DM/OM digestibility even when rumen fermentation indices are unchanged. In addition, post-ruminal lipid digestion and absorption (emulsification, micelle formation, and bile-mediated uptake) occur distal to the rumen and would not be reflected in our rumen measures. Late-gestation hormonal changes likely influence gastrointestinal motility across all groups, and thus cannot explain the between-group differences.

At the beginning of gestation, ewe fed flushing rations had higher protein digestibility due to the high crude protein content in flushing diet. These digestibility data were consistent with protein consumption, also being significantly different among treatments at the beginning of gestation. This increase in protein digestibility suggested that increasing the oil content in the ration does not affect the absorption of protein in the body. The lack of differences in crude protein digestibility at the end of gestation was consistent with no significant differences in protein consumption at the end of gestation. This pattern was also consistent with protein consumption in late gestation due to flushing treatment. The improvement in crude protein digestibility in the flushing treatments compared to the control, suggested that the addition of oil is used as an energy source for rumen microbes, which were the main source of protein for ruminants and have a higher digestibility level (Nur Atikah *et al*., 2018).

Although fat and protein digestability improved in the oil supplemented group, this did not translated into significantly higher ADG or feed efficiency. Although apparent digestibility improved, maternal BW, BCS, ADG, and FE were unchanged. Without direct measurements of fetal/neonatal or lactational outcomes, body composition, or energy balance markers, we cannot infer nutrient partitioning; thus, the present data should be viewed as digestive/fermentation responses rather than demonstrated productive benefits. This indicate that digestability alone may not determine growth performance especially in gestating ewe where energy and nutrient not only allocated directly for growth but also for fetal development. Although apparent digestibility increased, maternal growth metrics (BW, BCS, ADG, FE) were unchanged. During gestation, nutrients can be partitioned to conceptus growth, placental tissues, mammary development, or elevated maintenance costs rather than maternal accretion. We did not measure lamb birth weight, viability, or milk yield; therefore, we cannot ascribe a productive benefit to the higher digestibility observed. Alternatively, improved apparent digestibility may coincide with unmeasured metabolic costs (e.g., oxidation of absorbed fatty acids) or shifts in fecal/urinary energy that our design did not capture. Consequently, our results should be interpreted as maintenance of rumen function with improved apparent digestibility rather than evidence of beneficial nutrient allocation. As for FE, it ranged from 12.34 % to 15.36%. This feed efficiency rate indicated that about 4-17% of the total ration consumption per day is capitalized in the body to achieve an increase in the body weight of the breeding animal. The BCS describes the state of the body fat. It could describe the status of the nutritional adequacy status (Kenyon *et al*., 2014). The BCS from the beginning until the end of pregnancy met ideal BCS conditions for sheep is BCS 3 (Maurya *et al*., 2009). Other studies reported that BCS 3.00-3.50 had better reproductive performance in sheep compared to sheep with a BCS higher or lower than this range (Sejian *et al*., 2010). High and low BCS negatively affected ovulation, embryo loss, and conception rates which affect gestation success (Kenyon *et al*., 2014). Animals with a good BCS were not impaired in the production of reproductive hormones such as GnRH, estrogen, FSH and LH (Scaramuzzi *et al*., 2006). High body reserves before calving were very important to prevent cattle from the risk of negative energy balance after lambing (Kalyesubula *et al*., 2019). An ideal BCS also meant that animals have sufficient energy reserves to meet their maintenance needs as well as their reproduction, so they did not need to mobilize energy from body reserves which can reduce body weight.

While study repoorted the promising result with the digestibility, the certain limitations should be acknowledged. The relatively small sample size may limit statistical power, especially in detecting subtle differences. The body weight increase in this study may not fully reflect maternal tissue accretion, but the cumulative weight of the fetus, placental tissues, and related fluids. This raises limitation in interpreting growth resposes solely from the weight. Interestingly, despite the increase in digestibility, No-signifficant weight are observed, suggesting increasing nutrient efficiency without excessive maternal deposition. Further studies are needed to precisely partition maternal versus fetal growth during gestation.

The inclusion of palm oil and lemuru fish oil in flushing diets present a practical feeding strategy to improve nutrient digestibility in gestating ewes without negatively impacting on rumen fermentation. This may particularly useful in tropical production where dense energy diet are needed for reproductive efficiency. Additionally the use of locally available source of oil such as palm oil and lemuru fish oil may cause cost effective support for sustainable fat supplementation.

This trial was designed to evaluate digestive and rumen-fermentation responses within short supplementation ("flushing") windows, with n=5 per treatment, which restricts statistical power and the precision of effect estimates. Reproductive outcomes (ovulation/lambing rate, litter size), offspring metrics (birth weight, neonatal survival), and lactational outputs (milk yield) were not collected, and maternal body composition or energy-balance markers were not assessed; therefore, we cannot infer nutrient partitioning or downstream productive benefits. Changes in maternal BW/BCS during gestation may also partly reflect conceptus, placental tissues, and fluids, limiting interpretation of "growth." Accordingly, non-significant findings are interpreted as no clear difference detected within this design, not evidence of equivalence. Future studies with larger cohorts, longer follow-up, and prespecified reproductive/lactational endpoints are needed to confirm the magnitude and practical relevance of these responses.

CONCLUSION

In conclusion, Oil-enriched short-window supplementation (up to 4.2% palm and/or lemuru fish oil) improved apparent digestibility while maintaining rumen fermentation in gestating Garut ewes; within this design we did not detect changes in maternal BW, BCS, ADG, or FE. Effects were confined to the supplementation windows, with no evidence of carry-over beyond supplementation. Because reproductive, offspring, and lactational outcomes were not measured, these findings should be interpreted as digestive/fermentation responses, and adoption should depend on local cost and logistics rather than an expectation of higher lamb output. Larger, outcome-focused trials (birth weight/survival, milk yield, conception/lambing rates) are warranted to determine tangible productive benefits. This study demonstrates that short-term supplementation with a palm–fish oil blend safely improves digestibility without impairing rumen fermentation in gestating ewes, and underscores that digestibility improvements alone may not predict productive performance.

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NOVELTY STATEMENT

This study demonstrates that short-term (short-window) oil-enriched flushing using palm oil, lemuru fish oil, or their combination (up to 4.2% dietary dry matter) can improve apparent nutrient digestibility without disrupting rumen fermentation in gestating Garut ewes. The novelty lies in evaluating digestive and rumen responses during two critical reproductive phases (peri-mating and late gestation/early postpartum) rather than continuous supplementation. These findings provide new physiological insight into the safe use of mixed saturated and polyunsaturated lipid sources as a practical energy strategy for small ruminants under tropical conditions.

AUTHORS CONTRIBUTION

AN, LK, DAA, and KK conceived and designed the experiment. RIA, MS, and HH supervised and coordinated the research and provided clinical data. Statistical analysis was conducted by IHZ, AMD, and PIS. The initial draft of the manuscript was prepared by AN, DAA, IHZ, and AMD. All authors critically reviewed and approved the final version of the manuscript.

Ethical approval

This study was conducted at the Meat and Draught Animal Nutrition Laboratory, Department of Nutrition and Feed Technology, Faculty of Animal Science, IPB University. Twenty primiparous Garut ewes aged 12-14 months with body weight 28.92 ± 4.94 kg and BCS 2.69 ± 0.10 were divided into four treatments. All animal procedures were reviewed and approved by the Animal Care and Use Committee (ACUC) at IPB University No. 119-2018 IPB, and adhered to Indonesian animal welfare guidelines.

Generative AI and AI-assisted technology statement

The authors declare that no Generative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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